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Comparative in Vitro Evaluation of Hydrocortisone Acetate Permeation Through Porcine Skin and Synthetic Membranes Using Franz Diffusion Cells

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Abstract: *The evaluation of dermal drug permeation is a key requirement in the development, quality control, and regulatory assessment of topical formulations. Reliable in vitro models are essential to ensure reproducibility and biological relevance. This study aimed to compare the in vitro permeation profile of hydrocortisone acetate (HCA) through porcine skin and a synthetic membrane using Franz diffusion cells.In vitro permeation studies were performed using Franz diffusion cells maintained at 37 ± 0.5°C. A 1% hydrocortisone acetate ointment was applied to porcine skin and STRAT-M synthetic membranes. Samples were withdrawn at 15, 30, 45, and 60 minutes and analyzed by UV–Vis spectrophotometry at 241.5 nm. Drug concentrations were determined using a validated calibration curve (r^2 = 0.99). Experiments were conducted under controlled and reproducible conditions. The results show that hydrocortisone acetate permeation increased progressively over time for both membrane types. The STRAT-M membrane exhibited consistent and reproducible permeation profiles. In contrast, porcine skin showed greater variability, likely due to intrinsic biological differences and storage conditions. Despite this variability, overall permeation trends between the two models were comparable, with only slight differences in diffusion rates. In conclusion **b**oth porcine skin and synthetic membranes are suitable for in vitro permeation studies of topical formulations. While synthetic membranes provide higher reproducibility, porcine skin offers greater physiological relevance. The Franz diffusion cell method demonstrated robustness and suitability for routine evaluation of dermal drug delivery systems.*

Keywords: Hydrocortisone acetate, in vitro permeation, franz diffusion cells, porcine skin, strat-m membrane, topical formulations

INTRODUCTION

Transdermal and topical drug delivery systems have gained significant attention in modern pharmaceutical development due to their ability to provide localized and systemic therapeutic effects while minimizing

first-pass metabolism and systemic side effects. These systems are particularly advantageous for corticosteroids such as hydrocortisone acetate, which are widely used in the management of inflammatory skin conditions. A critical aspect in the development and evaluation of such formulations is the assessment of drug permeation across the skin barrier, which directly influences therapeutic efficacy and safety.

Human skin, particularly the stratum corneum, represents a highly effective barrier to drug penetration. Therefore, reliable and reproducible methods for evaluating dermal absorption are essential. Among available in vitro techniques, the Franz diffusion cell system remains the most widely accepted and utilized method for studying drug release and permeation from topical dosage forms. Since its introduction by Franz (1975), this technique has been extensively validated and continues to be considered the gold standard due to its simplicity, reproducibility, and ability to simulate physiological conditions. Recent studies have further confirmed its applicability in both research and regulatory settings (Kumar et al., 2023; Chen et al., 2021).

The selection of an appropriate membrane model is a crucial factor influencing the outcomes of in vitro permeation studies. Excised human skin is considered the most representative model; however, its use is limited by ethical concerns, variability, limited availability, and complex storage requirements. Consequently, alternative models such as animal skin and synthetic membranes have been widely adopted. Porcine skin is one of the most commonly used biological substitutes for human skin due to its close structural, biochemical, and functional similarities. These include comparable epidermal thickness, lipid organization, hair follicle density, and permeability characteristics. As a result, porcine skin has been extensively validated as a predictive model for human dermal absorption (Simon & Maibach, 2000; Jacobi et al., 2022). However, despite its advantages, porcine skin is associated with certain limitations, including variability due to animal age, anatomical site, and storage conditions, all of which may affect experimental reproducibility.

In contrast, synthetic membranes such as STRAT-M have been developed to overcome the limitations associated with biological tissues. STRAT-M is a multilayer polymeric membrane specifically designed to mimic the barrier properties of human skin. It offers several practical advantages, including consistent structure, ease of handling, long shelf life, and elimination of ethical concerns. Recent studies have demonstrated that STRAT-M provides reproducible permeation data and can serve as a reliable screening tool during early-stage formulation development (Uchida et al., 2015; Haq et al., 2020; Simon et al., 2020). In vitro permeation studies play a central role not only in formulation development but also in quality control and bioequivalence assessment of topical products. Regulatory agencies increasingly recognize these methods as alternatives to in vivo studies. The Organisation for Economic Co-operation and Development (OECD) has standardized skin absorption testing under Guideline 428, which outlines procedures for conducting in vitro permeation studies using diffusion cells. Furthermore, recent regulatory trends emphasize the importance of in vitro–in vivo correlation (IVIVC) and the use of validated laboratory models to support product approval and lifecycle management (OECD, 2020; EMA, 2022).

Hydrocortisone acetate (HCA) is a low-to-moderate potency corticosteroid commonly formulated in topical preparations. Its permeation behavior depends on multiple factors, including formulation

composition, membrane characteristics, and experimental conditions. Despite its widespread use, comparative data evaluating its permeation through biological and synthetic membranes under standardized conditions remain limited.

Therefore, the present study aims to comparatively evaluate the in vitro permeation profile of hydrocortisone acetate through porcine skin and STRAT-M synthetic membranes using Franz diffusion cells. By examining differences in permeation behavior, reproducibility, and practical applicability, this study seeks to assess the suitability of these membrane models for routine laboratory testing and pharmaceutical research. The findings are expected to contribute to the optimization of in vitro methodologies and support the selection of appropriate models in topical drug development.

The aim of this study was to comparatively evaluate the in vitro permeation profile of hydrocortisone acetate through porcine skin and a synthetic membrane (STRAT-M) using Franz diffusion cell methodology, in order to assess their suitability as models for dermal drug delivery studies.

MATERIALS AND METHODS

Materials

The following materials were used in this study: hydrocortisone acetate ointment (1%), hydrocortisone acetate reference standard, ethanol (50% v/v), and distilled water. Biological membrane samples consisted of porcine skin obtained from 8-week-old animals, while synthetic membranes (STRAT-M) were used as an alternative diffusion barrier. Permeation experiments were conducted using a Franz diffusion cell apparatus equipped with six cells (diffusion area diameter: 9 mm). Drug analysis was performed using a UV–Vis spectrophotometer.

Preparation of Standard Solutions

A primary stock solution was prepared by dissolving 250 mg of hydrocortisone acetate in 25 mL of 50% (v/v) ethanol. Subsequent serial dilutions were performed to obtain standard solutions with concentrations of 100, 50, and 40 µg/mL. The calibration curve was constructed by measuring absorbance at 241.5 nm and demonstrated good linearity with a correlation coefficient (r^2) of 0.99, confirming the suitability of the analytical method for quantification.

In Vitro Permeation Study

In vitro permeation studies were carried out using Franz diffusion cells under controlled experimental conditions. The receptor chamber was filled with 20 mL of 50% (v/v) ethanol, which served as the receptor medium, and maintained at a constant temperature of 37 ± 0.5°C to simulate physiological conditions.
Porcine skin and STRAT-M membranes were carefully mounted between the donor and receptor compartments, ensuring proper alignment and absence of air bubbles. Approximately 1 g of hydrocortisone acetate ointment (1%) was uniformly applied to the donor compartment. At predetermined time intervals (15, 30, 45, and 60 minutes), 1 mL samples were withdrawn from the receptor compartment and immediately replaced with fresh receptor medium to maintain sink conditions. The collected samples were analyzed using UV–Vis spectrophotometry at a wavelength of 241.5 nm.

Data Analysis

All experiments were conducted in triplicate ($n \geq 3$), and the results were expressed as mean values. Due to the limited sample size, comprehensive statistical analysis, including standard deviation and inferential statistical testing, was not performed. Instead, data were evaluated descriptively to assess permeation trends and compare the performance of the two membrane models.

RESULTS

Analytical Method Validation

The calibration curve for hydrocortisone acetate demonstrated strong linearity across the tested concentration range (40–100 µg/mL), with a correlation coefficient ($r^2 = 0.99$). This confirms the reliability and suitability of the UV–Vis spectrophotometric method for quantitative analysis.

In Vitro Permeation Profiles

Permeation data were collected over a three-week experimental period, and mean percentage permeation values were calculated for each sampling time point. The results for both porcine skin and STRAT-M synthetic membranes are summarized in Table 1.

Table 1. Mean Percentage Permeation of Hydrocortisone Acetate

Time (min)	Porcine Skin (%)	STRAT-M Membrane (%)
15	16.6	15.8
30	20.4	21.3
45	22.7	28.3
60	31.7	19.7

Comparative Permeation Analysis

Both membrane systems exhibited a time-dependent increase in hydrocortisone acetate permeation during the initial phase of the experiment (15–45 minutes), indicating effective diffusion across the barrier membranes. At 15 minutes, permeation values were comparable between porcine skin (16.6%) and STRAT-M membranes (15.8%), suggesting similar initial diffusion behavior.

At 30 minutes, a slight increase was observed in both systems, with STRAT-M membranes showing marginally higher permeation (21.3%) compared to porcine skin (20.4%). This trend became more pronounced at 45 minutes, where synthetic membranes exhibited a notably higher permeation (28.3%)

relative to porcine skin (22.7%). This may indicate reduced resistance to diffusion in the synthetic membrane during intermediate time points.

However, at 60 minutes, divergent permeation behavior was observed. Porcine skin demonstrated a continued increase in drug permeation (31.7%), consistent with sustained diffusion through a biologically complex barrier. In contrast, STRAT-M® membranes showed a decrease in permeation (19.7%), which may be attributed to saturation effects, altered membrane diffusion dynamics, or limitations in mimicking prolonged drug transport.

Variability and Reproducibility

Variability in permeation profiles was assessed qualitatively based on observations across the three-week study period. Porcine skin exhibited greater variability between experimental runs, likely due to inherent biological differences such as tissue heterogeneity, anatomical variation, and potential alterations caused by storage conditions.

In contrast, STRAT-M® membranes demonstrated more consistent and reproducible permeation behavior across repeated experiments. This highlights the advantage of synthetic membranes in reducing experimental variability and improving repeatability.

Table 2. Observed Variability Trends

Parameter	Porcine Skin	STRAT-M Membrane
Reproducibility	Moderate	High
Variability	Higher	Lower
Biological relevance	High	Moderate
Consistency over time	Variable	Stable

The results indicate that both porcine skin and STRAT-M membranes are capable of supporting in vitro permeation studies of hydrocortisone acetate. The comparable permeation values observed during the early time points suggest that synthetic membranes may serve as suitable initial screening tools.

However, the divergence observed at later time points highlights fundamental differences in diffusion behavior. The continued increase in permeation through porcine skin reflects its complex, multilayered structure, which allows for sustained drug absorption. Conversely, the decline observed in STRAT-M membranes suggests potential limitations in replicating long-term permeation dynamics.

Although the differences between membrane types appear moderate, the absence of formal statistical analysis limits definitive conclusions regarding significance. Future studies incorporating larger sample sizes and statistical testing (e.g., ANOVA) are recommended to validate these findings.

DISCUSSION

The present study provides a comparative evaluation of hydrocortisone acetate permeation through porcine skin and STRAT-M synthetic membranes using Franz diffusion cells. The findings confirm that both membrane models are suitable for in vitro permeation studies, although notable differences in diffusion behavior, reproducibility, and physiological relevance were observed.

The time-dependent increase in drug permeation observed in both membrane systems during the initial phase of the experiment (15–45 minutes) is consistent with the principles of Fick's law of diffusion, which describes passive diffusion as a function of concentration gradient across a membrane. This trend has been widely reported in dermal drug delivery studies and reflects the progressive establishment of steady-state conditions (Chen et al., 2021; Kumar et al., 2023).

Porcine skin demonstrated a continuous increase in permeation throughout the study period, reaching peak values at 60 minutes. This behavior reflects its complex multilayered structure, including the stratum corneum, viable epidermis, and dermis, which collectively influence drug diffusion kinetics. However, increased variability between experimental runs was observed. This variability can be attributed to biological factors such as inter-sample heterogeneity, anatomical differences, and potential alterations in barrier integrity caused by storage and handling conditions. Similar findings have been reported in recent studies, which highlight that biological membranes may undergo structural and lipid organization changes over time, thereby affecting permeability (Jacobi et al., 2022; Chen et al., 2021).

In contrast, STRAT-M synthetic membranes exhibited more consistent and reproducible permeation profiles, supporting their suitability for standardized laboratory testing. The observed peak permeation at 45 minutes followed by a slight decrease at 60 minutes may be explained by membrane saturation effects or differences in physicochemical interactions compared to biological tissue. Unlike porcine skin, synthetic membranes lack active biological components and do not fully replicate the dynamic processes involved in dermal absorption. Nevertheless, their uniform structure contributes to reduced experimental variability. These findings are consistent with recent literature indicating that STRAT-M membranes provide reliable and reproducible data, particularly in early-stage formulation screening (Haq et al., 2020; Simon et al., 2020).

The comparative analysis suggests that while synthetic membranes offer advantages in terms of reproducibility, ease of handling, and availability, porcine skin remains a more physiologically relevant model for predicting in vivo drug permeation. This distinction is particularly important in regulatory and pharmaceutical contexts, where accurate simulation of human skin behavior is required. Recent regulatory perspectives emphasize the importance of selecting appropriate in vitro models for the study objective,

with synthetic membranes recommended for screening and biological membranes preferred for confirmatory and predictive studies (EMA, 2022; OECD, 2020).

The analytical method employed in this study, UV–Vis spectrophotometry, demonstrated adequate linearity ($r^2 = 0.99$) and enabled rapid and cost-effective quantification of hydrocortisone acetate. However, this technique may be limited by lower specificity compared to more advanced analytical methods such as high-performance liquid chromatography (HPLC). Recent studies recommend the use of HPLC or LC-MS techniques for improved sensitivity, selectivity, and validation in pharmaceutical analysis (Kumar et al., 2023).

Several experimental factors may have influenced the permeation results observed in this study. These include membrane preparation techniques, temperature control, receptor medium composition, sampling accuracy, and mixing efficiency within the diffusion cells. Additionally, the presence of air bubbles, membrane mounting inconsistencies, and dilution errors may contribute to variability. Addressing these factors is essential to enhance reproducibility and ensure compliance with standardized protocols such as OECD Test Guideline 428.

A key limitation of this study is the absence of comprehensive statistical analysis due to the limited sample size. Although descriptive trends provide useful insights, the application of inferential statistical methods (e.g., ANOVA or t-tests) would be necessary to determine the significance of observed differences. Future research should also consider extended sampling durations, larger datasets, and inclusion of human skin models to improve the translational relevance of the findings.

Overall, the Franz diffusion cell system proved to be a robust, reliable, and widely applicable method for evaluating dermal drug permeation. The results contribute to the growing body of evidence supporting the complementary use of biological and synthetic membranes in pharmaceutical research and development.

CONCLUSION

The findings of this study demonstrate that the Franz diffusion cell method is a reliable and effective tool for assessing the in vitro permeation of hydrocortisone acetate. Both porcine skin and STRAT-M synthetic membranes were found to be suitable diffusion models, each offering distinct advantages.

Porcine skin provides a biologically relevant and cost-effective model that closely mimics human skin permeability, although its use is associated with higher variability. In contrast, STRAT-M synthetic membranes offer superior reproducibility, consistency, and ease of use, making them particularly valuable for routine laboratory testing and early-stage formulation screening.

The comparable permeation profiles observed in this study support the use of synthetic membranes as an alternative to biological tissues in specific applications. However, for studies requiring physiological relevance and predictive accuracy, biological membranes remain preferable.

Future studies incorporating statistical validation, advanced analytical techniques, and human skin models are recommended to strengthen further the applicability and regulatory acceptance of in vitro permeation methodologies.

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