A COMPARATIVE EVALUATION OF DIFFERENT MEMBRANES FOR THEIR DIFFUSION EFFICIENCY: AN IN VITRO STUDY

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ABSTRACT
The present study was undertaken to determine the diffusion rate of a drug from a semisolid dosage form using different membranes and to explore a membrane and to find out the comparative membrane identical to human skin with respect to diffusion rate of the drug from the selected dosage form. In the present study, Franz cell was used to carry out the diffusion study through fuzzy rat skin, hairless mouse skin, egg membrane, cellophane membrane and human skin. Diffusion rate of the drug was determined in 7.4 pH phosphate buffer for twelve hours, maintaining temperature to 37± 0.5°C. The drug retention in the was also determined at the end of the diffusion studies. The study concluded that the rate of drug diffusion through the fuzzy rat skin was identical to that of human skin. Minimum and maximum drug retention was occurred in cellophane and fuzzy rat skin membranes respectively.

Key words: Diffusion rate, semi permeable membrane, Franz cell.

INTRODUCTION
Alternative drug delivery systems like transdermal drug delivery systems are very useful for efficient delivery of drugs which undergoes extensive first pass metabolism or are susceptible to enzymatic and acidic degradation. Diffusion studies are one of the vital evaluation parameter which decides the efficiency of transdermal dosage form. There is a vast disparity in the diffusion rates of the drugs evaluated using different semipermeable membranes.¹,² A need arises to identify a model semipermeable membrane which would show identical diffusion rate of the drug comparable to human skin, and thus would help in identifying the true efficiency of a transdermal dosage form.³,⁴
MATERIALS AND METHODS

Materials

Salicylic acid was purchased from Sigma Aldrich, all the other chemicals used were of analytical grades. The animals use in the study was approved by animal ethical committee, Sigma Institute of pharmacy, CPCSEA registration no. 934/A/06/CPCSEA.

Experimental Methods

Calibration curve of Salicylic acid in pH 7.2 buffer

Calibration curve of Salicylic acid was prepared in pH 7.2 phosphate buffer using UV visible spectrophotometer (UV 1601, Shimadzu). 10 mg of drug was dissolved in 100ml of buffer to obtain the working standard of 100 µg/ml. Aliquots of 0.2 ml to 1.0 ml from the stock solution representing 2 to 10 µg/ml of drug were transferred to 10 ml volumetric flask and the volume was adjusted to 10ml with the solvent. Absorbance of the above solution were taken at 297 nm against the blank solution prepared in the same manner without adding the drug. A graph of absorbance Vs concentration was plotted and was found to be linear over a range of 2 to 10 µg/ml indicating its compliance with Beer’s law.

Preparation of salicylic acid ointment

Salicylic acid ointment was prepared employing lavigation technique wherein required dose of finely sifted salicylic acid through 120# sieve was gradually added in the prepared ointment emulsifying base containing emulsifying wax, white soft paraffin and liquid paraffin.

Pretreatment procedures for different selected membranes [5,6,7]

Human skin: Human skin obtained from dermatological department S.S.G hospital, Baroda was used. After surgical excision of the skin, the fatty tissues and the blood vessels were removed and the skin was stored at 4°C in phosphate buffer of pH 7.4 containing some antibiotics like penicillin and streptomycin to prevent degradation. In the present study preservation was done by adding gentamycin (0.16mg/ml). The skin was then sent to the dermatology department where it was dermatoned into 0.5mm thickness.

Egg membrane: The membrane was treated with 0.1N HCl over 36hrs so that the acid reacts with the calcium and aids the removal of the outer shell of the egg membrane.
reaction is indicated by the appearance of bubbles. Finally the separated membrane was washed with distilled water for several times.

**Cellophane membrane:** Cellophane membrane no. 300 purchased from Merck KGaA, Germany, was dipped in water for 24hrs and then in increasing concentrations of zinc chloride solution. The pore diameter of the membrane after the treatment is reported to be 80 microns\(^7\).

**Fuzzy rat skin:** Skin was excised and the fatty tissues and blood vessels underlying were removed and skin was stored in phosphate buffer pH 7.4 containing antibiotic gentamycin (0.16mg/ml) until further use.

**Hairless mouse skin:** Skin was excised and the fatty tissues and blood vessels underlying were removed and skin was stored in phosphate buffer pH 7.4 containing antibiotics like gentamycin (0.16mg/ml) until further use. Follicle free hairless skin was obtained by immersing the anaesthetizes mice into 60°C warm water for a minute followed by removal of the epidermis and healing for three months.

**In vitro evaluation of diffusion rate:** \(^8,9,10,11\)

![Schematic diagram of Franz diffusion cell](image)

**Figure 1**

Schematic diagram of Franz diffusion cell

The permeation kinetics of the drug was determined using a two compartment franz diffusion cell. Selected membranes were mounted between the two compartments of the diffusion cell and ointment equivalent to 10 mg of salicylic acid was applied on the drug releasing surface. Phosphate buffer pH 7.2 was used as the diffusion medium. The
diffusion profile of the drug was obtained by sampling 3ml of an aliquot from the receptor solution at predetermined intervals until 12 hours. The sampled aliquot was every time replaced by fresh diffusion medium of the same quantity. Throughout the studies temperature of 37± 0.5°C was maintained.

**Determination of drug retention**\cite{12,13,14}

The amount of the drug retained within the selected membrane was determined by cutting the membrane used for diffusion studies into small pieces and then sonicating it using ultra sonifier (EIE instruments, Delhi at 20,000Hz frequency) after immersing it in the phosphate buffer of pH 7.2 for 60 minutes. The resultant fluid was then filtered and drug content in the fluid was estimated at 297 λmax by using UV spectrophotometer.

**RESULTS AND DISCUSSION**

**In vitro evaluation of diffusion rate through different selected membranes**

The diffusion profile of salicylic acid ointment performed through egg and cellophane membrane showed that at the end of 12 hours nearly 75% of drug was getting diffused through the membranes as evident in Fig. 2,3. It was observed that the diffusion rate of drug through ointment was found to be 85% when human skin was taken as a membrane. A handsome variation in the diffusion rate of the drug through egg and cellophane membranes was observed when compared with human skin. Again the diffusion rate of the drug was found to be almost 85% in 12 hours when hairless mouse skin was used as a membrane. Whereas when fuzzy rat skin was used a membrane the diffusion rate was found to be 86% in 11 hours which was almost identical to the diffusion rate of the drug through human skin.

![Calibration curve of salicylic acid in pH 7.2 buffer](image)

*Figure 2*
Calibration curve of salicylic acid in pH 7.2 buffer.
**Figure 3**
Diffusion Profile of salicylic acid ointment: M1-Human skin, M2-Egg membrane

**Figure 4**
Diffusion profile of salicylic acid ointment: M1-Human skin, M3-Cellophane membrane
Figure 5
Diffusion Profile of salicylic acid ointment: M1-Human skin, M4-Rat fuzzy skin

Figure 6
Diffusion Profile of salicylic acid ointment: M1-Human skin, M5-hairless mouse skin

Determination of drug retention

The results of drug retention data as seen in Fig. 7, indicated that human skin and fuzzy rat skin showed maximum amount of drug retention that is 24 and 22% respectively. The hairless mouse skin showed almost 17% of drug retention. Whereas the drug retention was found to be as low as 3% and 4% in egg and cellophane.
membrane. The possible reason for the variation in drug retention levels may be the thickness of the membranes used. Since the thickness of human skin and fuzzy rat skin membranes is greater it retained more drug, while egg and cellophane membrane are comparatively thinner and showed less amount of drug retention.

Figure 7
Extent of drug retention in several membranes M1-Human skin, M2-Egg membrane, M3-Cellophane membrane, M4-Rat fuzzy skin membrane, M5-hairless mouse skin.

CONCLUSION

The results of the present study concluded a handsome variation in diffusion rate of salicylic acid through different membranes. Similarly, drug retention in different membranes showed remarkable difference. Out of all selected membranes fuzzy rat skin membrane showed the best results of drug diffusion rate and drug retention rate which can be correlated with human skin. Thus it was considered as a comparable model membrane to be used in place of human cadaver skin for determining the efficiency of a transdermal dosage form.
REFERENCES


