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Association of Permeability and Lipid Content of Membrane

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Background: Rat skin and goat *cul de sac* are mostly used in optimization of formulations as the model of human skin and *cul de sac*.

Aim: To explore the correlation between lipid content of rat skin and goat *cul de sac* and permeability.

Materials and methods: Find out wavelength maximum for Sapat plus malam®, Ciplox eye ointment® and chloramphenicol eye caps and the standard curve was also derived. *In vitro* studies using Cellophane® membrane and *ex vivo* studies using rat skin or goat *cul de sac* of the formulations. Permeability coefficient, % dislodgeable dose, lag time, diffusion parameter, and partition coefficient were found for both studies after six and a half hours of penetration studies. Student's unpaired t-test with equal variance was used to find any statistically significant difference in the *ex vivo* and *in vitro* diffusion transport studies at 95% level of confidence.

Results: Permeability coefficient of Sapat plus malam®, Ciplox eye ointment® and chloramphenicol eye caps were 0.000316 ± 0.0000625, 0.00416 ± 0.0001, 0.0034 ± 0.00004 for Cellophane® membrane and 0.0001 ± 0.000001, 0.002254 ± 0.0002, 0.00303 ± 0.0001 for *ex vivo* membrane in cm^2/min, respectively. For all three formulations, there were calculated t values which were higher than tabulated t values at 95% of confidence level ($P<0.05$).

Conclusion: Cellophane® membrane shows a better diffusion than rat skin or goat *cul de sac*. In the optimization of formulation, only Cellophane® membrane is advisable to use.

BACKGROUND

Most of the drugs delivered across topical or ocular route are effectively affected by lipid content of stratum corneum and conjunctiva respectively. Various membranes are used for *ex vivo* studies because human skin and eye are difficult to acquire due to ethical issues. The model Cellophane® membrane is the most commonly used among model membranes because of its nature. It is made of pure cellulose and it is free from fat content. Therefore, there could be no interference of lipid content during the drug penetration. For topical preparation rat skin and for ophthalmic preparation ocular goat *cul de sac* are also used because rat and goat are most freely available animals and they are easy to handle as well.[1,2] In the present study, a comparison was made of the permeability of well-known Indian market brands Sapat plus malam®, Ciplox eye ointment® and chloramphenicol eye caps in Cellophane® membrane and permeability through rat skin and goat *cul de sac,* respectively. The study concluded that there was a strong negative monotonic correlation between membrane lipid content and formulation's permeability.

AIM

The aim of this study was to explore the correlation between lipid proportion of rat skin and goat *cul de sac* and drug permeability.

MATERIALS AND METHODS

Sapat plus malam®, Ciplox eye ointment® and chloramphenicol eye caps were purchased from Sapat and Co. Pvt. Ltd, Mumbai, India, Cipla Ltd, Mumbai, India and Jyoti capsules, Kanpur, India, respectively. Cellophane® membrane was purchased from Angle trading, Rajkot, India. Methanol, sodium chloride, sodium hydroxide and potassium dihydrogen phosphate were purchased from Oxford lab, Mumbai, India. Rat skin was procured from the

animal house, School of Pharmacy, RK University, Rajkot, India. Goat eye were delivered from a local slaughterhouse for human feeding.

Preliminary studies

100 mg of Sapat plus malam® were soaked in 100 mL phosphate buffer pH 6.8 overnight. 100 mg of Ciplox eye ointment® and chloramphenicol eye caps were soaked in 100 mL methanol/ phosphate buffer (25:75% v/v) pH 6.8 and pH 7.4 overnight, respectively. Then they were filtered through filter paper of 11 µm pore size. The filtrate was scanned in 200–400 nm by Double Beam UV–visible Spectrophotometer (LT–2900, Labtronics (I) Pvt. Ltd., Ambala, India). The wavelength at which absorbance was maximal was considered as wavelength maximum for the prospective study. Standard curve was also derived at wavelength maximum.[3]

Organoleptic assessment and pH value

Particular organoleptic features of the formulations like appearance, homogeneity, texture were measured visually while pH of the formulations was measured by digital pH meter (335, Systronics, Ahmedabad, India).[4]

In vitro diffusion study

This study was performed by Franz diffusion cell (Durasil® (I) Pvt. Ltd; 3.14 cm^2 of effective diffusion area and 20 mL of receiver chamber capacity) using Cellophane® membrane. Cellophane® membrane was heated in 0.1N NaOH for half an hour to make it semipermeable having the pore size of 80 µm. It was mounted between the receiver and donor compartments of the cell.[5] Initially, the receiver compartment was filled with phosphate buffer pH 6.8, methanol/phosphate buffer pH 6.8 and methanol/phosphate buffer pH 7.4 for Sapat plus malam®, Ciplox eye ointment® and chloramphenicol eye caps (25:75% v/v), respectively and the donor chamber was empty. The receiver buffer was stirred at a speed of 150 rpm and assembled the apparatus on a magnetic stirrer with the hot plate (2MLH, Remi equipment, India) at 37 ± 1°C temperature. Aliquots were withdrawn at regular time intervals and analyzed for drug content by UV spectrophotometry.[4,6,7]

Ex vivo diffusion studies

This study was performed with the same Franz diffusion cell in the same manner but using abdominal rat skin and *cul de sac* of goat as membrane respectively.[8] The skin was extracted from the abdominal region of the rat. It was wiped with methanol and washed with tap water to remove adhering materials. It was mounted in between the two compartments of the Franz diffusion cell, so as the stratum corneum side was towards the donor chamber. Freshly excised goat ocular membrane was procured from local goat slaughterhouse for human feeding to laboratory in cold (2°C) 0.9% w/v saline within 3 h of slaughtering. No goat was separately killed for the study. The corneas were carefully dissected along with 4 cm^2 of the area, sclera tissue from the eyeball and washed with tap water to remove any adhering materials. It was mounted between the two chambers of the cell where the conjunctiva side was towards the donor chamber.[9] The whole study was approved by the Institutional Animal Ethics Committee (IAEC), New Delhi, India, under the reference number RKCP/COL/RP/16/74.[10]

Permeation data analysis

The cumulative amount of drug permeated through the membrane (mg/cm^2) against time curve was plotted for each formulation. The drug flux was found by dividing the slope of the graph linear portion with the effective diffusion cell area (mg/cm^2min). The permeability coefficient was derived by dividing drug flux by the initial concentration of the drug in the donor chamber. The lag time, i.e. the time at which drug release from formulation was also determined by extrapolating the curve to the abscissa.[11] The diffusion parameter was found from lag time by $\frac{1}{6}$(lag time). The time required for the release of more than 90 percentages of the drug (t_{90}) and to achieve MIC value (t_{MIC}) were noted. Drugs are targeted for local action, so t_{90} and t_{MIC} for *ex vivo* dynamics studies were not evaluated.[6,12] The remaining of the formulation on the membrane (dislodgeable dose) was put in a 100-mL glass beaker. The membrane and the used spatula were washed five times with respective phosphate buffer. The final volume was made to 10 mL with the same and the mixture was stirred (1000 rpm) for 1 h. One mL of it was transferred to a 10 mL volumetric flask and volume was adjusted with the same. The resulting solution was filtered through filter paper and the remaining amount of drug was quantified by the spectrometric method.[13] Partition coefficients were derived by the ratio of dislodgeable dose to permeable dose. Local accumulation (LAC) was derived by the ratio of drug retained in the membrane to the drug that penetrated.[14]

In vitro drug release kinetic study

Data treatment for all the formulations was done using the following models: zero-order kinetic, first-order kinetic, Higuchi, Korsmeyer/Peppa's, Hixson Crowell, Weibull, and Baker-Lonsdale. The equation of plot, correlation coefficient (R^2) value, slope of the plot, and sum of square residual was found. The model with the smallest sum of squared residual value was selected as best fit.[7,15]

Statistical analysis

Student's unpaired t-test with equal variance was used to find any statistically significant difference in the *in vitro* and *ex vivo* diffusion transport studies at 95% level of confidence.[12,16] All data were given as mean ± SD from five independent experiments.

RESULTS

Sapat plus malam® showed wavelength maximum at 295 nm in phosphate buffer pH 6.8, Ciplox eye ointment® and chloramphenicol eye caps showed wavelength maximum at 286 nm and 274 nm in methanol/phosphate buffer (25:75% v/v) pH 6.8 and 7.4, respectively. The calibration curves showed linearity in 8–50 µg/mL concentration ($R^2 \geq 0.98$). Permeation data showed better values for Cellophane® membrane than *ex vivo* studies (**Table 1**). After 6 and a half hours, there was more permeation of drug through Cellophane® membrane than *ex vivo* studies (**Fig. 1**). Permeability coefficients for Cellophane® membrane were higher than *ex vivo* studies (**Fig. 2**). Sapat plus malam®, Ciplox eye ointment®, and chloramphenicol eye caps had the minimum sum of square values in 0.73, 0.009, and 0.013 in Korsmeyer/Peppa's (0.45< release exponent = 0.4708 <0.89), first order, and Hixson Crowell model, respectively. For Sapat plus malam®, the calculated t value was 3.26, tabulated t value was 2.06 (pooled degree of freedom was 24), for Ciplox eye ointment®, calculated t value was 2.23, tabulated t value was 2.18 (pooled degree of freedom was 12) and for chloramphenicol eye caps, calculated t values was 2.41, tabulated t value was 2.06 (pooled degree of freedom was 24). In all three formulations, calculated t values were higher than tabulated t values, $P<0.05$, which was significant at 95% level of significance.

DISCUSSION

In vitro drug release profile of Sapat plus malam®, Ciplox eye ointment®, and chloramphenicol eye caps followed Korsmeyer/Peppa's (Non-Fickian transport), first order, and Hixson Crowell model, respectively.[17] Studies demonstrated that cumulative drug release was higher for *in vitro* studies than *ex vivo* studies, Cellophane® membrane had more diffusion, higher permeability coefficient, lower lag time, less drug accumulation, less dislodgeable dose, less diffusion parameter and less partition coefficient than rat skin or goat *cul de sac*. This was so because lipid content of the rat skin or goat *cul de sac* interferes with the permeation of drug and decreases its permeability.[18] There was also one reason that in formulations there was only white soft paraffin as the base no penetration enhancers were used. White soft paraffin is unable to break the lipid-lipid and lipid-protein bond of skin so there was less permeability of drug and high dislodgeable dose. There was no correlation between permeability coefficient of Cellophane® and that of rat skin or goat *cul de sac*.[19,20] Permeability coefficient of Sapat plus malam®, Ciplox eye ointment® had vast difference compared to that of chloramphenicol eye caps among *in vitro* and *ex vivo* studies. This is due to the fact that rat skin has higher lipid content than goat *cul de sac*.

CONCLUSION

Present investigation of the effect of permeation using different membranes for well-established brands in India concluded that researchers should not use rat skin or goat *cul de sac* for their optimization of the formulation. This is because of the following two reasons: each time the fat content of rat skin and goat *cul de sac* was found to be varying. Presence or absence of permeation enhancer(s) in the formulation. Therefore, misleading results will be obtained as compared to Cellophane® membrane. Moreover, Cellophane® membrane is more suitable to be used for the development of formulations as it has no lipid content.

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Table 1. Permeation data analysis of Sapat plus malam®, Ciplox eye ointment® and chloramphenicol eye caps

Parameters		Market brands		
		Sapat plus malam®	**Ciplox eye ointment®**	**Chloramphenicol eye caps**
Formulation	Drug (w/w)	8% salicylic acid and 1% tolnaftate	0.3% ciprofloxacin HCl	1% chloramphenicol
	White soft paraffin	q. s.	q. s.	q. s.
Organoleptic assessment	Appearance	Pleasant	Pleasant	Pleasant
	Homogeneity	Less homogeneous	Homogeneous	Homogeneous
	Texture	Fractured	Smooth	Smooth
pH		3 ± 0.6	7.4±0.6	7.4 ± 0.55
In vitro studies in Cellophane® membrane after 6 and a half hours	Dislodgeable dose (%)	60 ± 2	83 ± 3	9 ± 0.2
	Lag time (Sec)	30 ± 3	20 ± 2	850 ± 10
	t_{90} (min)	1020 ± 60	340 ± 12	635 ± 20
	t_{MIC}	130 ± 6 min	25 ± 5 Sec	231±11 Sec
	Permeability co-efficient (cm^2/min)	0.000316 ± 0.0000625	0.00416 ± 0.0001	0.0034 ± 0.00004
	Diffusion parameter (Sec^{-1})	0.0056 ± 0.0001	0.0083 ± 0.0002	0.0001 ± 0.000009
	Partition co-efficient	0.25 ± 0.02	1.4 ± 0.14	13.64 ± 0.6
Ex vivo dynamics studies after 6 and a half hours*	Dislodgeable dose (%)	89 ± 3	45 ± 2	92 ± 3
	Lag time (min)	31 ± 1	12 ± 0.5	32 ± 1.5
	Permeability co-efficient (cm^2/min)	0.0001 ± 0.000001	0.002254 ± 0.0002	0.00303 ± 0.0001
	Diffusion parameter (min^{-1})	0.0054 ± 0.0003	0.0139 ± 0.0005	0.0052 ± 0.0004
	LAC	1.91 ± 0.1	0.25 ± 0.001	5.4 ± 0.25

q. s. – quite sufficient; mean ± SD; n=5; **ex vivo* dynamics studies through rat skin for Sapat plus malam® and Ciplox eye ointment®; ocular *ex vivo* dynamics studies through goat *cul de sac* for chloramphenicol eye caps; t_{90} – time required for the release of more than 90 percentages of drug; t_{MIC} – time required to achieve MIC value.

AUTHORS' DISCLOSURES

Authors disclosed that there was neither conflict of interest nor ethical issues and no funding sources as well. Authors had no conflict on criticism of brands.

DISCLAIMER

Any opinions, findings, conclusions or recommendations expressed in this material are those of the corresponding author only.

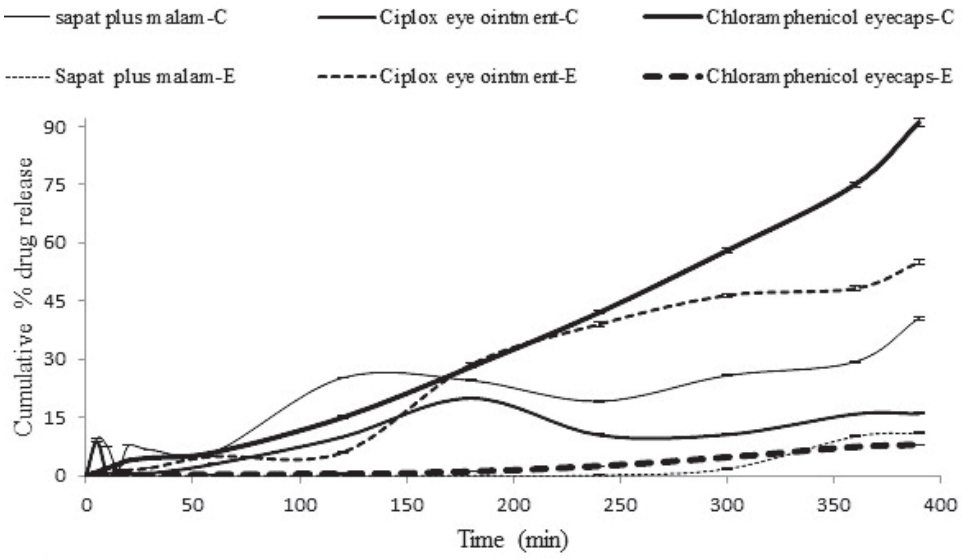

Figure 1. Diffusion studies; C: *in vitro* diffusion study through Cellophane® membrane; E: *ex vivo* diffusion study through rat skin or goat *cul de sac;* mean ± SD, n=5.

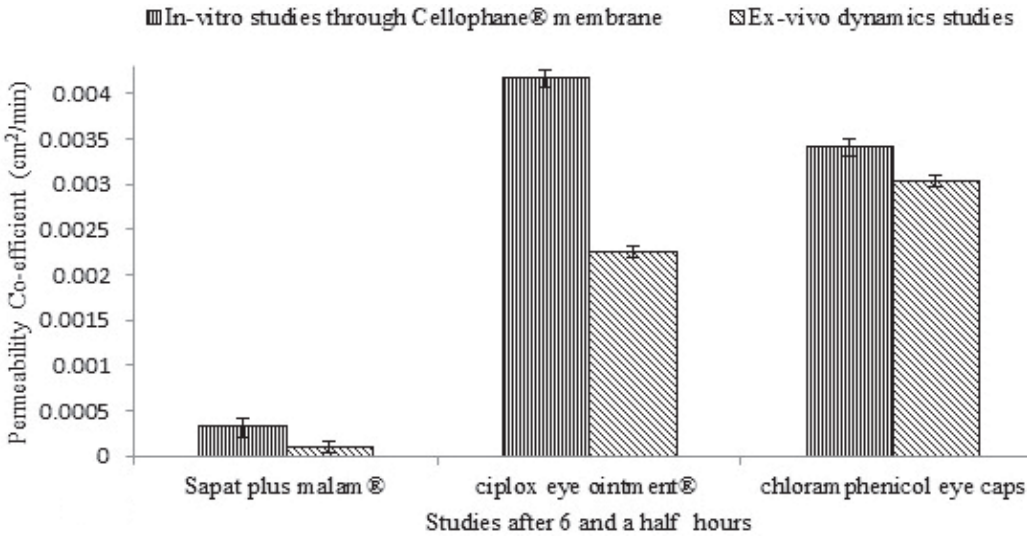

Figure 2. Permeability coefficient comparisons through different membranes after 6 and a half hours; mean ± SD, n=5.

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Взаимосвязь между проницаемостью и содержанием липидов мембраны

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Введение: Кожа крысы и слепая кишка козы применяются чаще всего для оптимизации препаратов в качестве моделей кожи человека и слепой кишки.

Цель: Исследование взаимосвязи между содержанием липидов кожи крысы и слепой кишки козы и их проницаемостью.

Материалы и методы: Установление максимально возможной длины волны для препарата Sapat plus malam®, глазной мази Ciplox ® и Хлорамфеникол капсул для глазного применения, а также получение стандартной кривой. Были проведены *in vitro* исследования на Cellophane® мембране и исследования *ex vivo* на коже крысы или слепой кишки козы данных препаратов. Коэффициент проницаемости, % остаточного количества (dislodgeable dose), промежуток времени (lag time), диффузионный коэффициент и коэффициент разделения были установлены и в обоих случаях в течение шести с половиной часов исследований на пенетрацию. Одновыборочный t–тест Стьюдента с равными дисперсиями был использован для установления статистически значимой разницы при *ex vivo* и *in vitro* исследованиях транспорта и диффузии при уровне статистической достоверности 95 % .

Ключевые слова: коэффициент проницаемости, диффузионное исследование, данные о проницаемости

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Результаты: Коэффициент проницаемости препарата Sapat plus malam®, глазной мази Ciplox® и Хлорамфеникол капсул для глазного применения составил, соответственно, 0.000316 ± 0.0000625, 0.00416 ± 0.0001, 0.0034 ± 0.00004 для Cellophane® мембраны и 0.0001 ± 0.000001, 0.002254 ± 0.0002, 0.00303 ± 0.0001 для *ex vivo* мембраны в $см^2$/мин. Для всех трёх препаратов были установлены T–критерии, превышающие табличные T–критерии, $P<0.05$ при уровне статистической достоверности 95 % .

Заключение: Cellophane® мембрана демонстрирует более хорошую диффузию по сравнению с кожей крысы и слепой кишкой козы. При оптимизации препарата рекомендуется применение исключительно Cellophane® мембраны.