International Journal of Pharmaceutics 697 (2026) 126884

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Advancing topical administration of sildenafil toward clinical translation: *ex vivo* glans permeation and pilot human pharmacokinetics

Greta Camilla Magnano [a,b,*], Stefano Dall'Acqua [c], Maurizio Pinamonti [d], Jelena Filipović-Grčić [e], Francesca Larese Filon [b], Dritan Hasa [a,**], Dario Voinovich [a]

[a] Department of Chemical and Pharmaceutical Sciences, University of Trieste, Italy
[b] Clinical Unit of Occupational Medicine, University of Trieste, Italy
[c] Department of Pharmaceutical Science and Pharmacology, University of Padova, Italy
[d] Department of Pathology, Cattinara Hospital, Trieste, Italy
[e] Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia

ARTICLE INFO

Keywords:
Sildenafil citrate
Topical formulation
Bull glans
In-vivo study
Pharmacokinetics

ABSTRACT

Sildenafil citrate, a selective phosphodiesterase type 5 (PDE5) inhibitor, is widely used to treat erectile dysfunction; however, its oral administration is associated with systemic adverse effects and reduced bioavailability due to first-pass metabolism. This study evaluated the potential for the topical delivery of sildenafil to the glans in the form of a cream formulation. *In vitro* permeation assays on bull glans tissue, which lacks a *stratum corneum*, showed that the tested formulation achieved moderate permeation (17.2 ± 1.61 μg/cm^2) and tissue retention (382 ± 80.5 μg/cm^2), supporting its suitability for subsequent *in vivo* testing. A pilot study in healthy male volunteers (n = 8) demonstrated low but detectable salivary sildenafil concentrations following topical application, with mean concentrations rising initially and then remaining relatively stable over the 2–4 h observation period. Population pharmacokinetic modelling using a one-compartment model with first-order absorption and elimination yielded an absorption rate constant of k_a = 1.20 h^{-1} and a model-derived t_{max} of approximately 1.9 h. Absolute salivary concentrations remained in the low ng/mL range, indicating limited systemic exposure. Histological analysis confirmed close structural similarity between bull and human glans tissue, supporting the translational relevance of the *ex vivo* model. Overall, these findings support the feasibility of developing a locally acting topical alternative to oral PDE5 inhibitors.

1. Introduction

Erectile dysfunction (ED) is a highly prevalent condition that significantly affects quality of life and interpersonal relationships. Phosphodiesterase type 5 (PDE5) inhibitors remain the first-line pharmacological therapy for ED due to their well-established efficacy (Nicolosi et al., 2006; Porst, 2004; Sussman, 2004). Sildenafil exerts its effect by selectively inhibiting PDE5, the enzyme responsible for the degradation of cyclic guanosine monophosphate (cGMP) in corpus cavernosum smooth muscle, (Moreland et al., 1998; Andersson and Wagner, 1995; Lau and Adaikan, 2006), thereby enhancing nitric oxide–mediated vasodilation and facilitating erection in response to sexual stimulation (Ghofrani et al., 2006; Langtry and Markham, 1999; Thompson et al., 2001). Despite its clinical success, oral administration of sildenafil presents several limitations, including extensive first-pass metabolism, resulting in interindividual variability in systemic exposure. Moreover, systemic administration may be associated with adverse effects, including headache, flushing, nasal congestion, and cardiovascular effects related to systemic vasodilation (Nichols et al., 2002; Osman et al., 2006; Zinner, 2007). These limitations have stimulated growing interest in alternative routes of administration that could reduce systemic exposure while preserving therapeutic efficacy (Dos Anjos and Alonso, 2008). Transdermal drug delivery represents an attractive non-invasive alternative to oral dosing, offering potential advantages such as avoidance of first-pass metabolism, reduced peak-related adverse effects, and the possibility of targeted local action. However, an effective transdermal delivery is often hindered by the barrier function of the *stratum corneum*, which severely restricts drug

* Corresponding author at: Department of Chemical and Pharmaceutical Sciences, University of Trieste, Italy.
** Corresponding author.
E-mail addresses: gmagnano@units.it (G.C. Magnano), dhasa@units.it (D. Hasa).

https://doi.org/10.1016/j.ijpharm.2026.126884
Received 21 January 2026; Received in revised form 23 March 2026; Accepted 13 April 2026
Available online 15 April 2026

permeation across most skin surfaces. Specifically to sildenafil, various formulation strategies, including penetration enhancers (Verma and Pathak, 2010), lipid-based carriers, and nanostructured systems have been explored to overcome limitations related to its transdermal administration (Abla et al., 2023; Alazzo et al., 2024; Atipairin et al., 2020; Abdelalim et al., 2020b). Nevertheless, none of these approaches has so far translated into an approved topical sildenafil product. The glans penis constitutes a particularly promising anatomical site for localised drug delivery (Alnajjar et al., 2012; Shabbir et al., 2011). Unlike keratinized skin, the glans is covered by a mucosal epithelium that lacks a *stratum corneum*, is highly hydrated, and is richly vascularised. These features confer substantially higher permeability compared with conventional skin, making the glans an attractive target for topical administration aimed at local absorption with limited diffusion barriers. From a functional perspective, the glans is particularly relevant due to its anatomical proximity to the underlying erectile tissues (Goldstein et al., 2001). Local administration at this site can therefore facilitate the establishment of pharmacologically active concentrations directly in tissues involved in erectile function, potentially enhancing the nitric oxide–cGMP signaling pathway while limiting systemic exposure (Mohamed et al., 2023). Sildenafil is a potent and selective inhibitor of phosphodiesterase type 5 (PDE5), with reported inhibitory activity in the low nanomolar range ($IC_{50} \approx 3$–10 nM) (Wallis, 1999). Achieving local concentrations within or above this range is therefore expected to be sufficient to elicit a pharmacological effect at the target tissue. Despite this rationale, systematic experimental evaluation of sildenafil delivery directly to glans tissue remains scarce. In our previous work (Magnano et al., 2024), we developed and screened different topical sildenafil formulations using porcine skin and a 3D human foreskin model, eventually identifying a liposomal cream as the most promising candidate in terms of permeation performance and formulation stability. Despite the robust *in vitro* foundation, in that study, we did not address mucosal delivery to the glans or the translational relationship between *ex vivo* permeation and *in vivo* systemic exposure. The present study was therefore designed as a direct progression of that work, with the primary objective of evaluating the previously developed formulation on glans tissue using a physiologically relevant *ex vivo* model, complemented by a pilot human pharmacokinetic assessment following topical application. Bull glans tissue was selected as an *ex vivo* surrogate due to its close anatomical and histological similarity to human glans, a similarity that was verified by histological analysis. The glans penis represents the distal expansion of the corpus spongiosum surrounding the urethra, a structural organisation that is conserved across mammalian species. Comparative anatomical studies of penile architecture have demonstrated similar fundamental organisation of erectile tissues among mammals, despite species-specific morphological adaptations (Hsu et al., 2005). Veterinary anatomical descriptions confirm that in bovine species the penis is likewise composed of corpora cavernosa and a corpus spongiosum surrounding the urethra and extending distally to form the glans (Amselgruber and Sinowatz, 1992). By combining *ex vivo* permeation and tissue-retention studies with a pilot *in vivo* salivary pharmacokinetic evaluation and pharmacokinetic modelling, this work provides an experimental basis for assessing mucosa-targeted topical sildenafil delivery. Specifically, this work aims to (i) characterise the balance between local tissue deposition and systemic uptake of sildenafil after glans application, (ii) explore the temporal relationship between *ex vivo* permeation behaviour and systemic appearance of the drug, and (iii) provide an experimental basis for further investigation of mucosa-targeted topical sildenafil formulations.

2. Materials and methods

2.1. Materials

In this work, all chemicals used were of analytical grade. Acetonitrile (ACN), formic acid (FA) and ethanol (EtOH) were purchased from Sigma-Aldrich (St. Louis, USA), while potassium dihydrogenphosphate (KH_2PO_4), sodium chloride (NaCl) and sodium hydrogenphosphate (Na_2HPO_4) were obtained from Carlo Erba (Italy). A Millipore purification pack system was used to produce MilliQ water. The physiological solution, used as the receptor fluid was obtained by the dissolution of KH_2PO_4 (0.19 g); Na_2HPO_4 (2.38 g) and NaCl (9 g) in 1 L of MilliQ water with a final pH of 7.35.

2.2. Formulation of the sildenafil cream

Based on our previous formulation screening and optimisation study (Magnano et al., 2024), in the present work, the liposomal cream was selected as the topical vehicle for sildenafil citrate. Such formulation is based on a preformed commercial transdermal cream and incorporates penetration-enhancing excipients, including isopropyl myristate and isopropyl palmitate. The qualitative composition of the cream, as provided by the manufacturer, is reported in Table 1. Sildenafil citrate was incorporated into the cream base using a mechanical unguator (Gako Unguator®, Farmalabor, Italy). Briefly, 1.950 g of sildenafil citrate was added to 25 mL of the cream base and mixed for 6 min at 1000 rpm to obtain a homogeneous formulation. The resulting cream was stored at room temperature and protected from light until use. Drug content was determined to confirm uniformity and accurate dosing. An aliquot of 0.5 mL of the cream (corresponding to a theoretical sildenafil citrate dose of 38.6 mg) was dispersed in 6.0 mL of a water/ethanol mixture (50:50, v/v) and stirred continuously for 4 h to ensure complete extraction. The dispersion was then filtered through a 0.45 μm PTFE membrane filter (Whatman®), and sildenafil concentration was quantified by HPLC as described in Section 2.5. The content of sildenafil citrate in the tested formulation was experimentally measured, corresponding to 38.12 ± 0.19 mg per 0.5 mL of product (mean ± SD, n = 3).

2.3. Glans tissue preparation

Samples of bull penis were sourced from a slaughterhouse, and the glans tissue was carefully dissected, wrapped in gauze, and stored at –25 °C. On the day of the experiment, the tissue was cut into square sections of 4 cm^2 and thawed in a physiological solution. The thickness of the penile tissue (1.2 ± 0.01 mm) was measured using a micrometric caliper. Prior to the permeation experiments, tissue integrity was evaluated by measuring the transepidermal water loss (TEWL), since this is a non-invasive parameter commonly used for assessing the barrier function of skin and mucosal tissues (Alexander et al., 2018; Machado et al., 2010; Miwa et al., 2006). The bull glans samples used in this study showed TEWL values of approximately 22 g/m^2/h, consistent with an intact mucosal barrier.

2.4. In-vitro absorption study of sildenafil cream through glans tissue

The permeation test was performed in Franz static diffusion cells following the OECD guidelines (OECD, 2004). Tissues from the glans were mounted between the upper (donor) and lower (receptor) chambers of static Franz diffusion cells. The absorption study was assessed using the protocol detailed in our previously work (Magnano et al., 2024). Specifically, 0.5 mL of cream formulation (approximately 38.6 mg of drug) was applied on the glans tissue and the experiments were conducted at 32 °C, which corresponds to the physiological temperature of the skin surface. The effective diffusion area in the Franz cell was 1.00 cm^2, resulting in a sildenafil theoretical dose normalised to the exposed surface of 38.6 mg/cm^2. This amount ensured infinite dose conditions, allowing the maintenance of a constant drug concentration in the donor compartment throughout the permeation experiment. At the end of the assay, the glans tissue was rinsed three times with 1.0 mL of water (MilliQ). Then, by using a scalpel the tissue was cut in little pieces, immersed in 5.0 mL of MilliQ water, stirred for 15 h and diluted 1:10 in

Table 1
Composition of the sildenafil formulation as reported by the manufacturer.

Composition
Sildenafil citrate, isopropyl myristate, glyceryl monostearate, lecithin, sorbic acid, PEG-40 stearate, cetyl alcohol, stearic acid, benzoic acid, isopropyl palmitate, simethicone, stearyl alcohol, potassium sorbate, EDTA, urea, butylated hydroxytoluene (BHT), carbomer, hydrochloric acid.

MilliQ water before quantification by HPLC. Sildenafil extraction from the entire tissue was carried out at room temperature for 15 h. Extraction efficiency was evaluated using a full mass-balance approach by comparing the applied dose with the sum of the amount permeated into the receptor compartment and the amount recovered from the glans tissue. Recovery (%) was calculated as the ratio between the total recovered amount and the applied dose.

$$\text{Recovery}\,(\%) = \frac{\text{total recovered amount}}{\text{applied dose}} \times 100$$

The sildenafil contents registered in the receptor compartment and accumulated within the bull glans tissue, was quantified using HPLC, following the same method reported in our previous study (Magnano et al., 2024).

2.5. High performance liquid chromatography (HPLC)

Sildenafil concentrations were determined by UV-HPLC analysis as previously described in our recent work (Magnano et al., 2024). Specifically, analyses were performed on an Agilent 1260 system with diode-array detection using an InfinityLab Poroshell 120 C18 column (3.0 × 100 mm, 4 μm) at 23 °C. The mobile phase consisted of acetonitrile and 0.1% formic acid in water (30:70, v/v) at 0.4 mL/min. The injection volume was 10 μL and detection was set at 254 nm. Sildenafil eluted at 4.8 ± 0.02 min within an 8.0 min run. The method provided an LOD (limit of detection) of 0.04 μg/mL and an LOQ (limit of quantification) of 0.12 μg/mL.

2.6. Permeability calculations of sildenafil samples

The cumulative content of permeated drug (dQ, μg) was plotted as a function of time (dt, s). The linear portion of the slope, representing to the steady-state (Hopf et al., 2020) was adopted to determine the flux as described in Eq. (1). The formulation was applied under infinite dose conditions to ensure a constant drug concentration in the donor compartment and allow reliable determination of permeation parameters (Niedorf et al., 2008). Non-occlusive conditions were selected to better approximate typical topical application, as occlusion is known to enhance skin permeation (Treffel et al., 1992).

$$J = \frac{dQ}{A*dt} \tag{1}$$

where A denotes the surface area of the diffusion barrier (cm^2). The obtained flux value was then to employed to determine the P_{app} (apparent permeability coefficient) as outlined in Eq. (2):

$$P_{app} = \frac{J}{C_d} \tag{2}$$

P_{app} (cm/s) refers to the apparent permeability coefficient, while J (μg/cm^2 per s) represents the flux at the steady state and C_d indicates the drug concentration in the donor compartment (μg/cm^3).

2.7. Histological analysis

The glans tissues were fixed in formalin for 2 h at room temperature, subsequently dehydrated, and embedded in paraffin wax to facilitate transverse sectioning. Sections of 4 μm thick were obtained using a microtome and subsequently stained with a hematoxylin and eosin (H&E) staining kit (Vector Laboratories Inc., USA). Staining durations were set at 5 min for hematoxylin and 1 min for eosin. The stained slides were analysed using a D-Sight slide scanner (Menarini Diagnostics, Italy). The thickness of the glans tissue was measured across six microscopic fields using ImageJ software (National Institutes of Health, USA).

2.8. Human pilot trial methodology

The *in vivo* study was approved by the Human Research Ethics Committee of the University of Trieste (n°3-31/3–25) and was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines. Eight healthy male volunteers, non-smoking, aged between 24 and 65 years participated in the trial. About 50 mg of sildenafil base corresponding to 0.9 mL of cream was applied locally to the human penis/glans. The applied dose (0.9 mL of cream corresponding 50 mg of sildenafil citrate) was selected to represent the nominal strength of the formulation and to allow detection of systemic exposure. The cream was self-applied by the volunteer and gently spread to ensure full and uniform coverage of the glans surface. Volunteers were instructed to wash their hands and the glans prior to application and to avoid urination, washing, or sexual activity until the last saliva sample was collected. At time intervals of 15, 30, 60, 90, 120, and 240 min after the application, 3 mL of saliva samples were collected and the samples were centrifuged for 15 min at 1000 rpm and the resulting supernatant was stored at −80 °C. Participants were instructed not to drink water or other fluids immediately before and during sample collection. To the best of our knowledge there are no reported human pharmacokinetic studies where the plasma and/or salivary concentrations of sildenafil have been reported after application of a topical formulation. The sampling timepoints (15–240 min) were therefore selected based on the known pharmacokinetic profile of sildenafil (after oral administration), in which peak systemic concentrations typically occur within 1–2 h (Nichols et al., 2002). To determine sildenafil concentrations in saliva, 500 μL of saliva sample was mixed with 500 μL of methanol containing benzanilide (5 μg/mL), as the internal standard (IS). The mixture was then extracted with 5 mL of ethyl acetate by vortexing. Phase separation was achieved by centrifugation, after which the organic layer was collected and evaporated using a vacuum centrifuge at 40°C. The resulting residue was re-dissolved in 250 μL of methanol, sonicated, and centrifuged for 10 min. The final solution was analysed by HPLC coupled with tandem mass spectrometry (HPLC-MS/MS).

2.9. Pharmacokinetic data evaluation

Pharmacokinetic evaluation was conducted using salivary concentration–time data from eight healthy adult volunteers. Data processing and modelling were performed in R (RStudio Version 2025.09.2 + 418; Posit Software, PBC), using base R functions and tidyverse packages for data organisation, visualisation, and numerical fitting. As saliva contains only the unbound fraction of sildenafil present in systemic circulation, salivary concentrations were used as a non-invasive surrogate to monitor the temporal pattern of systemic exposure following topical administration. Due to the extensive plasma protein binding, salivary levels are typically lower than plasma concentrations (Tracqui and Ludes, 2003), and therefore reflect the free circulating fraction rather than local tissue concentrations in the glans. Given the sparse sampling design and the exploratory nature of this pilot study, pharmacokinetic

evaluation focused on characterising the absorption phase following topical administration, while systemic disposition parameters were fixed according to established oral sildenafil pharmacokinetic data.

Salivary concentration–time profiles were described using a one-compartment model with first-order absorption and first-order elimination (Bateman function). The elimination rate constant (k_e) was fixed based on literature values corresponding to a plasma half-life of approximately 4 h, while the absorption rate constant (k_a) and individual scaling factors (Si), representing relative saliva/plasma partitioning and apparent exposure, were estimated via nonlinear least-squares regression (nls function). Model evaluation included diagnostic plots (DV–PRED, DV–IPRED), residual analyses, and visual predictive checks generated from model-based simulations. These diagnostic evaluations indicated that the selected structural model adequately described the observed salivary concentration–time profiles. *In vitro* permeation data for the cream formulation were normalised (Q/Q_{max}) and compared with the model-derived *in vivo* absorbed fraction ($F_{abs} = 1 - e^{-k_a t}$) to provide an exploratory comparison of the temporal profiles of *ex vivo* permeation and systemic appearance following topical application.

2.10. Data analysis

The results are presented as the amount of drug permeated per unit area of skin (μg/cm^2). Data from the skin absorption studies are reported as mean ± standard error of the mean (SEM).

3. Results

3.1. Permeation and tissue distribution of sildenafil from topical cream through glans tissues

Sildenafil permeation from the cream formulation increased progressively over the 4-hour experiment, with drug levels in the receptor fluid (RF) rising in a time-dependent increase (Fig. 1A). At the end of the permeation study, the cumulative amount of sildenafil that crossed the glans tissue reached **17.2 ± 1.61 μg/cm^2**, with a steady-state flux of $7.52 \times 10^{-4} \pm 2.13 \times 10^{-4}$ μg/cm^2*s and an apparent permeability coefficient P_{app} of $1.96 \times 10^{-8} \pm 0.48 \times 10^{-8}$ cm/s (Table 2).

Drug distribution within the glans tissue was also evaluated after 4 h of exposure. It is important to highlight that the glans tissue lacks the *stratum corneum* layer; therefore, sildenafil content was quantified across the full membrane thickness. The tested formulation exhibited a retained amount of 382 ± 80.5 μg/cm^2 within the tissue (Fig. 1B and Table 2). Additionally, the full absorbed recovered amount (Q_{abs}) was calculated utilizing Equation (3).

$$Q_{abs} = Totalglanstissue + RF \quad (3)$$

where "total glans tissue" represents the cumulative amount of sildenafil recovered from the entire glans and "RF" refers to the sildenafil amount recovered from the receptor fluid. The corresponding Q_{abs} value was 396 ± 71.4 μg/cm^2 for the cream (Table 2), indicating that such formulation effectively promotes both measurable retention within the glans tissue and permeation into the receptor fluid over the 4-hour exposure period.

3.2. Morphological analysis of glans tissue

Histological evaluation confirms the typical structure of bull glans tissue, as depicted in Fig. 2. Hematoxylin and eosin (H&E) staining clearly delineates three distinct layers: (i) the epithelial lining

Table 2
J (Flux), P_{app} (apparent permeability coefficients), content of sildenafil in RF, amount of sildenafil retained within the entire glans tissue after 4 h of exposure to each topical formulation, and the corresponding Q_{abs} value. The applied dose at time 0 (expressed as formulation amount per exposed surface area) was 38.6 mg/cm^2. Results are reported as mean ± SEM, n = 6.

Sample	J (μg/cm^2*s)	P_{app} (cm/s)	Sildenafil in RF at 4 h (μg/cm^2)	Total glans tissue at 4 h (μg/cm^2)	Q_{abs} (Total tissue + RF) (μg/cm^2)
Formulation	$7.52 \times 10^{-4} \pm 2.13 \times 10^{-4}$	$1.96 \times 10^{-8} \pm 0.48 \times 10^{-8}$	17.2 ± 1.61	382 ± 80.5	396 ± 71.4

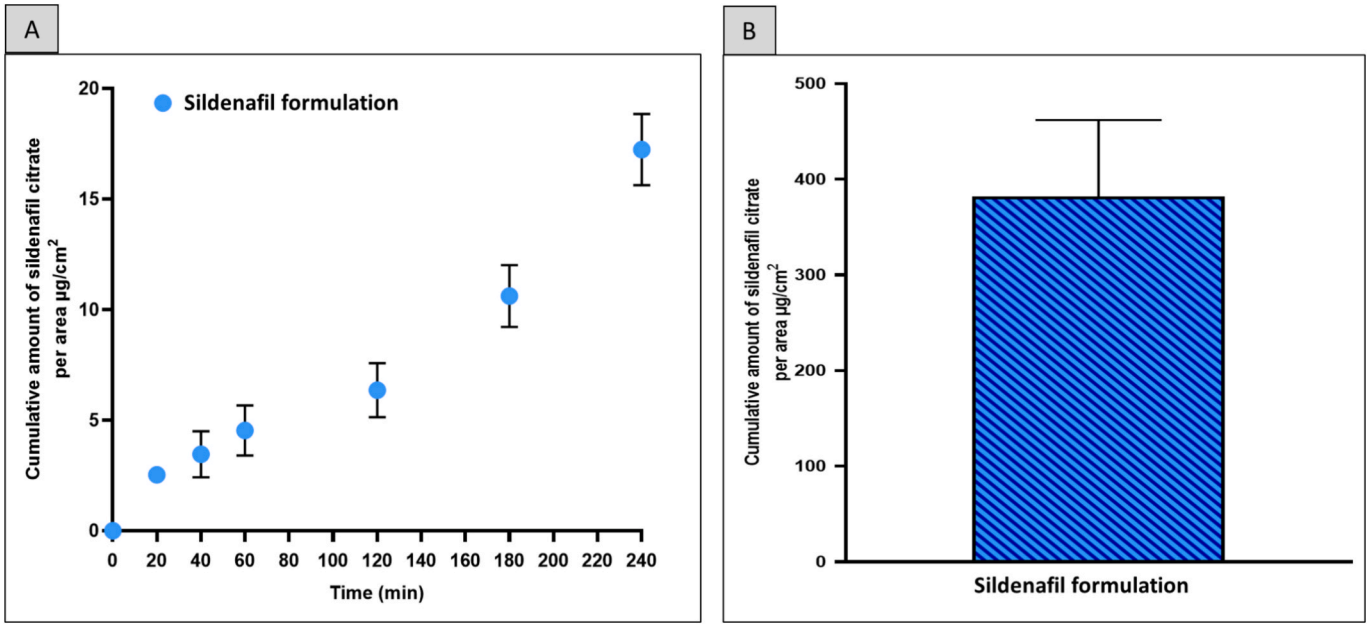

Fig. 1. (A) Amount of sildenafil (μg/cm^2) that permeated into the receptor compartment over time from formulation applied to glans tissue. Data are presented as mean ± standard error of the mean (SEM), n = 6. (B) Retention of sildenafil (μg/cm^2) in the total glans tissue following 4-hour exposure to the topical formulation. The administered dose was 38.6 mg/cm^2. Results are expressed as mean ± SEM, n = 6.

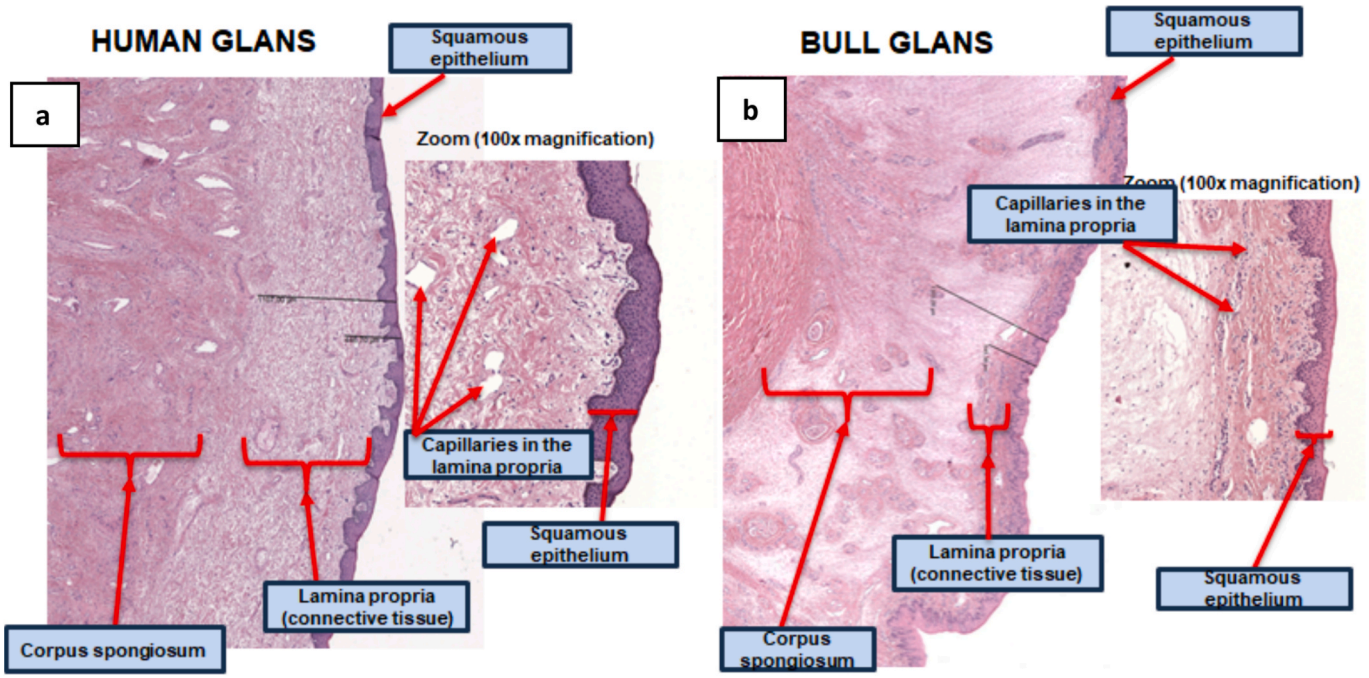

Fig. 2. Hematoxylin and Eosin (H&E) staining of two glans tissues. (a) Representative section illustrating the general tissue architecture of human glans (b) Representative section illustrating the general tissue architecture of bull glans. Corresponding bright-field microscopy image. Scale bar = 100 μm.

(squamous epithelium), (ii) the underlying lamina propria, and (iii) the highly vascularised corpus spongiosum (Thakar and Dugi, 2013). The absence of a *stratum corneum* in the epithelial layer suggests a higher permeability, which is particularly relevant for topical drug delivery applications. The bull glans tissue appears structurally similar to human glans tissue, supporting its suitability as a model for permeability and drug diffusion studies. The well-organised architecture observed in Fig. 3 supports its use in evaluating transdermal formulations and drug absorption dynamics.

3.3. Pharmacokinetic evaluation

Salivary concentration–time profiles obtained from eight healthy volunteers are characterised from an initial stage (first 30 min after topical administration), where sildenafil concentrations increase, followed by aplateau phase during the later sampling period (up to 2–4 h) (Fig. 3A). Interindividual variability was moderate, and mean salivary concentrations remained in the low ng/mL range throughout the study period, consistent with minimal systemic exposure after topical delivery to the glans. A one-compartment model with first-order absorption and elimination adequately described the pooled salivary data (Fig. 3B). The elimination rate constant (k_e) was fixed based on established oral pharmacokinetic parameters ($t_{1/2} \approx 4$ h), while the absorption rate constant (k_a) was estimated from the concentration–time profiles ($k_a = 1.20\ h^{-1}$). The model-derived time to maximum concentration ($t_{max} \approx 1.9$ h) was within the observed range. Individual scaling factors (Si), representing saliva/plasma partitioning and apparent exposure, showed moderate variability, reflecting both physiological differences in topical

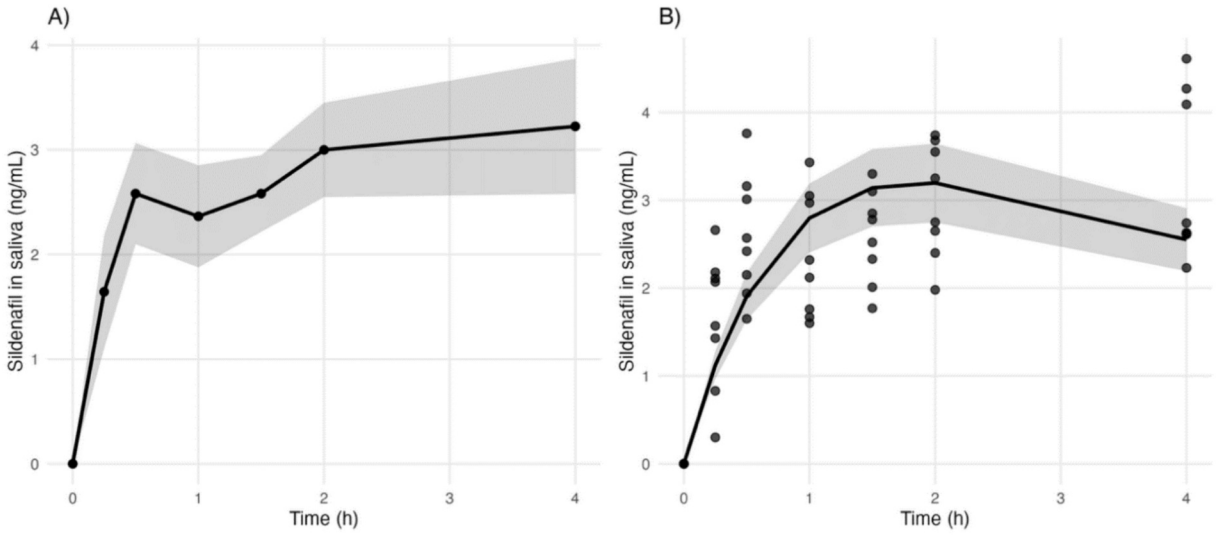

Fig. 3. (A) Mean salivary concentration–time profile of sildenafil after topical administration, with 95% confidence intervals (CI). (B) Individual salivary concentration–time profiles are overlaid with model-predicted mean values and corresponding 95% confidence intervals. Both panels show a rapid initial rise, followed by relatively stable concentrations with minor fluctuations for up to 4 h, indicating a prolonged absorption phase and low systemic exposure after topical application.

absorption and the known variability in salivary excretion of sildenafil.

Goodness-of-fit evaluations showed acceptable agreement between observed concentrations and both population and individual predictions (Fig. S1). Standardised residuals were symmetrically distributed around zero, with no evidence of heteroscedasticity or time-dependent bias, confirming the suitability of the structural model for describing the pooled salivary pharmacokinetic data.

To examine a possible correlation between *ex vivo* permeation and the systemic appearance of sildenafil after topical administration, the normalised *ex vivo* permeated fraction (F_perm) was compared with the model-derived *in vivo* absorbed fraction (F_abs) over the 0–4 h interval (Fig. 4). The two profiles showed distinct kinetic characteristics. *Ex vivo* permeation increased progressively, reaching approximately 25% of the maximal fraction at 1 h and about 65% at 3 h, whereas F_abs rose more steeply and approached its maximum within 2–3 h.

4. Discussion

The current study aimed to evaluate the permeation profile and pharmacokinetics of sildenafil citrate from a liposomal formulation through bull glans tissue, providing insights for topical administration with both local and systemic pharmacological effects.

Targeted topical delivery of sildenafil allows efficient local pharmacological activity in glans tissue while enabling partial systemic absorption, as supported by pharmacokinetic data in human volunteers. Although the formulation is applied topically to the glans, the objective is to enable systemic absorption of sildenafil through this highly permeable tissue as an alternative to the conventional oral administration, with the potential of reduced dose-related side effects (Abdelalim et al., 2020a; Atipairin et al., 2020; Hamzehnejadi et al., 2022; Abdelalim et al., 2020b). Moreover, the formulation tested in this study was selected based on the optimal permeation results obtained in our previous work (Magnano et al., 2024), where such topical cream demonstrated effective sildenafil penetration using both porcine ear skin and a 3D skin equivalent model. In the present study, the cream was tested directly on bull glans tissue, exhibiting a favourable permeation profile and substantial drug retention within the tissue, likely due to its specific composition, including penetration enhancers that improve sildenafil solubilisation and diffusion through the glans tissue. Similarly, the study of Mohamad et al. (Mohamad et al., 2022) demonstrated that the incorporation of Pluronic F127 as a penetration enhancer led to a 1.7-fold increase in skin permeability. Also Osman et al. (Osman et al., 2023) showed that gel formulations based on HPMC and PEG 400, used as permeation enhancers, achieved higher permeation profile compared to other types of gelling agents. Additionally, also the physicochemical properties of the topical cream, such as viscosity, solubility, and molecular size, play a crucial role in enhancing sildenafil permeation. In this regard, previous mechanistic studies have shown that increasing apparent drug solubility through enabling formulations does not necessarily lead to a proportional increase in permeation, since membrane flux is mainly governed by the unbound (free) drug fraction, which represents the driving force for diffusion (Tzanova et al., 2023). It is important to underline that, to the best of our knowledge, this is the first research work to evaluate a topical sildenafil formulation applied directly to glans tissue in order to simulate a real-life scenario. The bull glans tissue, unlike porcine skin or reconstructed human 3D skin models, lacks a *stratum corneum* and it presents highly hydrated. Such differences may alter drug partitioning and diffusion dynamics. Moreover, the different lipid composition, enzymatic activity, and vascularisation of keratinized skin compared to glans tissue may affect both drug absorption kinetics and local drug distribution. These findings emphasize the importance of selecting appropriate tissue models for transdermal delivery studies, especially when targeting glans surfaces with distinct permeability properties. However, histological analysis of the bull glans tissue revealed a typical structure, with a squamous epithelial layer, basement membrane, and lamina propria (Cubilla et al., 2001). It also confirmed the absence of a *stratum corneum* layer, which aligns with the observed high permeability and drug retention. This absence of a barrier layer is particularly advantageous for drug delivery applications, as it allows more efficient transdermal absorption (Trommer and Neubert, 2006; Phatale et al., 2022). This structural similarity between bull and human glans tissue validates the bull model as an appropriate system for studying transdermal drug delivery, particularly for drugs intended for localised action. Topical application of sildenafil in human volunteers resulted in detectable but low salivary concentrations in all subjects, confirming minimal systemic exposure. Currently, no specific target concentration of sildenafil in glans tissue has been established to achieve therapeutic effects. In our *ex vivo* study using Franz diffusion cells, approximately 380 ± 80.5 μg/cm^2 (≈1% of the applied dose) (Table 2) remained in the glans tissue, while only a minor fraction (17.2 ± 1.61 μg/cm^2, 0.04% of the dose) permeated through the tissue. *In vivo*, topical application led to low but detectable salivary concentrations, confirming minimal systemic exposure. The concentration–time profiles showed a rapid initial rise followed by a gradual increase during the later sampling period (2–4 h). Modelling with a one-compartment structure and first-order absorption and elimination yielded an absorption rate constant of $k_a = 1.20$ h^{-1} and a model-derived time to maximum concentration of approximately 1.9 h. These parameters fall within the range of observed salivary concentrations and are consistent with the highly vascularised nature of glans tissue, which allows rapid removal of permeated drug. Importantly, although the fractional absorbed profile (F_abs) increased more rapidly than the normalised permeation profile, absolute salivary concentrations remained in the low, ng/mL range, over an order of magnitude lower than those typically reported after oral administration (Tracqui and Ludes, 2003). This indicates that only a small proportion of the administered dose enters systemic circulation, supporting the concept of targeted topical delivery with reduced systemic exposure. Comparison of normalised *ex vivo* permeation (F_perm) with the model-derived *in vivo* absorbed fraction revealed distinct kinetic behaviours: permeation increased progressively, whereas F_abs rose more steeply and reached its maximum earlier. This divergence likely reflects the rapid vascular uptake characteristic of mucosal surfaces rather than diffusion-limited absorption. Taken together, the *ex vivo* and *in vivo* data suggest that the tested formulation enables efficient

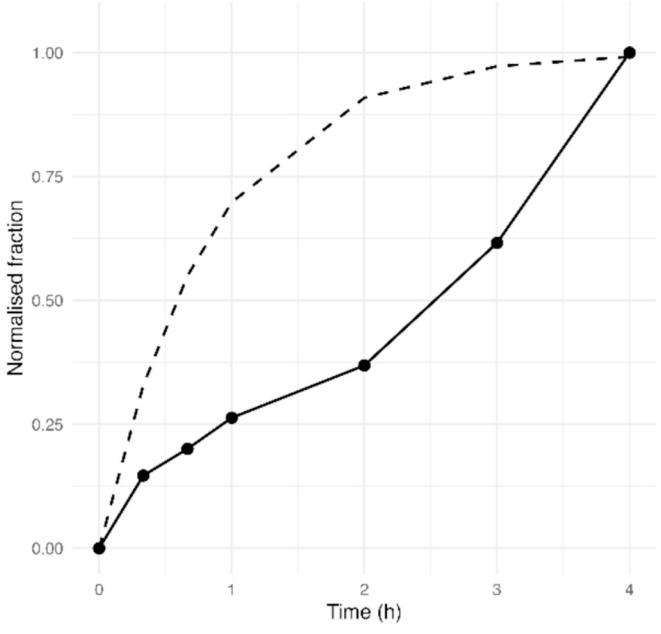

Fig. 4. Overlay of the normalised ex vivo permeation profile (F_perm, solid line) and the model-derived *in vivo* absorbed fraction (F_abs, dashed line) for the topical formulation over the 0–4 h interval.

delivery of sildenafil into glans tissue with limited systemic exposure. These findings highlight the importance of mucosal-specific formulation strategies and support further investigation of topical sildenafil for local therapeutic applications. Some limitations should be also acknowledged. The *in vivo* study involved a small number of healthy volunteers and relied on salivary concentrations to monitor the systemic appearance of sildenafil. While saliva predominantly reflects the unbound fraction of sildenafil and captures temporal absorption patterns, it does not provide a direct measure of plasma concentrations. In addition, the study is not designed to assess pharmacodynamic outcomes. These limitations are inherent to the exploratory nature of this work.

5. Conclusion

This study demonstrates direct evidence that a glans-targeted topical formulation of sildenafil can achieve a favourable balance between local tissue exposure and minimal systemic uptake. Building on our previous formulation-screening work, the present results demonstrate that the selected liposomal cream effectively permeates glans mucosa and is significantly retained within the tissue, while a small fraction of the applied dose reaches the systemic circulation. The use of bull glans tissue as an *ex vivo* model proved to be physiologically relevant, as supported by its close histological similarity to human glans and the ability to distinguish between local deposition and *trans*-tissue permeation. The pilot human salivary pharmacokinetic data support these findings, showing that topical application results in detectable but low systemic exposure. Overall, the observed permeation behaviour, tissue retention, and limited systemic exposure support the feasibility of mucosa-targeted sildenafil delivery as a strategy for topical therapy, potentially reducing systemic adverse effects. These results provide an experimental basis for further investigation of locally acting PDE5 inhibitor formulations targeting mucosal tissue. Future studies involving larger volunteer cohorts, extended pharmacokinetic sampling, and clinical efficacy assessments will be essential to confirm these preliminary results and establish the therapeutic potential and safety profile of such formulations.

Funding support

The authors have no relevant financial disclosures.

CRediT authorship contribution statement

Greta Camilla Magnano: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Stefano Dall'Acqua:** Investigation. **Maurizio Pinamonti:** Writing – review & editing, Investigation. **Jelena Filipović-Grčić:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Francesca Larese Filon:** Funding acquisition. **Dritan Hasa:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Dario Voinovich:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge Marco Di Luca for his help in the experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2026.126884.

Data availability

Data will be made available on request.

References

Abdelalim, L.R., Abdallah, O.Y., Elnaggar, Y.S.R., 2020a. High efficacy, rapid onset nanobiolosomes of sildenafil as a topical therapy for erectile dysfunction in aged rats. Int. J. Pharm. 591 (décembre), 119978. https://doi.org/10.1016/j.ijpharm.2020.119978.

Abdelalim, L.R., Elnaggar, Y.S.R., Abdallah, O.Y., 2020b. Oleosomes encapsulating sildenafil citrate as potential topical nanotherapy for palmar plantar erythrodysesthesia with high *ex vivo* permeation and deposition. AAPS PharmSciTech 21 (8), 310. https://doi.org/10.1208/s12249-020-01862-2.

Abla, K.K., Mneimneh, A.T., Allam, A.N., Mehanna, M.M., 2023. Application of box-behnken design in the preparation, optimization, and *in-vivo* pharmacokinetic evaluation of oral tadalafil-loaded niosomal film. Pharmaceutics 15 (1), 173. https://doi.org/10.3390/pharmaceutics15010173.

Alazzo, A., Alfahad, M., Jasim, M.H.M., Al-Zidan, R.N., 2024. Microemulsion: a potential approach for transdermal delivery of sildenafil citrate. Military Med. Sci. Lett. 93 (3), 255–263. https://doi.org/10.31482/mmsl.2023.031.

Alexander, H., Brown, S., Danby, S., Flohr, C., 2018. Research techniques made simple: transepidermal water loss measurement as a research tool. J. Invest. Dermatol. 138 (11), 2295–2300.e1. https://doi.org/10.1016/j.jid.2018.09.001.

Alnajjar, H.M., Lam, W., Bolgeri, M., Rees, R.W., Perry, M.J.A., Watkin, N.A., 2012. Treatment of carcinoma *in situ* of the glans penis with topical chemotherapy agents. Eur. Urol. 62 (5), 923–998. https://doi.org/10.1016/j.eururo.2012.02.052.

Amselgruber, W., Sinowatz, F., 1992. Zur mikrovaskularisation des penis vom stier (*Bos Taurus*)*. Anat. Histol. Embryol. 21 (4), 285–305. https://doi.org/10.1111/j.1439-0264.1992.tb00461.x.

Andersson, K.E., Wagner, G., 1995. Physiology of penile erection. Physiol. Rev. 75 (1), 191–236. https://doi.org/10.1152/physrev.1995.75.1.191.

Atipairin, A., Chunhachaichana, C., Nakpheng, T., Changsan, N., Srichana, T., Sawatdee, S., 2020. Development of a sildenafil citrate microemulsion-loaded hydrogel as a potential system for drug delivery to the penis and its cellular metabolic mechanism. Pharmaceutics 12 (11), 1055. https://doi.org/10.3390/pharmaceutics12111055.

Cubilla, A.L., Piris, A., Pfannl, R., Rodriguez, I., Agüero, F., Young, R.H., 2001. Anatomic levels: important landmarks in penectomy specimens: a detailed anatomic and histologic study based on examination of 44 cases. Am. J. Surg. Pathol. 25 (8), 1091–1104. https://doi.org/10.1097/00000478-200108000-00016.

Anjos, J.L., Alonso, A., 2008. Terpenes increase the partitioning and molecular dynamics of an amphipathic spin label in stratum corneum membranes. Int. J. Pharm. 350 (1–2), 103–112. https://doi.org/10.1016/j.ijpharm.2007.08.024.

Ghofrani, H.A., Osterloh, I.H., Grimminger, F., 2006. Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. Nat. Rev. Drug Discov. 5 (8), 689–702. https://doi.org/10.1038/nrd2030.

Goldstein, I., Payton, T.R., Schechter, P.J., 2001. A double-blind, placebo-controlled, efficacy and safety study of topical gel formulation of 1% Alprostadil (Topiglan) for the in-office treatment of erectile dysfunction. Urology 57 (2), 301–305. https://doi.org/10.1016/S0090-4295(00)00936-5.

Hamzehnejadi, M., Tavakoli, M.R., Abiri, A., Ghasempour, A., Langarizadeh, M.A., Forootanfar, H., 2022. A review on phosphodiesterase-5 inhibitors as a topical therapy for erectile dysfunction. Sex. Med. Rev. 10 (3), 376–391. https://doi.org/10.1016/j.sxmr.2022.02.002.

Hopf, N.B., Champmartin, C., Schenk, L., et al., 2020. Reflections on the OECD guidelines for *in vitro* skin absorption studies. Regul. Toxicol. Pharm. 117 (novembre), 104752. https://doi.org/10.1016/j.yrtph.2020.104752.

Hsu, G.-L., Lin, C.-W., Hsieh, C.-H., et al., 2005. Distal ligament in human glans: a comparative study of penile architecture. J. Androl. 26 (5), 624–668. https://doi.org/10.2164/jandrol.04145.

Langtry, H.D., Markham, A., 1999. Sildenafil: a review of its use in erectile dysfunction. Drugs 57 (6), 967–989. https://doi.org/10.2165/00003495-199957060-00015.

Lau, L.-C., Adaikan, P.G., 2006. Mechanisms of direct relaxant effect of sildenafil, tadalafil and vardenafil on corpus cavernosum. Eur. J. Pharmacol. 541 (3), 184–190. https://doi.org/10.1016/j.ejphar.2006.05.005.

Machado, M., Salgado, T.M., Hadgraft, J., Lane, M.E., 2010. The relationship between transepidermal water loss and skin permeability. Int. J. Pharm. 384 (1–2), 73–77. https://doi.org/10.1016/j.ijpharm.2009.09.044.

Magnano, G.C., Quadri, M., Palazzo, E., et al., 2024. 3D human foreskin model for testing topical formulations of sildenafil citrate. Int. J. Pharm. 649 (janvier), 123612. https://doi.org/10.1016/j.ijpharm.2023.123612.

Miwa, M., Nakajima, N., Matsunaga, M., Watanabe, K., 2006. Measurement of water loss in human nasal mucosa. Am. J. Rhinol. 20 (5), 453–455. https://doi.org/10.2500/ajr.2006.20.2938.

Mohamad, S.A., Mustafa, W.W., Salem, H., Elrehany, M., Rofaeil, R.R., Abdelkader, H., 2022. Physicochemical characteristics and *ex vivo* skin permeability for three phosphodiesterase 5 inhibitors (sildenafil, tadalafil and vardenafil): a proof-of-concept study for topical penile therapy. J. Drug Delivery Sci. Technol. 70 (avril), 103166. https://doi.org/10.1016/j.jddst.2022.103166.

Mohamed, S.A., Rofaeil, R.R., Salem, H., et al., 2023. Proniosomal gel-loaded phosphodiesterase inhibitors (sildenafil, vardenafil, and tadalafil): prospects for

topical penile therapy of tadalafil for treatment of erectile dysfunction. Gels 9 (8), 597. https://doi.org/10.3390/gels9080597.
Moreland, R.B., Goldstein, I., Traish, A., 1998. Sildenafil, a novel inhibitor of phosphodiesterase type 5 in human corpus cavernosum smooth muscle cells. Life Sci. 62 (20), PL309-18. https://doi.org/10.1016/S0024-3205(98)00158-1.
Nichols, D.J., Muirhead, G.J., Harness, J.A., 2002. Pharmacokinetics of sildenafil after single oral doses in healthy male subjects: absolute bioavailability, food effects and dose proportionality. Br. J. Clin. Pharmacol. 53 (s1). https://doi.org/10.1046/j.0306-5251.2001.00027.x.
Nicolosi, A., Buvat, J., Glasser, D.B., et al., 2006. Sexual behaviour, sexual dysfunctions and related help seeking patterns in middle-aged and elderly Europeans: the global study of sexual attitudes and behaviors. World J. Urol. 24 (4), 423–448. https://doi.org/10.1007/s00345-006-0088-9.
Niedorf, F., Schmidt, E., Kietzmann, M., 2008. The automated, accurate and reproducible determination of steady-state permeation parameters from percutaneous permeation data. Altern. Lab. Anim. 36 (2), 201–213. https://doi.org/10.1177/026119290803600209.
OECD, 2004. *Guidance Document for the Conduct of Skin Absorption Studies*. OECD Series on Testing and Assessment. OECD. https://doi.org/10.1787/9789264078796-en.
Osman, M.A., El Maghraby, G.M., Hedaya, M.A., 2006. Intestinal absorption and presystemic disposition of sildenafil citrate in the rabbit: evidence for site-dependent absorptive clearance. Biopharm. Drug Dispos. 27 (2), 93–102. https://doi.org/10.1002/bdd.487.
Osman, S., Yaseen, A., Mohammed, A., 2023. Formulation and characterization of different topical hydrogels loaded with sildenafil citrate. Al-Azhar J. Pharm. Sci. 68 (2), 28–42. https://doi.org/10.21608/ajps.2023.332165.
Phatale, V., Vaiphei, K.K., Jha, S., Patil, D., Agrawal, M., Alexander, A., 2022. Overcoming skin barriers through advanced transdermal drug delivery approaches. J. Control. Release 351 (novembre), 361–380. https://doi.org/10.1016/j.jconrel.2022.09.025.
Porst, H., 2004. Phosphodiesterase type-5 inhibitors: a critical comparative analysis. EAU Updat. Ser. 2 (2), 56–63. https://doi.org/10.1016/j.euus.2004.03.007.
Shabbir, M., Muneer, A., Kalsi, J., et al., 2011. Glans resurfacing for the treatment of carcinoma *in situ* of the penis: surgical technique and outcomes. Eur. Urol. 59 (1), 142–147. https://doi.org/10.1016/j.eururo.2010.09.039.
Sussman, D.O., 2004. Pharmacokinetics, pharmacodynamics, and efficacy of phosphodiesterase type 5 inhibitors. J. Am. Osteopath. Assoc. 104 (3 Suppl 4), S11–S15.
Thakar, H.J., Dugi, D.D., 2013. Skin grafting of the penis. Urol. Clin. N. Am. 40 (3), 439–448. https://doi.org/10.1016/j.ucl.2013.04.004.
Thompson, C.S., Mumtaz, F.H., Khan, M.A., et al., 2001. The effect of sildenafil on corpus cavernosal smooth muscle relaxation and cyclic GMP formation in the diabetic rabbit. Eur. J. Pharmacol. 425 (1), 57–64. https://doi.org/10.1016/S0014-2999(01)01077-9.
Tracqui, A., Ludes, B., 2003. HPLC-MS for the determination of sildenafil citrate (Viagra (R)) in biological fluids. Application to the salivary excretion of sildenafil after oral intake. J. Anal. Toxicol. 27 (2), 88–94. https://doi.org/10.1093/jat/27.2.88.
Treffel, P., Muret, P., Muret-D'Aniello, P., Coumes-Marquet, S., Agache, P., 1992. Effect of occlusion on in vitro percutaneous absorption of two compounds with different physicochemical properties. Skin Pharmacol. Physiol. 5 (2), 108–113. https://doi.org/10.1159/000211027.
Trommer, H., Neubert, R.H.H., 2006. Overcoming the stratum corneum: the modulation of skin penetration. Skin Pharmacol. Physiol. 19 (2), 106–121. https://doi.org/10.1159/000091978.
Tzanova, M.M., Nguyen, L., Moretti, F., et al., 2023. Interpreting permeability as a function of free drug fraction: the case studies of cyclodextrins and liposomes. Eur. J. Pharm. Sci. 189 (octobre), 106559. https://doi.org/10.1016/j.ejps.2023.106559.
Verma, P., Pathak, K., 2010. Therapeutic and cosmeceutical potential of ethosomes: an overview. J. Adv. Pharm. Technol. Res. 1 (3), 274. https://doi.org/10.4103/0110-5558.72415.
Wallis, R.M., 1999. The pharmacology of sildenafil, a novel and selective inhibitor of phosphodiesterase (PDE) type 5. Folia Pharmacol. Jpn. 114 (supplement), 22–26. https://doi.org/10.1254/fpj.114.supplement_22.
Zinner, N., 2007. ORIGINAL RESEARCH—ED PHARMACOTHERAPY: do food and dose timing affect the efficacy of sildenafil? A randomized placebo-controlled study. J. Sex. Med. 4 (1), 137–144. https://doi.org/10.1111/j.1743-6109.2006.00400.x.