The Effect of pH and Organic Ester Penetration Enhancers on Skin Permeation Kinetics of Terbutaline Sulfate From Pseudolatex-Type Transdermal Delivery Systems Through Mouse and Human Cadaver Skins

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ABSTRACT

The purpose of this research was to prepare a pseudolatex transdermal delivery system for terbutaline sulfate and to evaluate the effect of pH and organic ester penetration enhancers on permeation kinetics of terbutaline sulfate through mice abdominal skin and human cadaver skin. An increase in the permeation flux by increasing pH was observed. The distribution coefficient of terbutaline sulfate between 1-octanol and buffers of different pH values was also pH-dependent. Furthermore, the change of the permeability coefficient with pH correlated well with the distribution coefficient by a 2-degree polynomial equation. The permeation profile and related kinetic parameters of terbutaline sulfate was determined in presence of 3 ester-type permeation enhancers incorporated in the films, viz methyl laureate, isopropyl lanolate, and isopropyl myristate. Among the 3, the more pronounced enhancing effect was obtained with isopropyl myristate, regarding the permeation flux, permeability coefficient, and diffusion coefficient. This was attributed to solubility parameter of isopropyl myristate being closer to the solubility parameter of human skin, and such a pronounced enhancing effect was probably caused by its passage across the skin barrier through the lipid pathway.

KEYWORDS: permeation, terbutaline sulfate, enhancer, isopropyl myristate, human cadaver skin

INTRODUCTION

The extent of the bioavailability of terbutaline sulfate after oral administration is 7% to 26%. This decrease in the percentage is due to a high first-pass metabolism. The terminal half-life in healthy subjects is 17 hours and the biological half-life 3.6 hours.[1] Terbutaline is a β_2-receptor agonist similar to epinephrine. Nevertheless, a change from a catechol-like structure to a resorcinol-like structure and the use of a bulky amino substituent make it β_2-selective, unlike epinephrine.[2] Incomplete absorption from the gastrointestinal track and a fairly large first-pass metabolism make terbutaline a suitable candidate for transdermal delivery.

There are reports describing the use of Eudragit RL and Eudragit RS pseudolatex transdermal delivery systems as well as other dosage forms for controlled release of drugs.[3-4] Such pseudolatex may be easily prepared by the solvent-change technique, which consists of dissolving the polymer in a water-miscible organic solvent or a mixed water-miscible organic solvent system followed by dispersion in deionized water with the help of agitation. The organic solvent is then removed to leave stable latex. Once the product is dry, pseudolatex is obtained. These pseudolatex films are neither extremely hydrophobic nor extremely hydrophilic. Therefore, varying the ratio of the polymers in the composition of pseudolatices provides an additional method of controlling the thermodynamic environment of the drug inside the latex and also provides a way to control its release characteristics. It is believed that to increase the release of an ionic drug, for example terbutaline sulfate, the latex proportion of RL polymer must be increased.[5]

The number of species of terbutaline sulfate in aqueous solution is 3, and it has 3 pK_a values: 8.8 (due to the phenolic hydroxyl), 10.1 (due to the amino group), and 11.2 (due to the aromatic hydroxyl group).[6] This indicates that there must be an optimum pH for the higher fraction of the drug remaining as nonionic species, promoting maximum skin partition and permeation rate. Based on this hypothesis, a transdermal drug delivery system has been fabricated in this investigation to incorporate buffers of different pH values (varying from 4.2 to 5.6), which will instantly neutralize the acid mantle of the skin at the site of permeation of the drug. Furthermore, the effect of 3 ester-class chemical enhancers, namely methyl laurate,

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isopropyl lanolate, and isopropyl myristate, on the skin permeation rate of terbutaline sulfate have been studied.

MATERIALS AND METHODS

Materials

Eudragit RS-100, Eudragit RL-100, Eudragit NE 30D, and Eudraflex was provided by Röhm GmbH & Co KG (Darmstadt, Germany). Terbutaline sulfate USP was supplied by Wochhard Ltd (Aurangabad, India) and isopropyl myristate by Aldrich Chemical Co (Milwaukee, WI). Methyl laurate, isopropyl lanolate, and acetone was obtained from Loba Chemie Pvt Ltd (Mumbai, India). EDTA was supplied by Sigma Chemical Co (St Louis, MO). Monobasic sodium phosphate and dibasic sodium phosphate was purchased from Merck (Darmstadt, Germany). All other reagents were of analytical grade. Stock solutions were prepared fresh when required.

Swiss albino mice, 6 to 8 weeks old, were supplied by BN Ghosh (M/S BN Ghosh & Co, Kolkata, India). Human abdominal skin, obtained from ventral part of forearm of a 35-year-old male collected within 8 hours of death, was supplied by the Medical College and Hospital Authority (Calcutta, India).

Determination of Distribution Coefficient

The distribution coefficient was determined between saline phosphate buffer and 1-octanol at an ambient temperature of 37 ± 1°C, following the method of Leo et al.[7] Equal volumes (10 mL) of saline phosphate buffer and 1-octanol were added to 10 mg of accurately weighed terbutaline sulfate in a glass stopped tube. The mixture was shaken by a test tube shaker (wrist action shaker) for 24 hours at an ambient temperature of 37 ± 1°C. The drug concentration in aqueous and in 1-octanol phase were spectrophotometrically estimated at 270 nm. Distribution coefficients were calculated using the Equation 1[8]:

$$DC = \frac{(Ca - Cb)}{Cb} \quad (1)$$

where DC is the distribution coefficient and Ca and Cb represent the drug concentration in the aqueous layer at the beginning and at equilibrium, respectively.

Determination of the Partition Coefficient Between Mouse Skin Epidermis, Human Epidermis, and Saline Phosphate Buffer

The partitioning of the drug between saline phosphate buffer and skin epidermis was determined by the method reported by Valia et al[9] and Tojo et al.[10] A piece of whole, excised albino mouse and excised human cadaver skin was weighed accurately (90 to 100 mg) and put into separate glass stopped test tubes containing 10 mL of drug (50 mg) solution in saline phosphate buffer. The pieces of skin were equilibrated with the buffer for 24 hours at 37 ± 1°C. The solution was filtered and spectrophotometrically estimated at 270 nm. The experiment was performed in triplicate and the average calculated.

Dose Design

To achieve an effective plasma concentration of terbutaline sulfate (7 ng/mL), the required permeation rate through the skin was calculated to be 61.6 $\mu g/cm^2$ h. Using pharmacokinetic parameters, the permeation rate was calculated by the equation described by Guy et al.[11]

Preparation of the Pseudolatex Films

First, the backing membrane was prepared as follows. The Eudragit NE 30D suspension was air dried and crushed into small pieces. The polymer (250 mg) was dissolved in 5 mL of acetone, poured onto aluminum foil with an area of 4.8 cm^2, and dried overnight at 30°C. Films were then fabricated using the polymer Eudragit RL-100 and RS-100 alone or in different combinations (ie, F1 using Eudragit RL-100, F2 using equal parts Eudragit RL-100 and RS-100, F3 using 1 part Eudragit RL-100 and 2 parts RS-100, F4 using 1 part Eudragit RL-100 and 3 parts RS-100, F5 using 2 parts Eudragit RL-100 and 3 parts RS-100, F6 using RS-100, and F7 using 1 part Eudragit RL-100 and 1.7 parts RS-100). In all cases, the polymers were dissolved in 7 mL of a mixture of acetone and phosphate buffer solution (pH 7.4), containing 17.5 mg of terbutaline sulfate along with 0.1 mL of Eudraflex as a plasticizer. The resulting solution was cast over the backing membrane and dried in a vacuum at 40°C for 12 to 16 hours. Films containing 2% penetration enhancer, viz methyl laureate, isopropyl lanolate, and isopropyl myristate, were prepared in a similar manner as described above. The resulting films contained 3.65 mg of terbutaline sulfate per squared centimeter. After the initial physicochemical studies, formulation F3 and F4, prepared with and without penetration enhancer, were taken for skin permeation study. The buffer (pH 7.4) was used because it exhibited an optimum skin partition coefficient for terbutaline sulfate.

Measurement of Skin Thickness[12]

The abdominal thickness of human cadaver skin and albino mouse skin was measured microscopically after staining with hematoxylin-eosin. A wax block of skin was prepared by using steel molds. Molten wax was poured into the steel mold from a paraffin dispenser heated between 68°C

and 70°C. The skin was pushed down to the bottom of the mold so that it was positioned on the cutting surface. After cooling, the block was removed by slightly reheating the mold. The wax block was held on the holder of the microtome. The sections were cut 5- to 7-μm thick with a dispersible microtome blade. The section was transferred to a slide and affixed (egg albumin was applied to the site). The slide was put in a caplin jar containing hematoxylin for 10 minutes, then rinsed with isopropyl alcohol, and kept under running water for 5 to 7 minutes. The slide was dipped 30 times in a caplin jar containing 1% eosin and then rinsed with isopropyl alcohol. The slide was then allowed to dry completely and kept in a caplin jar containing xylene for 7 to 10 minutes. The slide was air dried and observed under a microscope; thickness was measured using a micrometer. The thickness was found to be $1.83 \times 10^{-2} \pm 0.14$ cm and $2.3 \times 10^{-2} \pm 0.29$ cm, respectively, after 3 measurements.

Determination of the Saturation of Terbutaline Sulfate

A saturated terbutaline sulfate solution mixed in phosphate saline buffer with different pH values (6.8, 7.0, 7.4, and 8.0) was prepared by equilibrating the excess terbutaline sulfate with the vehicle for 24 hours. The temperature of the solution was maintained at 30°C using a circulating water bath. The sample was filtered and appropriately diluted to estimate the saturation solubility of terbutaline sulfate. The terbutaline sulfate concentration was found to be 7.789 mg ml^{-1}, 8.243 mg ml^{-1}, 9.329 mg ml^{-1}, and 10.448 mg ml^{-1}, respectively.

Permeation Studies of Mouse and Human Cadaver Abdominal Skin[13]

Preparation of Mouse Abdominal Skin

The pretreated abdominal skin of albino mice was used in the Keshary-Chien diffusion cell. Hairs from the abdominal region were carefully removed with fine forceps. A full thickness of skin was taken out and then trimmed to remove the fatty material. Finally, the epidermal skin was taken and examined microscopically to ensure the integrity of the stratum corneum.[14]

Preparation of Human Cadaver Abdominal Skin

Human cadaver abdominal skin was dermatomed carefully, and the sample was then treated with deionized distilled water; the skin was then treated in a 5% wt/vol solution of EDTA for 8 hours. The epidermis was then separated carefully from the dermis with forceps. The epidermis was again washed with distilled water and kept spread on a cellophane sheet. The skin was then stored in a freezer at −20°C until further use. Before the experiment, the skin was taken out and thawed until it reached room temperature and was kept soaked in phosphate buffer solution for 1 hour. It was gently blotted dry with filter paper. The integrity of the skin was tested microscopically before use in the Keshary-Chien cell to detect any histologic change.[15] No significant histologic changes were observed.

The prepared mouse and human cadaver epidermis was fastened carefully between the donor and receptor compartment in the Keshary-Chien diffusion cell so that the stratum corneum surface faced the donor side. The active diffusion area was 3.14 cm^2. The receptor compartment was then filled with 12 mL of phosphate saline buffer solution (pH 6.8, 7.0, 7.4, and 8.0), containing 0.002% gentamycin as an antibacterial agent serving as the elution medium. The temperature of the elution medium was thermostatically controlled at 37 ± 1°C by a surrounding water jacket connected to a thermostatic bath and a peristaltic pump. The receptor solution was stirred magnetically at 500 rpm during the experiment.[16] The skin was allowed to equilibrate with the receptor solution for 30 minutes. A blank analysis of the receiver solution was conducted to ensure the absence of any interfering substances before adding the donor solution. The donor compartment contained 1 mL of terbutaline sulfate-saturated solution. Aliquots from the receptor solution were withdrawn periodically (up to 38 hours) and were spectrophotometrically estimated at 270 nm. The experiments were repeated 6 times.

The film sample was fixed on the skin securely so that the stratum corneum was located on the film side in between the donor and receptor compartment of Keshary-Chien cell. Both the effective skin area and the area of the film sample were 3.14 cm^2. The experiment was performed as described above and was repeated in 6 times.

Skin Irritancy Test[17]

The pseudolatex patch containing the drug and a piece of cotton wool soaked in saturated drug solution were placed on the back of albumin rats, secured firmly in place with adhesive plaster. An aqueous solution of 0.8% formalin was applied as a standard irritant. The animals were observed and scored for 7 days for any sign of edema and erythema.

Stability Studies[13]

The formulations were stored at 25°C, 40°C, and 50°C for 3 months. The samples were withdrawn every week, and the amount of intact drug remaining was estimated. It was reported that terbutaline sulfate solution discolored at a low pH due to oxidation and does not discolor at a stable

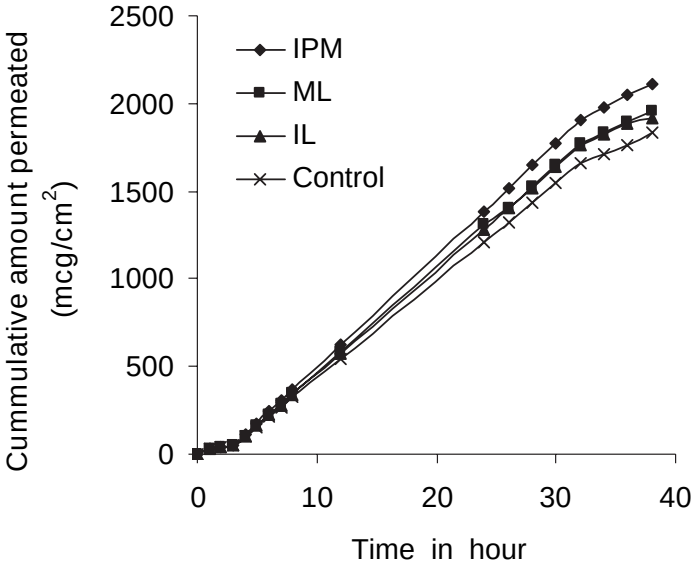

Figure 1. In vitro skin permeation profile of terbutaline sulfate through albino mice epidermis from formulation F3 with and without enhancer using phosphate buffer (pH 7.4).

higher pH.[6] It was also reported that terbutaline sulfate solution was stable up to 120 days at 25°C.[18]

RESULTS AND DISCUSSION

The steady-state permeation flux was determined from the slope of the linear portion of the cumulative amount of permeation (Q) versus time (t) plot shown in Figure 1 and 2. The lag time (t_L) was determined by extrapolating the linear portion of Q versus t curve to the abscissa. It has been shown that linearity of such a plot is not achieved until $t_L = 6$.[19] The partition coefficient (PC) of terbutaline sulfate was calculated by Equation 2[20]:

$$PC = \frac{Cs - Ceg}{Ceg} \times \frac{1000}{We} \quad (2)$$

where, Cs, Ceg, and We are the initial concentration of terbutaline sulfate in phosphate buffer solution (milligrams per milliliter), equilibrium concentration (milligrams per milliliter), and weight (milligrams) of the epidermis, respectively. The dry weight of the epidermis was considered for calculating the partition coefficient.

The permeability coefficient (P) was calculated using the relation derived from Fick's 1st law of diffusion, which is described in Equation 3.[21]

$$P = \frac{J}{C} \quad (3)$$

where J is the steady-state permeation flux and C is the initial concentration.

Table 1. The Relationship Between the pH and the Distribution Coefficient of Salbutamol Sulphate in 1-Octanol/Phosphate Buffer at 37 ± 1°C

pH	DC	(1/DC) × 10^3	(1/[H^+]) × 10^{-5}
6.8	45.20	22.12	100.00
7.0	166.11	6.02	25.11
7.4	432.90	2.31	10.00
8.0	909.09	1.3	6.30

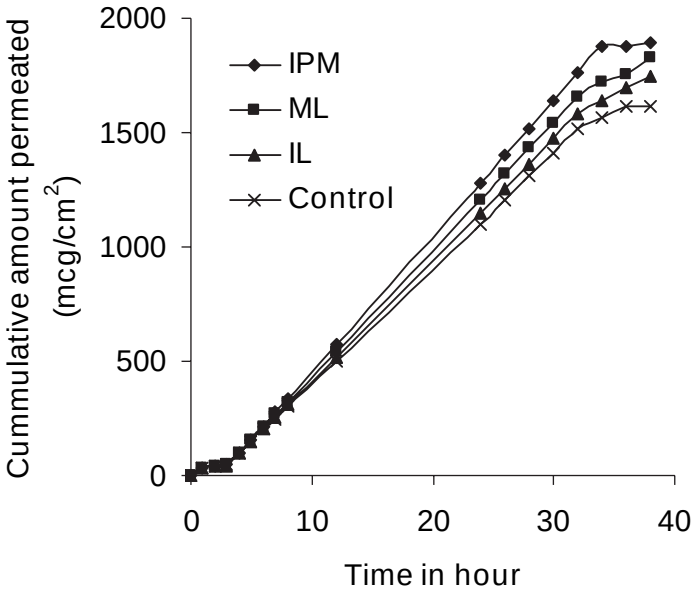

Figure 2. In vitro skin permeation profile of terbutaline sulfate through human cadaver epidermis from formulation F3 with and without enhancer using phosphate buffer (pH 7.4).

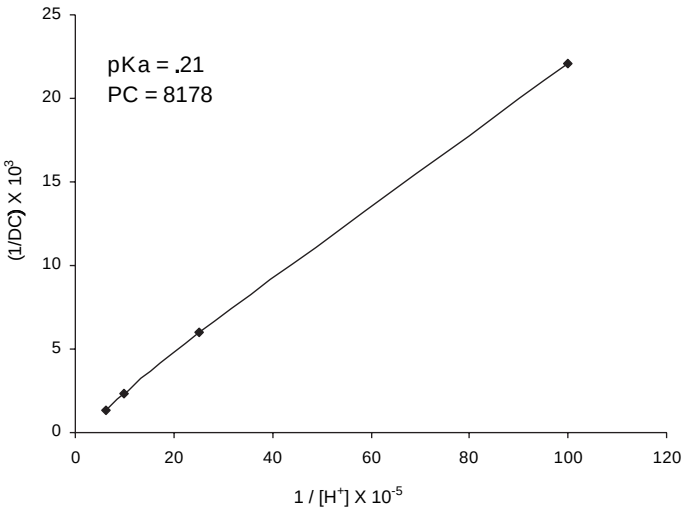

Figure 3. Double reciprocal plot of 1/DC and 1/[H+] for the determination of partition coefficient and the pK_a of terbutaline sulfate.

Table 2. Diffusion Flux (J) and Lag Time (t_L) of Terbutaline Sulphate From Formulation F3 and F4 at Different pH Values*

Formulation Code	Different pH	Diffusion Flux (J) [(μg/(cm^2 h)]	Lag Time(t_L) (h)
F3	6.8	60.00 ± 0.54	2.95 ± 0.12
	7.0	64.32 ± 0.92	3.1 ± 0.15
	7.4	69.95 ± 0.78	3.2 ± 0.21
	8.0	75.00 ± 0.87	3.3 ± 0.18
F4	6.8	55.19 ± 0.82	2.9 ± 0.14
	7.0	59.99 ± 0.91	2.95 ± 0.16
	7.4	64.93 ± 0.72	3.1 ± 0.14
	8.0	69.94 ± 0.98	3.2 ± 0.18

*Standard deviation is between parentheses. $n = 6$.

The diffusion coefficient was calculated using the relation derived from Fick's 2nd law of diffusion, which is described in Equation 4.[22]

$$DC = \frac{h^2}{6L} \quad (4)$$

where h is the thickness of the skin and L is the lag time.

The enhancement factor (E) was calculated as the ratio of the permeation rate of terbutaline sulfate from the formulation with enhancer to the permeation rate from the controlled system without the enhancer factor. The enhancement factor was calculated by a method given by Yu et al[23]: enhancement factor = [(normalized skin permeation rate) enhancer]/[(normalized skin permeation rate) control]

The relationship between the pH and distribution coefficient of terbutaline sulfate in 1-octanal/phosphate buffer at 37 ± 1°C are presented in Table 1. A double reciprocal plot of 1/DC versus 1/[H^+] results in a straight line, as shown in Figure 3. This agrees with the relationship[9]:

$$\frac{1}{DC} = \frac{1}{PC} + \frac{Ka}{PC[H^+]} \quad (5)$$

where K_a is the dissociation constant of terbutaline sulfate and PC is the partition coefficient. Therefore, from the slope and the intercept, pK_a and PC were calculated and found to be 4.21 and 8178, respectively.

As shown in Table 2, formulation F3 showed a higher diffusion rate (J) than formulation F4, mixed with phosphate buffer balance at a different pH value; the highest flux rate was at pH 8.0 for both the formulations. Although the flux values at pH 7.4 were slightly less than that at pH 8, however, the pH 7.4 buffer was selected for further study, being a physiologic pH along with the formulation F3.

As shown in Table 3, varying partition coefficients of terbutaline sulfate in the presence of different enhancers implied that the solubility of the drug in the epidermis had altered. Therefore, it may be assumed that these enhancers have acted through the nonpolar rout to increase the transport of terbutaline sulfate across the barrier. The increase in the experimental partition coefficient is in an agreement with the calculated value. The measure rate-limiting factor for permeation of many drugs through the epidermis is lipids, and their removal is known to reduce the barrier

Table 3. Permeation Flux (J), Experimental and Calculated Partition Coefficient K_1 and K_2, and the Best-Fit Equation for the Permeation Plot of Terbutaline Sulphate From Formulation F3 From Albino Mice and Human Cadaver Epidermis at Phosphate Buffer Solution (pH 7.4) With and Without Enhancers*

Skin Condition	Enhancers	Permeation Flux (J) [μg(cm^2 h)]	Experimental Partition Coefficient K_1†	Calculated Partition Coefficient K_2‡	Best-Fit Equation for Permeation Plot	Regression Coefficient
Albino Mice Epidermis	Control (without enhancer)	55.19	4.651 ± 0.97	4.475	Q = 55.18t—119.07	0.998
	Isopropyl lanolate	58.81	5.038 ± 0.64	4.835	Q = 58.89t—113.20	0.998
	Methyl laurate	59.99	5.315 ± 0.93	4.948	Q = 59.97t—132.39	0.998
	Isopropyl myristate	63.57	5.684 ± 1.05	5.442	Q = 63.56t—138.87	0.998
Human Cadaver Epidermis	Control (without enhancer)	50.03	5.19 ± 0.52	4.746	Q = 50.07t—98.89	1
	Isopropyl lanolate	52.63	5.40 ± 0.63	5.179	Q = 52.59t—113.99	0.999
	Methyl laurate	55.19	5.98 ± 0.93	5.624	Q = 55.18t—119.20	0.999
	Isopropyl myristate	59.13	6.342 ± 0.98	6.109	Q = 59.03—139.22	0.999

*Standard deviation is between parentheses, $n = 6$.
†Estimated using Equation 2.
‡Calculated from the relation $K_1 = Ph/D$, where h is the thickness of the barrier.

Table 4. Lag Time (t_L), Diffusion Coefficient (DC), Permeability Coefficient (P), and Enhancement Factor (E) for Formulation F3 From Albino Mice Epidermis and Human Cadaver Epidermis Permeation of Terbutaline Sulphate From Phosphate Buffer Solution (pH 7.4) With and Without Enhancers*

Skin Condition	Enhancers	Lag Time(t_L) (h)	Diffusion Coefficient (DC × 10^{-6}) ($cm^2\ h^{-1}$)	Permeability Coefficient ($P \times 10^{-3}$) ($cm^2\ h^{-1}$)	Enhancement Factor (E)
Albino Mice Epidermis	Control (without enhancer)	2.90 ± 0.22	3.040 ± 0.61	5.915 ± 0.33	1.00
	Isopropyl lanolate	2.94 ± 0.26	2.999 ± 0.42	6.303 ± 0.15	1.06
	Methyl laurate	2.95 ± 0.29	2.989 ± 0.81	6.430 ± 0.74	1.08
	Isopropyl myristate	3.05 ± 0.21	2.890 ± 0.71	6.814 ± 0.56	1.15
Human Cadaver Skin	Control (without enhancer)	2.70 ± 0.32	2.067 ± 0.21	5.363 ± 0.42	1.00
	Isopropyl lanolate	2.80 ± 0.34	1.994 ± 0.53	5.642 ± 0.39	1.05
	Methyl laurate	2.90 ± 0.20	1.924 ± 0.73	5.916 ± 0.44	1.10
	Isopropyl myristate	2.94 ± 0.24	1.898 ± 0.46	6.339 ± 0.74	1.18

*Standard deviation is between parentheses. $n = 6$.
P and D were calculated from the individual steady-state flux illustrated in Figures 1 and 2.

property of the epidermis.[24] Table 4 summarizes the effect of enhancers (viz isopropyl myristate, methyl laurate, and isopropyl lanolate) on the permeability coefficient of terbutaline sulfate and also the lag time, diffusion coefficient, and enhancement factor. Among the various ester types of enhancers studied, a higher permeability coefficient and enhancement factor (E) were obtained with isopropyl myristate.[25]

There was no significant difference in lag time in both albino mice and human cadaver epidermis. The presence of enhancer increases the enhancement factor up to 1.15 and 1.18 times with isopropyl myristate in albino mice and human cadaver epidermis, respectively. The enhancement capacity of isopropyl myristate is due to its interaction with the lipid component of stratum corneum. This happens because of the lower values of the Hildebrand solubility parameter (δ) of isopropyl myristate (δ = 8.02), which is nearer to that of the human skin (δ = 10.5). The enhancement effect of isopropyl myristate can also be manifested by virtue of its intermediate polar nature, isopropyl myristate being partitioned as a result, into both the lipid and polar phase of the skin. The animals subjected for primary skin irritation test did not show any sign of edema or erythema while observing for a period of 7 days.

All the formulations exhibited good stability at all the stage conditions. The patches were dipped in about 5 mL of a hydroalcoholic mixture for about 1 hour and sonicated for 15 minutes to extract terbutaline sulfate from the matrix. The same procedure was followed for placebo patches. The sample was filtered and appropriately diluted to estimate terbutaline sulfate spectrophotometrically.

CONCLUSIONS

The effect of phosphate buffer solutions of different pH values and organic ester permeation enhancers was studied. The change of the permeability coefficient of terbutaline sulfate with pH follows a 2-degree polynomial equation. Comparative epidermal release profiles show that epidermal flux through the skin was better in the case of formulations containing isopropyl myristate as a permeation enhancer. The desires permeation profile can be achieved with an alteration in the buffer and permeation enhancer concentration.

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REFERENCES

1. Leferink J, van den Berg W, Wagemaker-Engels I, Kreukniet J, Maes RAA. Pharmacokinetics of terbutaline, a β_2-sympathomimetic, in healthy volunteers and asthmatic patients. *Arzneimittelforschung.* 1982;32:159-164.

2. Insel PA, Mohan LC, Motulsky HJ, Stoolman LM, Koachman AM. Time dependent decreases in binding affinity of agonist for β-adrenergic receptors of intact S_{49} lymphoma cells. A mechanism of desensitization. *J Biol Chem.* 1983;258:13597-13605.

3. Thassu D, Vyas SP. Controlled transdermal mucolytic delivery system. *Drug Dev Ind Pharm.* 1991;17:561-576.

4. Shaw JE, Cramer HP, Gale R. Rate-controlled transdermal therapy utilizing polymeric membranes. In: Kydonious AF, Berner B, eds. *Transdermal Delivery of Drugs.* Boca Raton, FL: CRC Press; 1987;102-116.

5. Chang RK, Price JC. Preparation and preliminary evaluation of Eudragit RL and RS pseudolatices for controlled drug release. *Drug Dev Ind Pharm.* 1989;15:361-372.

6. Ahuja AS, Ashman J. Terbutaline sulphate. In: Florey K, ed. *Analytical Profile of Drug Substance.* New York, NY: Academic press; 1990:603-625.

7. Leo A, Hansch C, Elkins D. Partition coefficients and their uses. *Chem Rev.* 1971;71:525-616.

8. Chiang CH, Lai JS, Yang KH. The effect of pH and chemical enhancers on the percutaneous absorption of indomethacin. *Drug Dev Ind Pharm.* 1991;17:91-111.

9. Valia KH, Tajo K, Chien YW. Long term permeation kinetics of estradiol. Part-3, kinetic analysis of the simultaneous skin permeation and bioconversion of estradiol esters. *Drug Dev Ind Pharm.* 1985;11:1133-1173.

10. Tojo K, Chiang CC, Chien YW. Drug permeation across the skin: effect of penetrant hydrophilicity. *J Pharm Sci.* 1987;76:123-126.

11. Guy RH, Hadgrft J. Pharmacokinetic interpretation of plasma levels of clonidine following transdermal delivery. *J Pharm Sci.* 1985;74:1016-1018.

12. Bhattacharya A, Ghosal SK. Permeation kinetics of ketotifen fumarate along and in combination with hydrophobic permeation enhancers through human cadaver epidermis. *Boll Chim Farm.* 2000;139:177-181.

13. Murty SN, Hiremath SRR. Physical and chemical enhancers in transdermal delivery of terbutaline sulphate. *AAPS PharmSciTech.* 2001;2:1-5.

14. Durrhim H, Flynn GL, Higuchi WI, Behl CR. Permeation of hairless mouse skin I: experimental method and comparison with human epidermal permeation by alkanol. *J Pharm Sci.* 1980;69:781-786.

15. Raykar PV, Fung MC, Anderson BD. The role of protein and lipid domains in the uptake of solutes by human stratum corneum. *Pharm Res.* 1998;5:140-150.

16. Kakkar AP, Gupta A. Gelatin based transdermal therapeutic system. *Indian Drugs.* 1992;29:308-312.

17. Panigrahi L, Ghosal SK. Formulation and evaluation of pseudolatex transdermal drug delivery system of terbutaline sulphate. *Ind J Pharm Sci.* 2002;1:79-82.

18. Das Gupta V, Parasrampuria J, Gardner SN. Chemical stability of isoetharine hydrochloride, metaproterenol sulphate and terbutaline sulphate after mixing with normal saline for respiratory therapy. *J Clin Pharm Ther.* 1988;13:165-169.

19. Shah JC. Analysis of permeation data: evaluation of the lag time method. *Int J Pharm.* 1993;90:161-169.

20. Saket MM, James KC, Kellaway IW. Partitioning of some 21 alkyl esters of hydrocortisone and cortisone. *Int J Pharm.* 1984;21:155-166.

21. Aslani P, Kennedy RA. Studies on diffusion in alginate gels I. Effect of cross-linking with calcium or zinc ions on diffusion of acetaminophen. *J Control Release*. 1996;42:75-82.

22. Pefile SC, Smith EW, Albrecht CF, Kruger PB. Release of rooperol tetra-acetate from topical bases: in vitro studies using silicone membrane. *J Control Release.* 1998;161:237-243.

23. Yu JW, Chien T, Chien YW. Transdermal dual controlled delivery of testosterone and estradiol. I. Impact of system design. *Drug Dev Ind Pharm.* 1991;17:1883-1904.

24. Scheuplein R, Ross L. Effect of surfactants and solvents on the permeability of epidermis. *J Soc Cosmet Chem.* 1970;22:853-873.

25. Mandal SC, Bhattacharyya M, Ghosal SK. In vitro release and permeation kinetic of pentazocine from matrix-dispersion type transdermal drug delivery system. *Drug Dev Ind Pharm.* 1994;20:1933-1941.