

A study on the effect of inorganic salts in transungual drug delivery of terbinafine

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Abstract

Objectives The poor success rate of topical therapy in nail disorders is mainly because of the low permeability of keratinized nail plates. This can be overcome by utilizing potent perungual drug penetration enhancers that facilitate the drug permeation across the nail plate. This study evaluated the efficacy of inorganic salts in enhancing the trans-nail permeation using a model potent antifungal agent, terbinafine hydrochloride.

Methods Permeation studies were carried out across human cadaver nail in a Franz diffusion cell using terbinafine solution (1 mg/ml; pH 3). Preliminary studies were carried out to assess the effect of salts (0.5 M) on the terbinafine permeation into and through the nail. Further, the influence of salt concentration (0.25–3 M) on permeation, the mechanism for the enhancement and the suitability of developing a formulation were also studied.

Key findings Terbinafine permeation (3–5 fold) through the nail and drug load (4–7 fold) in the nail were enhanced significantly when salts were used at 0.5 M concentration. Increase in salt concentration up to 1 M increased the permeation, which decreased with further increase in salt concentration (>1 M). Mechanistic studies revealed that the enhanced permeation by salts was mainly due to their ability to increase the nail hydration and also to increase the thermodynamic activity of the drug. The cumulative amount of terbinafine permeated at 24 h from the formulated gel ($9.70 \pm 0.93 \mu\text{g}/\text{cm}^2$) was comparable with that of a solution ($11.45 \pm 1.62 \mu\text{g}/\text{cm}^2$).

Conclusions Given the promising results from the permeation and drug load studies, it was concluded that inorganic salts could be used as potent transungual permeation enhancers.

Keywords hydration; nail; permeation enhancer; salts; terbinafine

Introduction

The most common causes of nail diseases are fungal infections and psoriasis. Onychomycosis, fungal infection of the nail, is the most prevalent (approximately 50%) of all nail diseases. Dermatophytes are the main causative organism for onychomycosis, while non-dermatophytic molds and yeasts are also involved.^[1] Existing treatment modalities for onychomycosis include oral delivery and topical application. Topical drug delivery is the most appropriate therapy as it offers higher patient compliance and better proximity to the infected site and avoids potential systemic adverse effects.^[2,3] Currently, topical formulations are available as nail lacquers, creams, ointments, gels, solutions and lotions.^[4] However, the efficiency of these formulations is limited due to their inability to deliver a therapeutically effective amount of drug into and across the impermeable nail plate. Therefore, this therapy is limited for the treatment of superficial and minor subungual onychomycosis.^[5,6]

Topical delivery of drugs by the usage of permeation enhancers is well known. A wide variety of proven permeation enhancers are available and are used in enhancing drug delivery across the skin. The skin contains about 15% of lipids arranged as lamellae in the intercellular space. Most skin penetration enhancers act by modulation of lipid structures. The nail plate has less lipid content (<1%) than the skin.^[7] The major component of the nail plate is keratin. Enhancers of skin drug penetration that act predominantly on the keratinocyte pathway are relatively few, thus it is obvious that most skin permeation enhancers are unsuccessful in transungual drug delivery. Therefore, research is being carried out to screen novel entities for their potential transungual permeation enhancement ability. Many chemical agents, such as acetylcysteine, urea, salicylic acid, 2-n-nonyl-1,3-dioxalane,

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mercaptoethanol, thioglycolic acid and glycolic acid, have been screened for their unequal permeation enhancement capacity.^[8–12] Reports on the capability of these agents to enhance transungual delivery are inconsistent.^[2] Recently Malhotra and Zatz^[10] reported the ineffectiveness of keratolytic agents, even at high concentration, when delivered from gel formulation. The authors also observed the failure of sodium metabisulfite to enhance penetration. Also, the efficiency of acetylcysteine was limited only on the dorsal nail layer and it was inactive in the intermediate and lower nail layers.^[8]

It is well known that the cure rates with existing topical therapy remain much lower than those achieved by systemic antifungal therapy. Successful treatment of nail diseases by the topical route can be achieved by identifying potent perungual penetration enhancers to deliver drugs across the nail plate in effective amounts. It is also known that the efficiency of penetration enhancers vary with different formulation conditions and with different drugs. Therefore, one needs to identify the most appropriate enhancers for the given drug and formulation. In this direction, we developed a high-throughput technique for screening penetration enhancers, called TransScreen-N. It is a rapid microwell plate-based method that involves two different treatment procedures – simultaneous exposure treatment and sequential exposure treatment. The technique was developed to screen permeation enhancers for the delivery of terbinafine hydrochloride, a potent antifungal agent, which is the current treatment of choice in onychomycosis. During this process the efficiency of inorganic salts to act as perungual permeation enhancers was discovered. The drug load in nail pieces soaked in formulations containing salts as enhancers (sodium sulfite, sodium phosphate, potassium phosphate, calcium phosphate and ammonium carbonate) were found to be significantly higher than the control. TransScreen-N is a screening method only and the results are suggestive. The method does not reveal the drug permeation profile, flux, lag time or the possible mechanisms. Encouraged by results that suggested the potential permeation enhancement efficiency of salts in TransScreen-N, further research on this phenomenon was undertaken. The objective of this study was to evaluate the efficacy of inorganic salts in enhancing the transungual delivery of terbinafine hydrochloride.

Materials and Methods

Materials

Terbinafine hydrochloride was procured from Uquifa (Jiutepec, Mexico). Sodium sulfite, sodium phosphate monobasic, potassium phosphate monobasic, calcium phosphate monobasic and ammonium carbonate were purchased from Sigma (MO, US). Human cadaver nails (second, third and fourth finger nails), both male and female with varying thickness (0.4–0.7 mm), were procured from Science Care (AZ, US) and were stored at 4°C and used within a week. All solutions were prepared in de-ionized water.

Analytical method

The amount of terbinafine in the samples was quantified by high-performance liquid chromatography (HPLC) system

(Waters 1525) with an autosampler (Waters, 717 plus) consisting of a Phenomenex C18 (2) 100 R analytical column (4.6 × 150 mm; Luna 5.0 μm) and a variable wavelength dual λ absorbance detector (Waters 2487). The mobile phase consisted of aqueous solution (0.096 M triethyl amine, 0.183 M orthophosphoric acid) and acetonitrile (60 : 40) adjusted to pH 2 with orthophosphoric acid. Elution was performed isocratically at 32°C at a flow rate of 1.0 ml/min. The injection volume was 20 μl and the column effluent was monitored at 224 nm. The method was validated by determination of linearity, precision and accuracy. The range for the calibration curve was 2–1000 ng/ml (R² = 0.99). The coefficient of variation and the accuracy (relative mean error) was in the range of 1.03–6.08% and –0.54 to –6.96%, respectively.

Preliminary in-vitro permeation studies

Nails were cleaned and adherent tissue was removed with a pair of scissors and scalpel and the nail was rinsed in water. Each of the nail plates was soaked in water for 1 h and mounted on a nail adapter (PermeGear, PA, US). The whole assembly was sandwiched between the two chambers of a Franz diffusion cell (Logan Instruments Ltd, NJ, US). Terbinafine hydrochloride solution (500 μl, 1 mg/ml with 0.5 M enhancer) was placed in the donor compartment and the receiver chamber was filled with 5 ml of acidified water (adjusted to pH 3 using 0.1 M hydrochloric acid or sodium hydroxide as the initial study data revealed that an ionic strength within 0.15 M does not affect the drug load and permeation). The enhancers used were ammonium carbonate, calcium phosphate, potassium phosphate, sodium phosphate and sodium sulfite. The active permeation area was 0.2 cm². The solution in the receiver compartment was stirred at 600 rev/min with a 3-mm magnetic stir bar. Samples were withdrawn at regular intervals for a period of 24 h from the receiver compartment and analysed. Similarly, control experiments (formulation with drug and no enhancer) were run in parallel for comparison.

Amount of drug in nail

The amount of terbinafine loaded in the nail was determined after the permeation study (24 h). Briefly, after in-vitro diffusion studies the nail plates were washed by a standardized procedure using water and 95% ethanol, until the surface was free from drug. The active diffusion area was cut into small pieces, weighed and placed in screw-cap pyrex vials. Sodium hydroxide (1.5 ml of 1 M) was added and the vials were incubated for 24 h in a shaker water bath at room temperature to allow the nail to dissolve. Extraction of drug was carried out by a slight modification of the method described by Dykes *et al.*^[13] Briefly, after dissolving the nails in vials, 200 μl of 5 M hydrochloric acid was added to neutralize the mixture. Hexane (3 ml) was added to the vial to extract the drug and the vials were shaken manually for 30 min. The mixtures were transferred into centrifuge tubes and centrifuged at 4000 rev/min for 10 min. The hexane layer was collected, 1 ml of 0.5 M sulfuric acid–isopropyl alcohol (85 : 15) was added and the mixture was shaken vigorously for 30 min. The lower acidic aqueous layer, which holds the majority of terbinafine, was collected separately and the amount of drug in the nail was determined. This

extraction procedure was validated by spiking different drug concentrations (2–20 $\mu\text{g/ml}$) into sodium hydroxide solution in which the nail was previously dissolved. The recovery was found to be $84 \pm 7\%$.

The amount of terbinafine diffused into the peripheral region (which was not in contact with the formulation or enhancer) was determined by dissecting the peripheral nail area (4–5 mm surrounding), which was washed, dried and weighed. The amount of drug was determined as described above.

Solubility measurements

A normal equilibrium solubility determination was carried out by the method of Okumara *et al.*^[14] An excess amount of terbinafine hydrochloride was added and dissolved in a measured amount of solution containing different concentrations of sodium phosphate (0, 0.25, 0.5, 1.0, 2.0 and 3.0 M) in a glass vial to obtain a saturated solution. The pH of the solution was adjusted to pH 3 or 5 (using 0.1 M HCl or 0.1 M sodium hydroxide) and was monitored occasionally throughout the study period. The system was stirred for 24 h at room temperature and kept at rest for 1 h to assist the attainment of equilibrium. The solution was then filtered through a membrane filter (pore size 0.22 μm) and, after dilution, the solubility was determined.

Effect of salt concentration on permeation

Permeation of terbinafine in the presence of different concentrations of sodium phosphate (0.25, 0.5, 1, 2 and 3 M) was carried out as described previously in preliminary in-vitro permeation studies. The donor drug concentration was 1 mg/ml (pH 3). The amount of drug permeated after 24 h was measured by HPLC.

Effect of pretreatment on permeation

Pretreatment of nails was carried out by soaking the nail plates in 0.5 M sodium phosphate solution (pH 3) for 24 h. The nail plates were then washed with 10 ml of water (pH 3) five times and mounted on a nail adaptor. Drug solution (500 μl ; 1 mg/ml; pH 3) was placed in the donor compartment. For control trials, the nail was soaked in water (pH 3). Permeation studies were carried out as described in preliminary in-vitro permeation studies.

Effect of pH

The influence of pH on the permeation of drug was assessed using 1 mg/ml of terbinafine hydrochloride solution (pH 5) in the absence of salt. Permeation was carried out as described previously in preliminary in-vitro permeation studies and the amount of terbinafine permeated after 24 h was determined.

Permeation studies using dialysis membrane

Permeation studies were carried out across the dialysis membrane (MWCO 1000, 7 Spectra/Por; Spectrum Laboratories, Inc., CA, US) with varying concentration of sodium phosphate (0.25, 0.5, 1, 2 and 3 M). The membrane was mounted between the donor and receiver chamber of a Franz diffusion cell (Logan Instruments Ltd., NJ, US). Drug–enhancer solution (500 μl ; 1 mg/ml; pH 3) was placed in the donor compartment while the receiver chamber was filled

with 5 ml of the respective sodium phosphate solutions without drug (pH 3), to maintain uniform osmotic pressure. The permeation area was 0.64 cm^2 . The solution in the receiver compartment was stirred at 600 rev/min with a 3-mm magnetic stir bar. Samples were withdrawn at regular intervals from the receiver compartment and analysed. Control experiments were run in parallel for comparison using terbinafine solution (1 mg/ml; pH 3).

Water uptake

Nail pieces (4 × 4 mm) were incubated at $37 \pm 0.5^\circ\text{C}$ for 24 h, weighed (W1) and soaked for 24 h in water (pH 3) or 0.5 M sodium phosphate solution (pH 3). Then the nail plates were removed and wiped with Kimwipe to remove the surface water. Nails were placed in a screw-cap Pyrex vial containing pre-weighed anhydrous sodium sulfate (W2) and kept at room temperature until the weight of sodium sulfate (W3) remained constant (48 h). The increase in the weight of sodium sulfate was noted and the percentage water uptake of the nail was calculated by the following equation.

$$\% \text{ Water uptake} = [(W3 - W2)/W1] \times 100 \quad (1)$$

Permeation using poloxamer gel

The poloxamer gel was prepared by a ‘cold process’. Drug solution (1 mg/ml; pH 3) was slowly added to poloxamer (21% w/v) with constant stirring at low temperature (4–5°C) until the polymers were uniformly dispersed. The required amount of permeation enhancer (0.5 M sodium phosphate) was incorporated into the drug dispersion while mixing, and the pH was readjusted to 3 using 0.1 M HCl. The dispersion was mixed and then kept in a refrigerator (4–5°C) overnight (12 h).

The drug content was determined by accurately weighing 1 g of each gel, which was diluted with ethanol (95%), filtered and assayed by HPLC. The drug content in the gel was found to be 0.84–0.89 mg/g.

Nail plates were soaked in water for 1 h and mounted on a nail adaptor. Five-hundred milligrams of the viscous gel (with or without enhancer) was placed in the donor compartment and the permeation studies were carried out for a period of 24 h, as previously described in preliminary in-vitro permeation studies.

Statistical analysis

Statistical analysis on the effect of different salts on the permeation of terbinafine through the nail, drug load in the nail and the influence of concentration of sodium phosphate on the drug permeation across the nail and dialysis membrane was performed using two tailed Kruskal–Wallis test (Graphpad Prism 5; Graphpad software, Inc., CA, US). In all cases, post-hoc comparisons of the means of individual groups were performed using Dunn’s test. The effect on enhancement of terbinafine permeation across nail and dialysis membrane with different concentrations of sodium phosphate was analysed using Mann–Whitney *U*-test. The data points provided in the graphs are an average of four trials. The error bars represents the standard deviation. $P < 0.05$ denoted significance in all cases.

Results

Figure 1 represents the effect of different inorganic salts at 0.5 M concentration on the permeation of terbinafine hydrochloride across the nail plate. It is evident that the permeation was significantly enhanced (3–5 fold, Kruskal–Wallis test, Dunn's test, $P = 0.008$) in the presence of salts

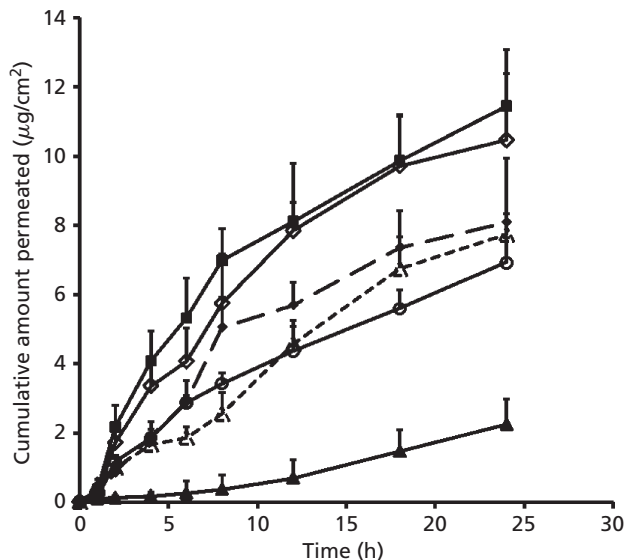


Figure 1 Permeation profile of terbinafine hydrochloride in the presence of different salts. Permeation was carried out using terbinafine hydrochloride solution (1 mg/ml; pH 3) and the salt concentration was 0.5 M. ▲, control; ◆, ammonium carbonate; ◇, potassium phosphate; ■, sodium phosphate; △, calcium phosphate; ○, sodium sulfite. Data are expressed as mean \pm SD, $n = 4$.

when compared with the control. The cumulative amount of drug permeated after 24 h with different salts (ammonium carbonate ($8.09 \pm 1.85 \mu\text{g}/\text{cm}^2$), sodium phosphate ($11.44 \pm 1.62 \mu\text{g}/\text{cm}^2$), calcium phosphate ($7.75 \pm 0.59 \mu\text{g}/\text{cm}^2$), potassium phosphate ($10.47 \pm 1.92 \mu\text{g}/\text{cm}^2$), sodium sulfite ($6.93 \pm 0.72 \mu\text{g}/\text{cm}^2$)) was found to be higher than the control ($2.24 \pm 0.72 \mu\text{g}/\text{cm}^2$). The profiles also indicated that the drug permeation was higher in the initial period (up to 12 h) and followed slow release afterwards, with all the enhancers studied. In contrast, control experiments exhibited much less permeation and the lag time to attain steady-state diffusion was ~ 8 h.

The drug load in the active diffusion area and peripheral area of the nail plate after 24 h of drug permeation studies with different enhancers and the control are represented in Figure 2. The inorganic salts enhanced the drug load in both the active diffusion area (4–7 fold, Kruskal–Wallis test, Dunn's test, $P = 0.011$) and peripheral area (5–10 fold, Kruskal–Wallis test, $P = 0.0275$).

Table 1 summarizes the solubility of terbinafine with different concentration of sodium phosphate at pH 3 and pH 5. The solubility decreased with increasing salt concentration at both pH 3 and pH 5.

In the next step, permeation studies were carried out to evaluate the effect of the concentration of sodium phosphate (0, 0.25, 0.5, 1.0, 2.0 and 3.0 M) on drug permeation across the nail plate using terbinafine solution (1 mg/ml pH 3), and the data obtained are presented in Figure 3. The drug permeation increased with increase in salt concentration up to 1 M and decreased with further increase in salt concentration (2–3 M) (Kruskal–Wallis test, $P = 0.001$). A similar effect was also observed when the permeation was carried out at different salt concentrations across the dialysis membrane (Figure 4).

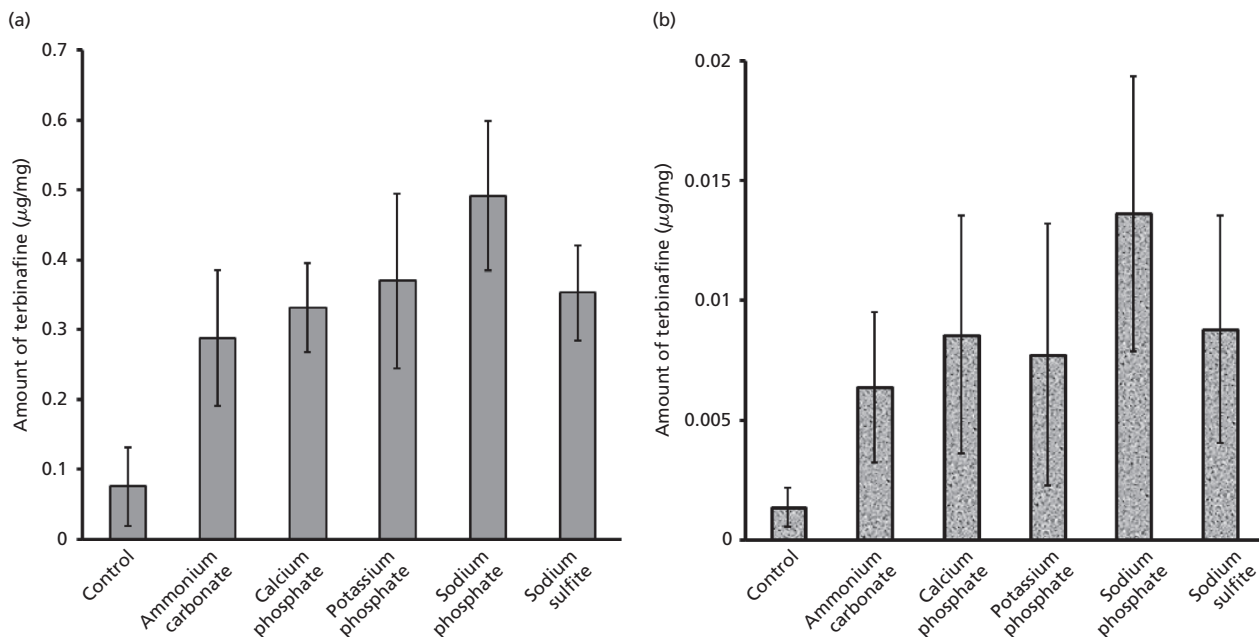
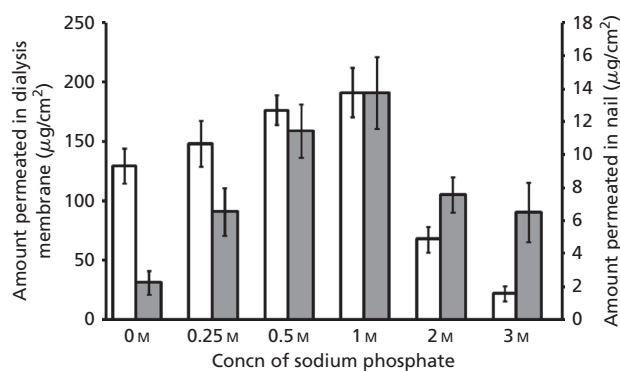


Figure 2 The amount of terbinafine hydrochloride loaded ($\mu\text{g}/\text{mg}$) in the nail diffusion area (a) and peripheral nail (b) during permeation studies in the presence of different salts (0.5 M) in 24 h. The diffusion area was 0.2 cm^2 . Data are expressed as mean \pm SD, $n = 4$.

Table 1 Solubility of terbinafine hydrochloride in water (pH 3 and 5) with different concentration of sodium phosphate

Concentration of sodium phosphate (M)	Terbinafine hydrochloride solubility (mg/ml)	
	pH 3	pH 5
0	1.57 ± 0.23	1.12 ± 0.16
0.25	1.41 ± 0.18	0.86 ± 0.10
0.5	1.19 ± 0.25	0.74 ± 0.14
1.0	0.98 ± 0.20	0.63 ± 0.08
2.0	0.79 ± 0.16	0.54 ± 0.12
3.0	0.69 ± 0.11	0.42 ± 0.11

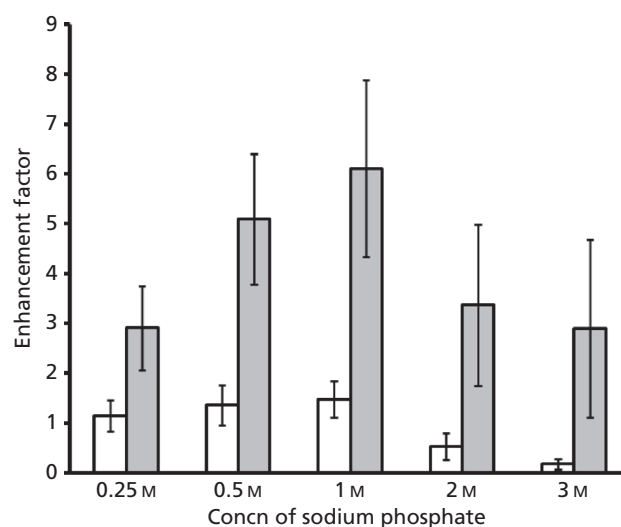
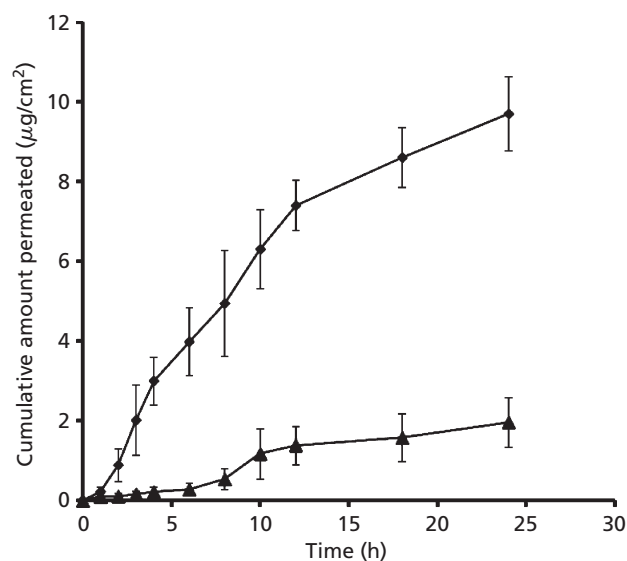
Solubility studies were carried out at room temperature (25 ± 1°C). Each value represents the mean ± SD, n = 5.

**Figure 3** Comparison of the cumulative amount of terbinafine permeated across the dialysis membrane and nail at the end of 24 h with different concentrations (0, 0.25, 0.5, 1.0, 2.0 and 3 M) of sodium phosphate. The diffusion area of the dialysis membrane and nail were 0.64 cm² and 0.2 cm², respectively. Open columns, dialysis membrane; shaded columns, nail. Data are expressed as mean ± SD, n = 4.

Mechanistic aspects on the enhancement of terbinafine permeation by inorganic salts were studied by pretreatment of nails with sodium phosphate solution (0.5 M for 24 h) and then followed by the permeation studies. The amount of terbinafine permeated after 24 h across the treated and untreated nail plate was 3.03 ± 0.52 and 2.39 ± 0.44 µg/cm², respectively. Water uptake study indicated a higher percentage water uptake by the nail in the presence of inorganic salt (36.34 ± 4.68%) when compared with control (26.27 ± 4.55%). The cumulative amount of terbinafine permeated after 24 h from the formulated gel containing 0.5 M sodium phosphate (9.70 ± 0.93 µg/cm²) was found to be comparable with the amount of drug permeated from solution (11.44 ± 1.62 µg/cm²) (Figure 5).

Discussion

Preliminary in-vitro permeation studies were carried out across the nail plate with different inorganic salts using terbinafine hydrochloride solution (1 mg/ml; pH 3). The concentration of all salts was kept at 0.5 M for the purpose of comparison. The donor vehicle was adjusted to pH 3 as the drug possesses high solubility at this pH as compared with

**Figure 4** Comparison of enhancement in terbinafine hydrochloride permeation across nail and dialysis membrane in the presence of different concentration of sodium phosphate. Enhancement was calculated with respect to control (1 mg/ml, pH 3 water). The donor constituted 1 mg/ml of terbinafine hydrochloride. Open columns, dialysis membrane; shaded columns, nail. Data are expressed as means ± SD, n = 4.**Figure 5** Effect of sodium phosphate on the permeation of terbinafine hydrochloride from poloxamer gel across the nail plate. The concentration of sodium phosphate used was 0.5 M. ♦, sodium phosphate; ▲, control. Data are expressed as mean ± SD, n = 4.

higher pH. From Figure 1 the ability of salts to enhance the permeation of terbinafine across the nail plate and reduce the lag time to attain steady-state diffusion is evident. All the salts were comparable in their enhancement efficiency (3–5 fold), although with sodium phosphate the difference to the control was significant (~5-fold enhancement). This is the first report to our knowledge on the enhancement effect of inorganic salts in transungual drug delivery.

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