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Enhancing Skin Permeation for Optimized Transdermal Drug Delivery

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Abstract. Enhancers, including hydrophilic solvents like propylene glycol and surfactants, play a crucial role in optimizing topical drug delivery by facilitating diffusion from the vehicle to the skin, thus enhancing therapeutic efficacy. Using *in vitro* diffusion cell technique, hairless mouse skin was tested with triamcinolone acetonide solution in phosphate buffers at different pH levels. The study assessed enhancers, such as urea, alcohols, dimethyl sulfoxide, dimethyl acetamide, propylene glycol, cetrimide and sodium lauryl sulfate. Results indicated that sodium lauryl sulfate is the most effective enhancer, significantly increasing the permeability coefficient relative flux (E_{pen}) 4.47. Most enhancers tested enhanced drug diffusion, with the notable exception of ambroxol HCl with relative flux (E_{pen}), 1.5, which had a least effect. The results demonstrate differential efficacy among the tested penetration enhancers, with sodium lauryl sulfate exhibiting significant potential to enhance transdermal drug delivery. The comparative analysis of relative flux values offers critical insights into the distinct permeation-enhancing capabilities of each agent, thereby contributing to the rational design of more effective transdermal drug delivery systems.

Keywords: skin, drug delivery, triamcinolone acetonide, enhancers

Introduction

Skin, the largest organ in non-aquatic species, covers an area of approximately 20,000 square centimeters in humans. Despite exposure to dehydrating environmental factors, the skin effectively retards water loss, maintaining a dynamic equilibrium within the body's internal tissues and organs. This critical barrier function is primarily attributed to the stratum corneum, a highly organized, multilayered structure of enucleated keratinocytes that constitutes the outermost layer of the skin (Dixena *et al*., 2025).

Percutaneous absorption occurs when materials penetrate from the external environment through the skin and into the bloodstream, a process essential for drug delivery through topical formulations. The stratum corneum permits transdermal diffusion *via* three principal pathways: the appendageal route (through sweat ducts and hair follicles), the intercellular route (between corneocytes) and the transcellular route (directly through the corneocytes) (Behera *et al*., 2024). Penetration enhancers function primarily by increasing the thermodynamic activity of the drug molecule, which serves as the fundamental driving force for transdermal permeation. Thermodynamic activity governs the drug's tendency to move across the skin barrier, whereas solubility is more related to the formulation characteristics and drug availability in the donor phase (Williams and Barry, 2012). It is therefore critical to distinguish between these roles when evaluating the mechanism and efficacy of transdermal enhancers. To enhance skin penetration, scientists and specialists recommend non-toxic agents that temporarily reduce the skin's impermeability, thus facilitating the diffusion of medications for skin treatment (Kumar *et al*., 2024). Accelerants, such as dimethyl sulfoxide (DMSO), are substances that stimulate drug penetration by altering the equilibrium and affecting the arrangement and interactions of various molecules. Anionic surfactants bind tightly to skin proteins, causing swelling of the stratum corneum (Alhallak *et al*., 2023).

Additionally, polar hydrophilic units and protein molecules interact within the protein structure, influencing the flow of water ions and hydrogen bonding. Understanding these mechanisms is crucial for optimizing drug delivery through the skin and developing effective topical treatments (Mahanty *et al*., 2023). Temperature and water association in the stratum corneum produce swelling and an increase in temperature, which in turn

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affects the layer's diffusion properties. Due to the variety in structure and characteristics of the skin, identifying enhancers is challenging. Some enhancers are used as co-solvents to maximize drug absorption through the skin (Parhi and Mandru, 2021). Literature classifies penetration enhancers into various types based on their mechanisms of action, including the promotion of trans epidermal water loss and the disruption of stratum corneum barrier properties to enhance drug permeation (Al Kury *et al*., 2022).

One such enhancer, pentyl acetate ($C_5H_{10}O_2$), is a liquid that can irritate the skin and mucous membranes, while also increasing drug absorption. It is also used as a solvent for waxes and lipids. Surfactants, categorized as cationic, anionic and nonionic, differ in their ability to enhance drug absorption. Cationic surfactants have the least ability to enhance drug absorption, while anionic surfactants perform better, with laurate ion being the most effective in enhancing the permeation of other solutes. Anionic surfactants bind firmly to skin proteins, causing swelling of the stratum corneum by opening protein filaments in different directions (Jiao *et al*., 2021). Nonionic surfactants, although showing minimal drug absorption, can alter the rate of bio-synthesis, arrangement and composition of the lipid layers, thereby affecting membrane structure. Compared to lipophilic and hydrophilic stability, nonionic surfactants play a significant role in these alterations (Cortes *et al*., 2021).

Triamcinolone acetonide (TA) was selected as the model molecule for evaluating transdermal delivery due to its well-characterized physicochemical properties, including moderate molecular weight, lipophilicity (log P ~2.5) and low aqueous solubility, which make it an ideal candidate for studying the efficacy of permeation enhancers. TA is a synthetic corticosteroid with potent anti-inflammatory and immunosuppressive activity, frequently used in the treatment of dermatological conditions. However, its therapeutic effectiveness through topical application is often limited by poor skin penetration, necessitating strategies to enhance its delivery across the stratum corneum. Using TA as a reference compound allows for comparative assessment of various chemical enhancers' ability to increase skin permeability, as it represents a class of pharmacologically active molecules that require enhanced transdermal transport for clinical efficacy (Ng *et al*., 2010).

Material and Methods

Triamcinolone acetonide (TA) supplied by (Squib Pakistan), urea (Searle), sodium lauryl sulphate (SLS) (Merck), cetrimide (Fluka) and ambroxol HCl (Merck), tetrazolium blue (Merck) and purified H_2O, was used in study. Tetrazolium blue test solution was prepared by 350 mg of tetrazolium blue dissolved in 100 mL ethanol 95% to produce 100 mL (Parhi and Mandru, 2021). Tetramethlyammonium hydroxide 10% trimethlyammonium test solution was prepared by dissolving 4 mL to 100 mL with ethanol 9% (Yu *et al*., 2021).

Diffusion cell. Small diffusion cells were fabricated with some modifications (Yang *et al*., 2021). The cells were cylindrical with a diameter of 1.90 cm. The two halves of the cells were designated as the donor section and the receptor section, with heights of 2.5 cm and 8.0 cm, respectively. The donor compartment could hold a volume of 2.0 mL, while the receptor compartment could accommodate 6.0 mL. The two halves were clamped together, with the lower half (receptor compartment) attached to a water jacket. This water jacket had an inlet and outlet for circulating water at a controlled temperature of 37 °C ± 0.5 °C, maintained by a thermoregulator (Erweka). The hairy side of the skin was positioned towards the upper cells and the setup was then placed on a magnetic stirrer.

Preparation of membrane. The membrane used was full-thickness skin obtained from hairless albino mice aged 6-12 weeks, were sacrificed in a desiccator using chloroform vapors for a few min. A rectangular piece of the abdominal skin was removed with surgical shears. Lipids and excess subcutaneous debris were carefully removed from the underside of the skin. For each experimental condition, transdermal permeation studies were performed using six replicates (n=6) per formulation, enhancer and pH condition to ensure statistical reliability. Each replicate involved the use of a separate piece of full-thickness abdominal skin carefully excised and standardized for area (2.5 cm^2) and thickness. The skin samples were mounted in diffusion cells and each enhancer was evaluated independently. The permeation data collected from each replicate were analyzed to determine mean values and standard deviations.

Permeation. The donor compartment was filled with 1.5 mL of the test solution containing 0.01% triamcinolone acetonide and 0.2% w/v enhancer and the experiment

was initiated. This solution, containing 150 µg of triamcinolone acetonide in phosphate buffer, was used as the test sample. The contents of the cells were agitated at different time intervals and samples were taken using a 1 mL automatic micropipette. To determine the effect of pH on the rate of permeability, phosphate buffers at pH 5.8 and 8.0, as well as solutions at variable pH, were used.

Sampling and extraction of drug. A 1 mL sample was transferred to a separating funnel and extracted three times with 5 mL of chloroform. The chloroform extracts were collected in a 25 mL volumetric flask and assayed using the tetrazolium blue method. The concentration of triamcinolone acetonide in the samples was determined from a standard curve prepared with known concentrations of the steroid (Natsheh and Touitou, 2020).

Standard curve. The standardized mixture was prepared by dissolving the reference standard triamcinolone acetonide in chloroform. A standard mixture was made by dissolving 10 mg of triamcinolone acetonide in chloroform in a 100 mL flask to produce a 100 mL solution. This solution was used to prepare further dilutions of 0.1, 0.2, 0.3, 0.4 and 0.5 µg/mL of triamcinolone in 25 mL flasks. To each flask, 1 mL of tetrazolium blue test mixture was added, followed by 1 mL of trimethylammonium hydroxide test solution and the volume was brought up to the mark with 95% ethanol. A reagent blank, consisting of 15 mL chloroform in a 2 mL flask and the other reagents, was prepared for correcting the absorbance values. The absorbance values were plotted against the corresponding concentrations.

Statistical analysis. All quantitative data obtained from permeation studies, total phenolic and flavonoid content assays, antioxidant activity (DPPH and ABTS) and anti-inflammatory and antimicrobial evaluations were expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine significant differences among multiple groups. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using GraphPad Prism (version 9.0, GraphPad Software Inc., USA).

Key transdermal delivery parameters such as flux (J), diffusion coefficient (D), enhancement ratio (E_{pen}) and lag time ($T_{la}g$) were calculated based on standard permeation models. The steady-state flux (J) was determined from the linear portion of the cumulative amount permeated (Q) versus time (t) plot using the formula: $J=dQ/dt$. The lag time ($T_{la}g$) was calculated by extrapolating the linear portion of the permeation curve back to the x-axis (time axis). The diffusion coefficient (D) was calculated using the equation:

$$D=h^2/6\times T_{la}g$$

where *h* represents the thickness of the skin membrane. The enhancement ratio (E_{pen}) was calculated by comparing the flux in the presence of enhancer to that of the control (without enhancer) using $E_{pen}= J_{control}/J_{enhancer}$.

Results and Discussions

The results demonstrate differential efficacy among the tested penetration enhancers. In the control group, the maximum permeation value of 0.81 was achieved after 6 h as shown in Table 1. Sodium lauryl sulphate had the highest permeation at 6 h with a value of 2.4, compared to urea at 2.006 and ambroxol HCl at 1.144. At the 3h time point, urea exhibited the highest permeation, whereas sodium lauryl sulfate demonstrated a delayed peak in permeation, reaching its maximum at 6 h. Urea exhibited a gradual increase in the flow of triamcinolone acetonide over time, likely due to the shrinkage of the skin upon contact and its mild keratolytic action. At pH 8.0, the rate constant was higher up to 4 h, showing a regular increasing effect on the rate constant of triamcinolone acetonide permeation.

Statistical summary of the chemical impact data for the control and various enhancers: urea, sodium lauryl sulphate, cetrimide and ambroxol HCl is provided in Table 2. Each group consists of six observations. The mean impact values indicate that urea (1.3692) and sodium lauryl sulphate (1.2723) have the highest average impacts, followed by cetrimide (0.7967), ambroxol HCl (0.6343) and the control group (0.3917). The standard deviation is highest for sodium lauryl sulphate (0.7043), suggesting greater variability, while cetrimide has the lowest standard deviation (0.2556), indicating more consistent results. The minimum impact values range from 0.14 in the control group to 0.277 for ambroxol HCl. Quartile analysis shows the distribution of impacts within each group, with Urea and sodium lauryl sulphate having the highest third quartile values (1.7017 and 1.6483, respectively). The maximum impacts observed were 2.4 for sodium lauryl

sulphate, 2.006 for urea and 1.144 for ambroxol HCl. These findings highlight the variable efficacy and consistency of the different chemical enhancers.

The relative impact of different chemicals—urea, sodium lauryl sulphate, cetrimide and ambroxol HCl compared with control at specific time points over a six hour period presented in Table 3. At hour 1, urea exhibited the highest impact (0.3375), followed by sodium lauryl sulphate (0.2454), cetrimide (0.2232) and ambroxol HCl (0.1287), with the control showing the lowest impact (0.0651). By hour 2, urea remained the most effective (0.3247), while sodium lauryl sulphate (0.2478) and ambroxol HCl (0.1580) increased slightly compared to hour 1. The trend continued at hour 3, where urea peaked at 0.3463, maintaining the highest impact among the enhancers. At hour 4, sodium lauryl sulphate showed a notable increase to 0.2689, while urea's impact slightly decreased to 0.3167. By hour 5, sodium lauryl sulphate (0.2896) and urea (0.2891) had similar impacts, with cetrimide and ambroxol HCl showing lower values. At 6 h, sodium lauryl sulphate reached its maximum impact (0.3333), surpassing urea (0.2786) and other chemicals. These results demonstrate the varying efficacy of the chemicals over time, with urea showing a strong early impact and sodium lauryl sulphate becoming more effective in the later hours.

Urea exhibited the highest relative impact initially, which decreased towards the end, while sodium lauryl

Table 1. Cumulative concentration of triamcinolone acetonide (µg/mL) over time across mouse skin at pH 8.0.

	1 h	2 h	3 h	4 h	5 h	6 h
Control	0.14±0.011	0.18±0.04	0.27±0.031	0.38±0.051	0.57±0.073	0.81±0.016
Urea	0.726±0.024	0.976±0.017	1.254±0.008	1.476±0.015	1.777±0.054	2.006±0.017
SLS	0.528±0.082	0.745±0.041	0.928±0.027	1.253±0.044	1.78±0.028	2.4±0.013
Cetrimide	0.48±0.021	0.63±0.022	0.67±0.011	0.98±0.28	1.18±0.062	0.84±0.021
Ambroxol HCl	0.277±0.013	0.475±0.011	0.499±0.051	0.571±0.022	0.84±0.034	1.144±0.043

Table 2. Relative chemical impact of enhancers and comparison in quartiles

	Control	Urea	Sodium lauryl sulphate	Cetrimide	Ambroxol HCl
Count	6.0	6.0	6.0	6.0	6.0
Mean	0.39166	1.36916666	1.27233333	0.79666666	0.6343333
Std deviation	0.25685	0.48266030	0.70433079	0.25555169	0.3090175
Min impact	0.14	0.726	0.528	0.48	0.277
25% 1st quartile	0.2025	1.0455	0.79075000	0.64	0.481
50% Median	0.325	1.365	1.0905	0.755	0.5349999
75% 3rd quartile	0.5225	1.70174999	1.64825	0.945	0.7727499
Max impact	0.81	2.006	2.4	1.18	1.144

F-statistic=3.22; *p*-value = 0.027.

Table 3. Relative impact of enhancers at specific time points

Time	Control	Urea	Sodium lauryl sulphate	Cetrimide	Ambroxol HCl
Hour 1	0.0651±0.003	0.3375±0.014	0.2454±0.074	0.2232±0.076	0.1287±0.055
Hour 2	0.0598±0.008	0.3247±0.012	0.2478±0.027	0.2095±0.054	0.1580±0.032
Hour 3	0.0745±0.011	0.3463±0.008	0.2562±0.053	0.1850±0.066	0.1378±0.052
Hour 4	0.0815±0.012	0.3167±0.017	0.2689±0.042	0.2103±0.082	0.1225±0.033
Hour 5	0.09270±0.011	0.2891±0.026	0.2896±0.042	0.1919±0.053	0.1366±0.032
Hour 6	0.1125±0.013	0.2786±0.043	0.3333±0.054	0.1166±0.073	0.1588±0.052

sulphate showed an increasing impact over time, culminating in the highest relative impact at the end, as illustrated in Fig 1.

Table 4 provides a detailed insight into the enhancing effect that various substances have on the flux of triamcinolone acetonide. The control achieved its maximum value of 0.67 after 6 h, while among the enhancers, sodium lauryl sulphate had the highest maximum value of 2.337 and the lowest was observed with ambroxol HCl 1.12. At pH 5.8, the rate increases gradually as the time intervals get longer. Over time, sodium lauryl sulphate decreases the rate constants of triamcinolone acetonide in acidic media, however, in basic solution, the rate constant at 6 h is higher than at 4 h and up to 2 h. At pH 5.8, the rate constant for triamcinolone acetonide under the influence of cetrimide and ambroxol HCl appears to drop steadily over time. Cetrimide elevated the permeability rate constant of Triamcinolone acetonide at neutral pH from two to six hours, reaching a maximum. The role of cetrimide increases steadily at basic pH up to 4 h, after which it declines up to six hours.

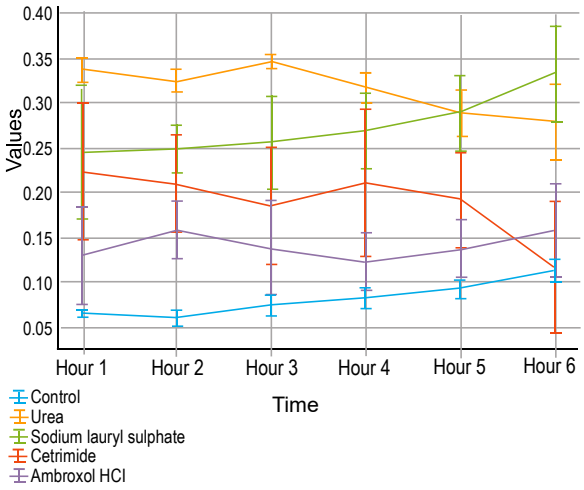


Fig. 1. Relative impact of each enhancer changes over time.

The impact of various enhancers across different quartiles, comparing the control group with urea, sodium lauryl sulphate, cetrimide and ambroxol HCl is illustrated in Table 5. Each group consisted of six observations. The mean impact values reveal that sodium lauryl sulphate exhibited the highest average impact (1.6158), followed by Urea (1.4120), Cetrimide (0.8322),

Table 4. Cumulative concentration of triamcinolone acetonide ug/mL through mice skin at pH 5.8

Time	Control	Urea	Sodium lauryl sulphate	Cetrimide	Ambroxol HCl
Hour 1	0.16±0.035	0.909±0.131	0.644±0.008	0.380±0.021	0.445±0.025
Hour 2	0.19±0.024	1.101±0.097	1.972±0.112	0.530±0.061	0.550±0.024
Hour 3	0.25±0.037	1.301±0.141	1.293±0.131	0.680±0.083	0.680±0.016
Hour 4	0.38±0.022	1.478±0.113	1.550±0.126	0.890±0.055	0.789±0.022
Hour 5	0.52±0.021	1.701±0.127	1.899±0.147	1.082±0.028	0.870±0.054
Hour 6	0.67±0.014	1.982±0.125	2.337±0.116	1.431±0.044	1.120±0.034

Table 5. Impact of enhancers in different quartiles at pH 5.8

	Control	Urea	Sodium lauryl sulphate	Cetrimide	Ambroxol HCl
Count	6.0	6.0	6.0	6.0	6.0
Mean	0.3617	1.4120	1.6158	0.8322	0.7423
Std deviation	0.2015	0.3936	0.5967	0.3855	0.2410
Min impact	0.16	0.909	0.644	0.38	0.445
25% 1st quartile	0.2050	1.151	1.3572	0.5675	0.5825
50% Median	0.315	1.3895	1.7245	0.785	0.7345
75% 3rd quartile	0.485	1.6452	1.9537	1.034	0.8497
Max impact	0.67	1.982	2.337	1.431	1.12

F-statistic=17.40; *p*-value=0.0006

ambroxol HCl (0.7423) and the control group (0.3617). The standard deviation was highest for sodium lauryl sulphate (0.5967), indicating greater variability in its impact, whereas ambroxol HCl had the lowest standard deviation (0.2410), suggesting more consistent results. The minimum and maximum impacts for each enhancer highlight the range of their effects, with sodium lauryl sulphate showing the broadest range (0.644 to 2.337). Quartile analysis further details the distribution of impact values, with the first quartile, median and third quartile providing insight into the central tendency and dispersion within each group. This detailed statistical summary underscores the variable efficacy of different enhancers and their potential applications.

Sodium lauryl sulphate shows the most substantial increase in permeability among all treatments, indicating it has the highest impact on the response. Cetrimide demonstrates a moderate increase, with a significant impact that is less pronounced compared to urea and sodium lauryl sulphate. Ambroxol HCl (at pH 6.55) shows the least increase among the treatments, with its impact being relatively lower compared to the other treatments as illustrated in Fig. 2.

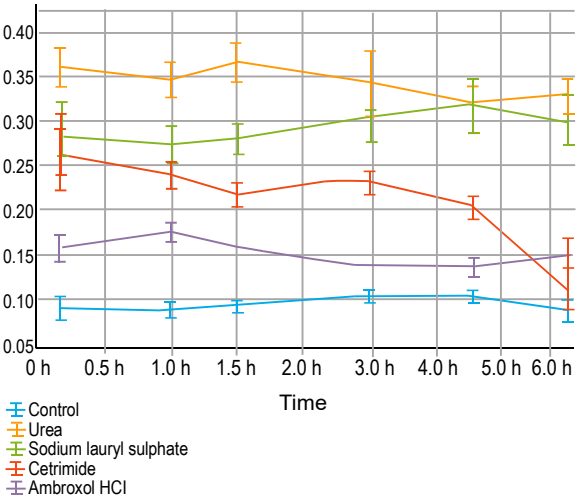


Fig. 2. Permeability impact of different chemicals over time at pH 5.8.

The cumulative concentration of triamcinolone acetonide (µg/mL) through mice skin at variable pH levels over a 6 h period is included in Table 6. The data shows that urea at pH 6.55 and sodium lauryl sulphate at pH 6.9 significantly enhance the permeation of triamcinolone acetonide compared to the control (pH 6.05). Urea exhibits a consistent increase, reaching a peak concentration of 2.317 µg/mL at 6 h. Sodium lauryl sulphate demonstrates the highest enhancement, with a cumulative concentration of 2.594 µg/mL at 6 h. Cetrimide at pH 6.85 also shows a notable increase, achieving 1.67 µg/mL at 6 h. In contrast, Ambroxol HCl at pH 6.55 has the least impact on permeation, with a maximum concentration of 0.890 µg/mL at 6 h. These results indicate that urea and sodium lauryl sulphate are the most effective enhancers for increasing the permeability of triamcinolone acetonide through mice skin, while ambroxol HCl has a relatively lower effect.

The control (pH 6.05) shows a gradual increase in response over time, starting from 0.18 µg/mL at 1 h and reaching 0.59 µg/mL at 6 h. Urea (pH 6.55) exhibits a steady and significant increase, beginning at 0.98 µg/mL and peaking at 2.31 µg/mL by 6 h, indicating a strong and consistent impact over time. Sodium lauryl sulphate (pH 6.9) demonstrates the most substantial increase among all treatments, starting at 0.69 µg/mL and reaching 2.59 µg/mL at 6 h, resulted in the highest impact on the response. Cetrimide (pH 6.85) shows a moderate increase, starting at 0.64 µg/mL and reaching 1.67 µg/mL by 6 h, with a significant impact that is less pronounced compared to urea and sodium lauryl sulphate. Ambroxol HCl (pH 6.55) displays the least

Table 6. Cumulative concentration of triamcinolone acetonide ug/mL through mice skin at variable pH

Time (h)	Control pH 6.05	Urea pH 6.55	Sodium lauryl sulphate pH 6.9	Cetrimide pH 6.85	Ambroxol HCl pH 6.55
1	0.18±0.021	0.984±0.023	0.691±0.055	0.644±0.078	0.290±0.011
2	0.29±0.035	1.161±0.028	0.848±0.068	0,844±0.085	0.410±0.015
3	0.38±0.028	1.370±0.014	1.031±0.087	0.970±0.066	0.520±0.021
4	0.43±0.017	1.682±0.036	1.327±0.065	1.188±0.049	0.690±0.018
5	0.48±0.027	1.964±0.048	1.826±0.088	1.388±0.068	0.797±0.031
6	0.59±0.019	2.317±0.036	2.594±0.072	1.674±0.064	0.890±0.027

increase among the treatments, starting at 0.29 µg/mL and reaching 0.89 µg/mL by 6 h, indicating a relatively lower impact compared to the other treatments.

In average rate of change in the concentration of triamcinolone acetonide for each treatment over the experimental period sodium lauryl sulphate (pH 6.9) treatment exhibits the highest average rate of change at 0.38 µg/mL/h, indicating the most substantial enhancement in permeation. Urea (pH 6.55) follows with an average rate of 0.26 µg/mL/h, demonstrating a significant but lesser impact compared to sodium lauryl sulphate. Cetrimide (pH 6.85) shows a moderate average rate of change at 0.20 µg/mL/h, reflecting a moderate enhancement in permeability. The control (pH 6.05) has the lowest average rate of change at 0.08 µg/mL/h, with minimal impact. Ambroxol HCl (pH 6.55) also shows a relatively low average rate of change at 0.12 µg/mL/h, indicating a smaller effect compared to other treatments as illustrated in Fig. 3. These findings indicate the varying efficacy of different treatments in enhancing the rate of permeation of triamcinolone acetonide through the skin.

Absorption rates of different enhancers at variable pH levels over specified intervals are summarized in Table 7. The data shows that sodium lauryl sulphate (pH 6.9) exhibits the highest absorption rate across all intervals, with an average rate of 0.38 µg/mL/h, indicating the most effective enhancement in permeation. urea (pH 6.55) also demonstrates significant absorption,

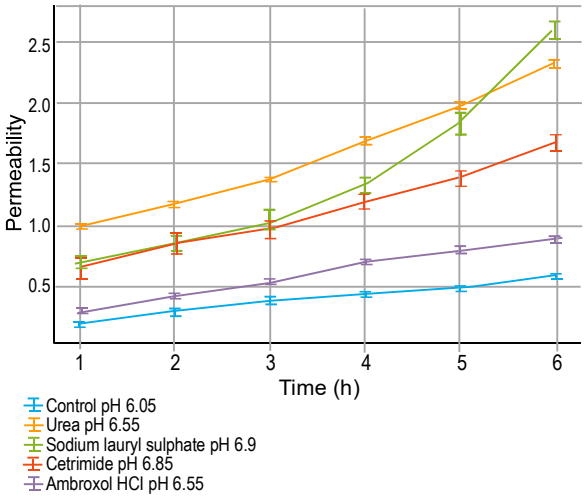


Fig. 3. Impact of various treatments over time at variable pH.

Table 7. Average rate of change for each treatment

Treatment	Average rate of change
Control (pH 6.05)	0.0820±0.01
Urea (pH 6.55)	0.2666±0.042
Sodium lauryl sulphate (pH 6.9)	0.3806±0.032
Cetrimide (pH 6.85)	0.2060±0.028
Ambroxol HCl (pH 6.55)	0.1200±0.041

with an average rate of 0.26 µg/mL/h, reflecting a strong but less pronounced impact compared to sodium lauryl sulphate. Cetrimide (pH 6.85) shows a moderate absorption rate with an average of 0.20 µg/mL/h, while Ambroxol HCl (pH 6.55) has a relatively lower average absorption rate of 0.12 µg/mL/h. The control (pH 6.05) has the lowest average rate of 0.08 µg/mL/h, suggesting minimal enhancement compared to the treatments. These findings highlight the varying efficacy of different enhancers in facilitating the absorption of triamcinolone acetonide through the skin at different pH levels.

Absorption of triamcinolone acetonide through hairless mouse skin with and without enhancers at pH 8.0 reveals that the presence of urea and sodium lauryl sulphate decreases the diffusion coefficient of triamcinolone acetonide across both sides of the neutral pH range. Notably, urea achieves this without a lag time, suggesting a higher degree of diffusivity throughout the pH spectrum. Cetrimide also enhances the rate of diffusivity, though its effect is less pronounced. In contrast, ambroxol HCl demonstrates an increase in triamcinolone Acetonide diffusivity primarily at higher pH values.

Sodium lauryl sulphate is identified as the most effective enhancer, demonstrating the highest diffusion coefficient (1.20 cm^2/h) and enhancement penetration (2.85) in Table 8. Urea also proves to be a strong enhancer, with a high diffusion coefficient (0.64 cm^2/h) and a flux of 0.52 $\mu g/cm^2/h$. In contrast, ambroxol HCl exhibits the least enhancement effect, characterized by the lowest diffusion coefficient (0.11 cm^2/h) and the longest lag time (1.40 h). Both the control and cetrimide show moderate effects, with cetrimide achieving a higher enhancement penetration (1.45) compared to the control (1.00). These results provide valuable insights into the relative effectiveness of each enhancer in facilitating the permeation of triamcinolone acetonide through mouse skin at pH 8.0.

The control, without any enhancer in Table 9, has a lag time (L) of 1 h, a diffusion coefficient (D) of 0.035 cm^2/h and a flux (J) of 0.10 $\mu g/cm^2/h$, with an enhancement penetration (E_{pen}) ratio of 1. Urea significantly reduces the lag time to 0.00 h and increases the diffusion coefficient to 0.580 cm^2/h, resulting in a flux of 0.20 $\mu g/cm^2/h$ and an enhancement penetration ratio of 1.99. Sodium lauryl sulphate also demonstrates a substantial effect with a lag time of 0.45 h, a diffusion coefficient of 0.960 cm^2/h and a flux of 0.32 $\mu g/cm^2/h$, yielding the highest enhancement penetration ratio of 3.10. Cetrimide has a lag time of 0.70 h, a diffusion coefficient of 0.490 cm^2/h and a flux of 0.20 $\mu g/cm^2/h$, with an enhancement penetration ratio of 1.93. Ambroxol HCl shows a lag time of 0.80 h, a diffusion coefficient of 0.099 cm^2/h and a flux of 0.12 $\mu g/cm^2/h$, resulting in an enhancement penetration ratio of 1.21. These results highlight the effectiveness of each enhancer in improving the flux and diffusion of triamcinolone acetonide through the skin (Li *et al*., 2019).

The control, without any enhancer, exhibits a lag time (L) of 1 h, a diffusion coefficient (D) of 0.040 cm^2/h and a flux (E) of 0.08 $\mu g/cm^2/h$, with an enhancement penetration (E_{pen}) ratio of 1. In Table 10 urea significantly enhances penetration, reducing the lag time to 0.00 h and increasing the diffusion coefficient to 0.540 cm^2/h, resulting in a flux of 0.26 $\mu g/cm^2/h$ and an enhancement penetration ratio of 3.29. Sodium lauryl sulphate shows a lag time of 0.40 h, with the highest diffusion coefficient of 1.000 cm^2/h and a flux of 0.36 $\mu g/cm^2/h$, yielding the highest enhancement penetration ratio of 4.47.

Table 8. Lag time and coefficient of triamcinolone acetonide ug/mL at pH 8.0

Enhancer	Lag time 'L' (h)	Diffusion coefficient 'D' (cm^2/h)	Flux 'J' ($\mu g/cm^2/h$)	E_{pen}
Control	1.00	0.044	0.1314±0.01	1
Urea	0.00	0.64	0.529±0.011	1.96
Sodium lauryl sulphate	0.40	1.20	0.3744±0.199	2.85
Cetrimide	0.50	0.40	0.1925±0.111	1.45
Ambroxol HCl	1.40	0.11	0.1610±0.01	1.23

Table 9. The ratio of flux with or without enhancer

Enhancer	Lag time 'L' (h)	Diffusion coefficient 'D' (cm^2/h)	Flux 'J' ($\mu g/cm^2/h$)	E_{pen}
Control	1.00	0.035	0.1048±0.01	1
Urea	0.00	0.580	0.2094±0.033	1.99
Sodium lauryl sulphate	0.45	0.960	0.3258±0.005	3.10
Cetrimide	0.70	0.490	0.2031±0.010	1.93
Ambroxol HCl	0.80	0.099	0.1270±0.0265	1.21

Table 10. Penetration of triamcinolone acetonide through hairless mouse skin with or without enhance at variable pH

Enhancer	Lag time 'L' (h)	Diffusion coefficient 'D' (cm^2/h)	Flux 'J' ($\mu g/cm^2/h$)	E_{pen}
Control	1.00	0.04	0.0814±0.006	1
Urea	0.00	0.54	0.2682±0.046	3.29
Sodium lauryl sulphate	0.40	1.00	0.3640±0.030	4.47
Cetrimide	0.50	0.56	0.2045±0.011	2.53
Ambroxol HCl	0.40	0.08	0.1231±0.018	1.50

Cetrimide demonstrates a lag time of 0.50 h, a diffusion coefficient of 0.560 cm^2/h and a flux of 0.20 $\mu g/cm^2/h$, with an enhancement penetration ratio of 2.53. Ambroxol HCl shows a lag time of 0.40 h, a diffusion coefficient of 0.080 cm^2/h and a flux of 0.12 $\mu g/cm^2/h$, resulting in an enhancement penetration ratio of 1.50. These results illustrate the varying degrees of effectiveness of each enhancer in facilitating the penetration of triamcinolone acetonide through the skin.

The control, without any enhancer, has a diffusion coefficient (D) of 0.00136 cm^2/h and a permeability coefficient (P) of 0.0002478 cm/h. Urea significantly increases both coefficients, with a diffusion coefficient of 0.490 cm^2/h and a permeability coefficient of 0.0004993 cm/h. Sodium lauryl sulphate exhibits a diffusion coefficient of 0.0030247 cm^2/h and a permeability coefficient of 0.0001187 cm/h. Cetrimide shows a diffusion coefficient of 0.0019444 cm^2/h and a permeability coefficient of 0.0004820 cm/h, while ambroxol HCl has a diffusion coefficient of 0.0017014 cm^2/h and a permeability coefficient of 0.0003011 cm/h. These results highlight the varying effects of different enhancers on the diffusion and permeability of Triamcinolone acetonide, with urea showing the most pronounced impact (Kapoor *et al*., 2017). Permeability coefficients of triamcinolone acetonide at various pH levels with the presence of different enhancers. Urea, cetrimide and sodium lauryl sulphate exhibit similar patterns in their partition coefficients, suggesting comparable effects on permeability.

The variations in triamcinolone acetonide permeability across different pH levels appear to be influenced by the permeability coefficients, which are elevated for urea, sodium lauryl sulphate and ambroxol HCl. This indicates that un-ionized molecules can more readily diffuse through the lipid membrane compared to ionized molecules, as the latter lacks the necessary free energy to effectively traverse the lipid barrier. Cetrimide and sodium lauryl sulphate, both ionic surfactants, have been studied for their effects on the percutaneous absorption of naproxen through excised human skin (Parhi and Mandru, 2021). Among these, cetrimide and sodium lauryl sulphate-anionic and cationic surfactants respectively-show a more significant impact on the flux of triamcinolone acetonide compared to ambroxol HCl. sodium lauryl sulphate's effect on enhancement is observed on both sides of the neutral pH range, a pattern similarly seen with cetrimide. Sodium lauryl sulphate's enhancing effect may be attributed to its strong binding to skin proteins, leading to excessive swelling of the stratum corneum. This swelling occurs due to cooperative binding of the surfactant to the protein, causing reversible conformational changes (Elsoud *et al*., 2017).

Cetrimide, on the other hand, may penetrate through the stratum corneum, progressively modifying the barrier properties and enhancing its own flux. It has been noted that anionic surfactants, owing to their detrimental effects or acanthotic action on the epidermis, facilitate the passage of metals and other substances through the skin (Ghezelbash *et al*., 2014). Ambroxol HCl effects on the skin penetration of chemicals is limited. Pharmacologically, Ambroxol HCl is primarily used as a mucolytic agent in the respiratory tract, making it challenging to ascertain its mechanism for enhancing triamcinolone acetonide absorption through the skin. Comparative analysis suggests that Ambroxol HCl behaves similarly to the control, exhibiting minimal or negligible influence on flux.

Table 11. Permeability coefficients and diffusion coefficients of triamicilone acetonide in the presence and absence of enhancer at pH 5.8

Enhancer	Diffusion coefficients D pH 5.8	Permeability coefficients P pH 5.8	Diffusion coefficients D pH 8.0	Permeability coefficients P pH 8.0
Control	0.00136	0.000247784	0.00136	0.000310867
Urea	0.490	0.0004993156	0.490	0.000613737
SLS	0.0030247	0.0001187355	0.003404	0.000895032
Cetrimide	0.0019444	0.0004819818	0.002722	0.000457455
Ambroxol HCl	0.00170139	0.0003010782	0.000472	0.000381887

D the diffusion coefficient cm^2/min and p the permeability coefficient cm*h-1

The flux rate decreases on both sides of the neutral pH range, which may be attributed to a reduced diffusion coefficient. Assuming the penetration process follows a first-order rate constant, the rate constant can be determined from semi logarithmic plots of the triamcinolone acetonide concentration at various time intervals using the following equation:

$$\text{Rate constant} = \log(Y_2\text{-}Y_1)/t_2\text{-}t1*2.303$$

Permeability rate constants observed at 2, 4 and 6 h offer insights into the behavior of enhanced triamcinolone acetonide absorption through hairless mouse skin at various pH levels. In the absence of enhancers, the rate constants for triamcinolone acetonide exhibit a consistent pattern across all pH levels, showing a decreasing trend as the pH shifts from acidic to basic. The rate constant reaches its highest value at the most basic pH and maintains this peak level up to 4 h.

Conclusion

This study evaluated the effects of various enhancers on the transdermal absorption of triamcinolone acetonide through hairless mouse skin at different pH levels. Lag time is a critical factor in interpreting results. Urea demonstrates an immediate diffusion of the drug, with no lag time, indicating a rapid enhancing effect. The permeability coefficient of triamcinolone acetonide in the presence of enhancers shows that sodium lauryl sulphate is the most effective enhancer compared to others within the pH range investigated. Most enhancers, when compared to the control, increase the drug's penetration. However, some enhancers, such as ambroxol HCl, exhibit minimal enhancing effects. The relative flux (E_{pen}) highlights significant differences in enhancing properties. Sodium lauryl sulphate shows the highest relative flux, making it the most effective enhancer, while ambroxol HCl shows the least enhancement in percutaneous absorption of triamcinolone acetonide. Variations in the rate constants are observed at different time intervals, reflecting changes in the rate of drug absorption over time.

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Conflict of Interest. The authors declare that they have no conflict of interest.

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