

SKIN PERMEATION ENHANCEMENT EFFECTS OF ASCORBIC ACID AND TRIETHYL CITRATE ON ROFECOXIB

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The enhancing effect of ascorbic acid and triethyl citrate (TEC) on the in vitro skin permeation of rofecoxib across rat epidermis was investigated. Skin pre-treatment with ascorbic acid and TEC at different concentrations, followed by application of rofecoxib gel, showed higher permeation flux than the control condition. The mechanism underlying this permeation enhancement was probed with fourier transform infrared spectroscopy (FTIR). The FTIR spectra of rat epidermis treated with ascorbic acid revealed that ascorbic acid at low concentration appears to interact with dermal keratin, whereas at higher concentration it appears to interact with both dermal proteins and lipids. The FTIR spectra of rat epidermis treated with TEC showed a decrease in peak heights for both asymmetric and symmetric C-H stretching absorbance, indicating a change in the fluidity of alkyl chains in the intercellular lipids in the stratum corneum (SC). The protein disruption effect of TEC was probably due to the solvation of keratin by the formation of hydrogen bonds between TEC hydroxyl groups and keratin chain C=O groups. Skin pre-treatment with different concentrations of permeation enhancers did not show any significant change in lag time in comparison to control. The amount of rofecoxib retained in the skin after skin pre-treatment with enhancers was found to be higher than in the experiment without skin pre-treatment. Scanning electron microscopy (SEM) confirmed the maintenance of skin integrity throughout the permeation experiment. The observed permeation enhancing effects of ascorbic acid and TEC in the present study indicate that a rapid percutaneous absorption of rofecoxib at effective therapeutic levels may facilitate faster anti-inflammatory activity.

Keywords: Rofecoxib, Ascorbic acid, Triethyl citrate, Skin pre-treatment, Rat epidermis

INTRODUCTION

The transdermal route for drug administration is limited by the barrier properties of the SC. To improve the permeation of drugs across the skin, the barrier properties of the SC may be manipulated by using skin permeation enhancers (Cooper 1984; Loftsson, Gildersleeve and Boder 1987). As a result, less potent drugs with higher daily dose requirements and those with less favourable physicochemical properties for permeation may also be considered for transdermal delivery. This study examined the permeation

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enhancement effects of ascorbic acid and TEC on the permeation of rofecoxib across rat epidermis.

Non-steroidal anti-inflammatory drugs (NSAIDs) play a major role in the management of inflammation and pain through inhibition of the enzyme cyclo-oxygenase (COX) in the pathway of prostaglandin synthesis. Rofecoxib exhibits greater selective inhibitory activity against the inducible isoform of cyclo-oxygenase (COX 2) than against COX 1, which is associated with gastrointestinal (GI) adverse effects. The major GI adverse effects of the less selective NSAIDs (upper GI perforations, ulcerations and bleeding) are recognised to be due to interference of the drug with the biosynthesis of prostaglandin and other arachidonic acid metabolites in the gastric mucosa. These common side effects can reduce patient compliance and discourage physicians from prescribing the drugs. Rofecoxib, a selective COX 2 inhibitor, was approved in the year 1999 by FDA with a view to reduce the incidence of GI adverse effects, as observed with other less selective NSAIDs (Soniwala *et al.* 2005). The oral bioavailability of rofecoxib is about 93% and the steady-state plasma concentration is reached at 3 to 4 days with multiple oral dose administration. However, the drug was withdrawn in 2004 after incidence of GI adverse effects and cardiac toxicities following oral administration. Rofecoxib is practically insoluble in water (4.6 mg/l) (Baboota, Dhaliwal and Kohli 2005), which may cause GI ulceration due to longer residence time in contact with gastric mucosa, resulting in dangerously high drug concentration. There are reports that demonstrate a significant decrease in gastric ulcerogenic activity of rofecoxib through administration of solid dispersions in polyethylene glycol (PEG) 6000, polyvinylpyrrolidone K 30, or Eudragit E 100, as well as treatment comprising an inclusion complex with β -cyclodextrin (Soniwala *et al.* 2005; Baboota, Dhaliwal and Kohli 2005). These formulations improve the aqueous solubility of the drug, thus enhancing its dissolution rate, thereby leading to a faster onset of action and reduced GI mucosal toxicity. On the other hand, the formulations described above may increase the risk of rofecoxib's systemic cardiac toxicity. Topical gel formulation using skin as the route of drug administration may prevent systemic side effects by confining drug concentrations / actions to the site of the origin of pain, rather than flooding all of the body tissues (Yum, Wang and Theeuwes 1991). Kesavanarayanan, Nappinnai and Ilavarasan (2007)

successfully formulated valdecoxib, another selective COX 2 inhibitor, as a carbopol 940 gel for effective and safe administration, resulting in a local analgesic effect with negligible systemic side effects. Das and Ahmed (2007) confirmed the feasibility of the topical gel formulation of rofecoxib. The anti-inflammatory activity of the gel formulation in carrageenan-induced hind paw oedema model reveals that rofecoxib slowly permeated from the gel formulation to the inflammation site. A maximum of 58.93% inhibition of oedema was observed at 6 h as compared to the maximum value of 65.51% inhibition of oedema with oral rofecoxib at 4 h. Rapid percutaneous absorption of rofecoxib at higher levels is required to achieve effective therapeutic plasma levels of 207–321 ng/ml for faster anti-inflammatory activity. Desai (2004) observed higher cumulative drug permeation through excised rat abdominal skin following treatment with a microemulsion gel containing a rofecoxib-PEG 4000 solid dispersion, when compared to treatment with a microemulsion gel containing neat rofecoxib. Faster anti-inflammatory activity was also observed with a microemulsion gel containing a rofecoxib-PEG 4000 solid dispersion, as compared to the conventional gel.

The present study aimed to investigate the enhancing effect of ascorbic acid and TEC on the *in vitro* skin permeation of rofecoxib from a gel formulation, across rat epidermis. The enhancers were applied as a pre-treatment at different concentrations. We attempted to evaluate the possible mechanisms of action of the compounds investigated as permeation enhancers.

Ascorbic acid is generally used as an antioxidant in cosmetic formulations. Topical ascorbic acid protects the skin against harm caused by exposure to sunlight. It neutralizes reactive oxygen species (free radicals), the highly reactive molecules produced by the interaction of sunlight, cell membranes, and other components of skin tissue. Vaddi *et al.* (2001a, 2001b) investigated the enhancing effect of ascorbic acid on the permeation of haloperidol across rat skin and human skin. They reported that ascorbic acid enhanced permeation by increasing the solubility of drug in the vehicle, thus providing a high concentration gradient across the skin.

TEC, a water-soluble plasticiser, is incorporated into pharmaceutical polymers to modify drug release from polymeric systems (Bodemeier and Paeratakul 1990) and to improve mechanical properties (Gutierrez-Rocca and McGinity 1994). It is typically used in

film coating to reduce brittleness and to enhance polymer coalescence for flexible film formation. Das, Ghosal and Bhattacharya (2006) reported on pseudolatex transdermal film containing Trazodone hydrochloride prepared from Eudragit RL100 and RS100, using TEC as plasticiser. The concentration of TEC in the film markedly affected the skin permeation properties of the drug. The release and corresponding permeation across mouse epidermis was faster at low and high TEC concentrations because of incomplete coalescence of the latex and leaching of the plasticiser.

METHODS

Materials

The rofecoxib sample was a gift from Alembic Pharmaceutical Ltd. (Vadodara, India). The other materials were kindly supplied by various manufacturers: sodium alginate, carbopol 940 (Loba Chemie Pvt. Ltd., Mumbai, India); glycerol (Qualigens Fine Chemicals, Mumbai, India); methyl paraben (Himedia Laboratories Pvt. Ltd., Mumbai, India); triethanolamine (Central Drug House Ltd., Mumbai, India); PEG 400 (Merck Ltd., Mumbai, India); sodium bromide (Loba Chemie Pvt. Ltd., Mumbai, India); and chloroform (Thomas Baker Chemicals Pvt. Ltd., Mumbai, India). All the chemicals were of analytical grade satisfying pharmacopoeial specifications.

Preparation of Rofecoxib Gel

Gels were prepared according to the method reported by Das and Ahmed (2007). In brief, the polymer mixture (4% w/w) consisted of sodium alginate and carbopol 940 at a 3:1 ratio in a mixture of water and glycerol (10% v/w) with 1% w/w rofecoxib. Methyl paraben at 0.2% w/w was added as preservative in the dispersion. The dispersion was stirred until homogeneous, then neutralised and made viscous using 4% v/w triethanolamine.

***In vitro* Permeation Studies**

The Institutional Animal Ethics Committee of Dibrugarh University, India approved all experiments with animals. The Wister male albino

rats of 150–200 g (M/S Ghosh Enterprises, Kolkata, India) were used for the experiments and maintained under controlled conditions with regard to temperature (25°C) as well as humidity (45%–60% RH). Animals had free access to water and food.

The *in vitro* permeation study was performed using Keshary-Chien glass diffusion cells and rat epidermis with intact SC as membrane. The method of epidermis preparation was based on our previous report (Das and Ahmed 2007). The epidermis was thoroughly washed with water, dried at room temperature under 25% RH, wrapped in aluminium foil and stored at $4 \pm 1^\circ\text{C}$ until further use.

For *in vitro* permeation studies, skins were allowed to hydrate for one hour before being mounted on the Keshary-Chien diffusion cell with the SC facing the donor compartment, with an effective diffusion area of 1.54 cm². The receptor compartment was filled with 19.5 ml of an aqueous PEG 400 solution (40% v/v); receptor phase was maintained at $37 \pm 0.5^\circ\text{C}$ to maintain the donor phase temperature at 32°C to 33°C . One gram of gel was placed on the SC side in the donor compartment and covered with aluminium foil to prevent drying. The amount of drug that permeated was determined spectrophotometrically at 257 nm by removing a 1 ml aliquot with a hypodermic syringe fitted with a 0.22 μm membrane filter, at designated time intervals for 8 h. The volume was replenished with the same volume of pre-warmed receiver solution to maintain sink conditions. Blanks were run for each set as described above with placebo gel and calculated accordingly.

Pre-treatment of Skin for Permeation Studies

The solutions for the pre-treatment were different concentrations of ascorbic acid in water (10% w/w, 20% w/w and 30% w/w) and TEC in PEG (10% v/v, 20% v/v and 30% v/v). The skin was pre-treated by placing 1 ml of the pre-treatment solution in the donor compartment of the diffusion cell. The receptor compartment was filled with PEG 400 (40% v/v) solution maintained at $37 \pm 0.5^\circ\text{C}$. The pre-treatment solution was removed by soaking it up with tissue paper; then the permeation experiment was carried out.

Determining the Amount of Rofecoxib Retained in the Skin

At the end of the *in vitro* permeation experiment, the skin sample was removed from the diffusion cell and washed with distilled water to remove the adhering gel, then blotted dry with tissue paper. The treated skin area was weighed and cut into small pieces, then extracted with 10 ml of PEG 400 (40% v/v) solutions for 24 h and filtered through a membrane filter (0.22 μm). The amount of rofecoxib in the sample was estimated spectrophotometrically at 257 nm.

Sample Analysis

The sample concentrations of rofecoxib were determined using UV spectrophotometry. In order to generate a calibration curve, an accurately weighed amount of rofecoxib was solubilised in the 40% v/v PEG 400 solution to obtain a primary standard in the concentration range of 10–80 $\mu\text{g}/\text{ml}$. The calibration curve was obtained by measuring absorbance at a predetermined λ_{max} of 257 nm with a Hitachi U-2001 UV-VIS spectrophotometer. The concentration of rofecoxib in test samples was calculated using the linear regression equation of the calibration curve (Absorbance = 0.0252 + 0.0117 \times Concentration, $R^2 = 0.9973$). The high value obtained as correlation coefficient (R^2) indicates the linearity of the calibration curve; the curve did not deviate significantly from the origin, as indicated by its low intercept value. The method was validated for accuracy and precision. When a standard drug solution was assayed repeatedly ($n = 6$), mean standard error (accuracy) and RSD (precision) were found to be 0.5% and 0.7%, respectively.

FTIR

Rat epidermis was treated with the relevant permeation enhancers and dried under vacuum, then stored in a desiccator to remove any traces of enhancer solution. The completely dried samples of rat epidermis were then studied by FTIR (Perkin Elmer, Japan) from KBR pellets.

SEM

Surface morphology of the rat epidermis before and after the permeation experiment was performed by SEM. The study was performed to detect any histological changes that occurred after the permeation experiment. The skin samples were sprinkled on one side of double adhesive tabs and coated with gold under vacuum to render them electrically conductive. The SEM photomicrographs were obtained at 15 kV using a JEOL JSM-6360 scanning electron microscope.

Permeation Data Analysis and Statistics

The steady state flux (J_{ss} , $\mu\text{g}/\text{cm}^2/\text{h}$) was calculated from the slope of the linear plot of the cumulative amount permeated per unit area (Q , $\mu\text{g}/\text{cm}^2$) as a function of time (t , h). The lag time (t_L , h) was determined from the x-intercept of the slope at steady state. The apparent diffusion parameter (D/h^2 , h^{-1}) was calculated from the following equation (Narishetty and Panchagnula 2004):

$$D/h^2 = 1/6t_L$$

where h (cm) is the thickness of the skin and D is diffusion coefficient within the skin (cm^2/h). The permeation-enhancing effect of the enhancer was calculated in terms of enhancement ratio (ER) using the following equation:

$$\text{ER} = J_{ss}$$

with permeation enhancer / J_{ss} without permeation enhancer.

Data are represented as mean \pm SD ($n = 3$). Statistical comparisons were made using Student's t -test at a significance level of $P < 0.05$ using MS Excel software.

RESULTS AND DISCUSSION

In the present work, the permeation enhancement effects of ascorbic acid and TEC on the penetration of rofecoxib through rat skin were studied. The enhancers were applied on the skin as pre-treatment.

Rofecoxib was delivered from a 4% w/w sodium alginate-carbopol gel.

Skin Pre-treatment with Ascorbic acid

The percutaneous penetration flux for rofecoxib increased significantly ($P < 0.05$) with skin pre-treatment with ascorbic acid as compared to control. The various penetration parameters are presented in Table 1. A linear Q versus t relationship was observed after an initial lag time. The high values of R^2 in Table 1 indicate the linearity of this relationship and that rofecoxib permeates through the intact rat epidermis at a constant rate.

Figure 1 depicts the effect of different pre-treatment concentrations of ascorbic acid on the flux of rofecoxib across the rat epidermis. The permeation of rofecoxib across the skin pre-treated with 10%, 20% and 30% w/w ascorbic acid was 1.06, 1.24 and 1.77 times greater, respectively, than that of the control. Although ascorbic acid increased the flux of rofecoxib at all pre-treatment concentrations in comparison to control, there was a decrease in the apparent diffusion parameter in a concentration-dependent manner. This might be due to the gradual increase in skin retention of rofecoxib, as observed in Table 1. An apparent increase of lag time was observed when the pretreatment concentrations of ascorbic acid increased. The increase in pretreatment concentrations might have increased the skin binding of rofecoxib, as indicated by the corresponding skin retention values at different pretreatment concentrations (Table 1). There was no significant change in lag time at all concentrations ($P < 0.05$) when compared to control.

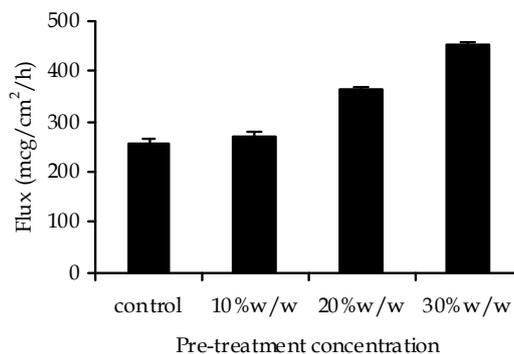


Fig. 1: Effect of skin pre-treatment with ascorbic acid at different concentrations on the permeation of rofecoxib across rat epidermis.

The skin permeation enhancement effect of ascorbic acid on rofecoxib can be attributed primarily to its physicochemical properties. Ascorbic acid melts at approximately 190°C (British Pharmacopoeia 2003), which is an indication of weak cohesiveness or self-association of the molecule. Furthermore, ascorbic acid's low molecular weight (176 Da) ensures its association or interaction with protein and lipid components of SC in a concentration-dependent manner during the skin pre-treatment, thereby altering barrier properties.

We sought to identify the mechanism of ascorbic acid enhancement using FTIR spectroscopy. The FTIR spectra of untreated rat epidermis showed characteristic absorption peaks (Fig. 2A) at approximately 2854 cm⁻¹ and 2924 cm⁻¹ due to symmetric and asymmetric C-H stretching vibrations of lipid components (Krishnaiah, Satyanarayan and Karthikcyan 2002; Wang, Yang and Heng 2003) and at approximately 1750-1550 cm⁻¹ due to C=O stretching vibrations of intracellular protein (Babita *et al.* 2006). The FTIR studies showed that 10% w/w and 20% w/w ascorbic acid in vehicle decreased the heights of C=O stretching absorbance peaks (Figs. 2B & 2C) for intracellular protein, indicating changes in protein conformation. The polar nature of ascorbic acid could favour its uptake into the corneocytes found on the surface of SC.

Table 1: Effect of skin pre-treatment with ascorbic acid and TEC on the permeation of rofecoxib from 4% w/w sodium alginate-carbopol 940 gel across rat epidermis.

Skin pre-treatment		Cumulative amount permeated at 8 h (Q, $\mu\text{g}/\text{cm}^2$)	Flux (J_{ss} , $\mu\text{g}/\text{cm}^2/\text{h}$)	Lag time (t_L , h)	ER	D/h ² ×10 (h ⁻¹)	Skin retention ($\mu\text{g}/\text{mg}$)	Best fit equation of permeation plot	Coefficient of correlation (R ²)
Control		1948.32 ± 91.45	254.63 ± 10.98	0.40 ± 0.01	1	4.17	0.392	Q = 254.63 t -107.16	0.9934
Ascorbic acid (% w/w)	10	2043.20 ± 89.20	268.9 ± 10.92	0.44 ± 0.12	1.06	3.79	0.405	Q = 268.90 t -120.78	0.9896
	20	2465.95 ± 93.21	365.97 ± 5.38	0.52 ± 0.17	1.24	3.20	0.438	Q = 359.97 t -215.05	0.9858
	30	3142.74 ± 115.20	451.57 ± 6.17	0.60 ± 0.14	1.77	2.78	0.511	Q = 451.57 t -278.36	0.9843
TEC (% v/v)	10	2064.20 ± 89.20	272.45 ± 5.17	0.41 ± 0.08	1.07	4.07	0.402	Q = 272.45 t -124.58	0.9914
	20	2629.29 ± 97.26	362.95 ± 8.99	0.48 ± 0.03	1.43	3.47	0.449	Q = 362.95 t -156.28	0.9935
	30	2541.12 ± 95.26	353.91 ± 8.99	0.45 ± 0.12	1.39	3.70	0.417	Q = 353.95 t -150.44	0.9929

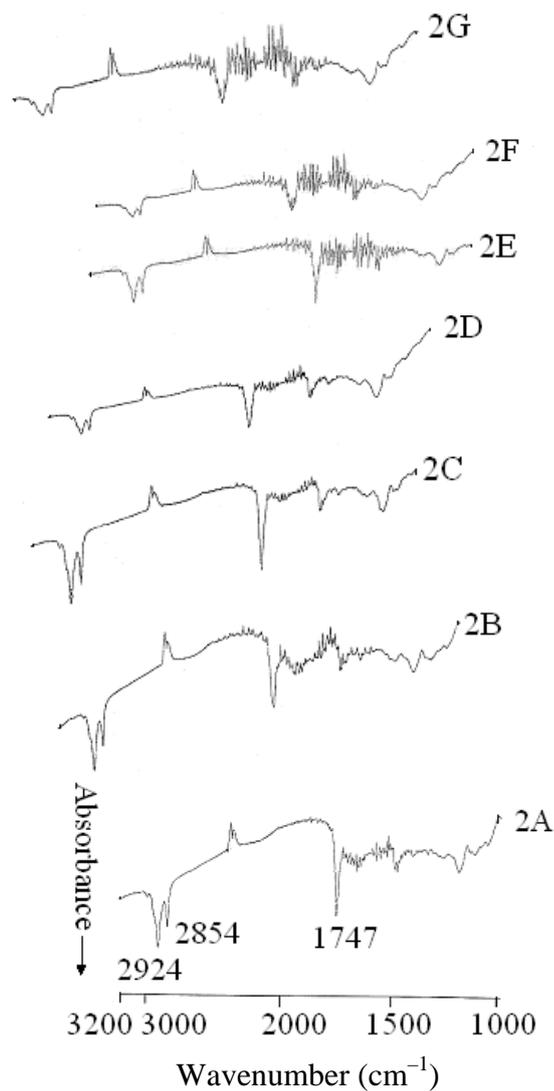


Fig. 2: FTIR spectra of rat epidermis without pre-treatment (2A); rat epidermis pre-treated with 10% w/w ascorbic acid (2B); rat epidermis pre-treated with 20% w/w ascorbic acid (2C); rat epidermis pre-treated with 30% w/w ascorbic acid (2D); rat epidermis pre-treated with 10% v/v TEC (2E); rat epidermis pre-treated with 20% v/v TEC (2F); and rat epidermis pre-treated with 30% v/v TEC (2G).

It may hydrate keratin in the corneocytes and occupy hydrogen-binding sites on the protein chains (Barry 1987), thereby reducing the bond between protein structures in the corneocytes. The dermal

keratins are rich in cysteine residues and strong disulfide cross-linkages may underlie the insoluble nature of this protein. In water, ascorbic acid cleaves the disulfide cross-linkages by reducing each cysteine to two cysteine residues. A decrease in the number of disulfide bridges increases the hydration of keratins (Lehninger, Nelson and Cox 1993; Martin, Bustamante and Chun 1994), which results in increased permeability. Due to the polar nature of ascorbic acid, it can also interact with the polar heads of the intercellular lipids and loosen the lipid packing. A dramatic decrease in peak heights and increase in peak areas for both lipid and protein components was observed in the FTIR spectra of rat epidermis pre-treated with 30% w/w ascorbic acid in vehicle (Fig. 2D). At this concentration, it may enter into the aqueous region of the rat epidermis lipid bilayer. As a result, hydrogen bonding with lipid polar heads could form large hydration shells, expanding the hydrophilic region between the polar heads. This could enhance lipid fluidity and reduce the resistance of the lipid barrier to the diffusion of rofecoxib (Goats and Knutson 1994; Cornwell and Barry 1994).

Skin Pre-treatment with TEC

The permeability of rofecoxib across rat skin pre-treated with TEC increased significantly ($P < 0.05$) in comparison to control. Maximum permeability was observed at a pre-treatment concentration of 20% v/v, beyond which a sudden decrease in permeability was observed. The different permeation parameters are presented in Table 1. A linear Q versus t relationship with high R^2 value at each pre-treatment concentration was observed after an initial lag. Figure 3 depicts the effect of different pre-treatment concentrations of TEC on the flux of rofecoxib across rat epidermis. The skin pre-treated with 10%, 20% and 30% v/v TEC showed 1.07, 1.43 and 1.39 times greater permeability of rofecoxib, respectively as compared to control. However, the apparent diffusion parameter decreased (Table 1) in a concentration-dependent manner. This may be due to the gradual increase in skin retention of rofecoxib with increasing concentrations of pretreatment solvent. This may explain the apparent increase in lag time with an increase in pre-treatment concentrations. Skin pre-treatment at different concentrations of TEC did not show any significant ($P < 0.05$) changes in lag time when compared with control.

The FTIR study elucidated the effect of skin pre-treatment with TEC in terms of the biophysical properties of SC. The physicochemical properties of TEC [water solubility 6.5%, MW 276 Da, BP 294°C, solubility parameter 8.6–9.5 (cal/cm³)^{1/2}] play a crucial role in the biophysical behaviour of SC. FTIR studies have shown that each TEC pre-treatment concentration reduced the heights of both symmetric and asymmetric C-H stretching absorbance peaks (Figs. 2E, 2F & 2G), indicating the fluidity of alkyl chains in intercellular lipids. Its lipophilicity and solubility parameters [$\delta = 8.6-9.5$ (cal/cm³)^{1/2}] are similar to the solubility parameter of the skin [10(cal/cm³)^{1/2}] (Sloan *et al.* 1986). Thus, it is probable that TEC was incorporated easily into the intercellular lipid bilayer during pre-treatment, disrupting its structure. The SC lipids are organised as highly-ordered to less-ordered lamellar sheets (Bouwstra *et al.* 1997), held together by van der Waals, electrostatic, hydrophobic and hydrogen bonding interactions (Moore and Rerek 2000). Ceramide is a major constituent of the SC lipid matrix and plays a crucial role in skin barrier function. The penetration of TEC into the intercellular lipid bilayer region during pre-treatment resulted in the formation of hydrogen bonds with ceramide head groups (between TEC carbonyl or hydroxyl groups and ceramide hydroxyl or carbonyl groups), breaking the interlamellar hydrogen bonding network in the lipid bilayer and thus disrupting barrier properties (Narishetty and Panchagnula 2004). The slight hydrophilic nature of TEC ensures its uptake by keratinised SC cells during pre-treatment. It may solvate keratin due to the formation of hydrogen bonds between TEC hydroxyl groups and keratin chain C=O groups, resulting in disruption of the protein structure. This idea might be confirmed by the FTIR spectra of rat epidermis pre-treated with different concentrations of TEC. The FTIR spectra (Figs. 2E, 2F & 2G) clearly show that there was a significant decrease in heights and increase in areas of C=O stretching absorbance peaks, which indicate protein disruption. The lipid fluidisation and protein disruption caused by TEC significantly reduced the resistance of the SC barrier to the diffusion of rofecoxib. However, the deviation from the expected effect of flux as the concentration of TEC increased from 20% v/v to 30% v/v (Table 1 & Fig. 3) is difficult to explain and may be attributed to the low drug content in the gel formulation used for the diffusion study as compared to the others.

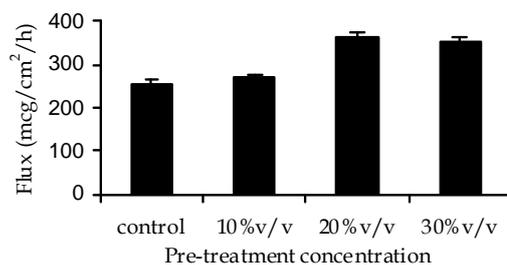
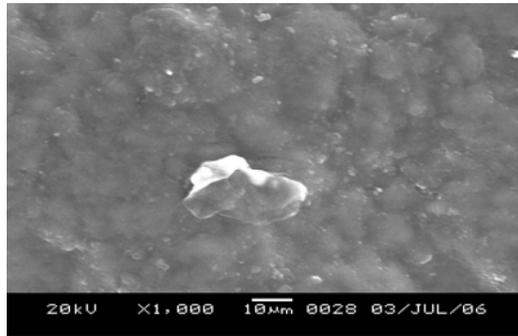


Fig. 3: Effect of skin pre-treatment with TEC at different concentrations on the permeation of rofecoxib across rat epidermis.

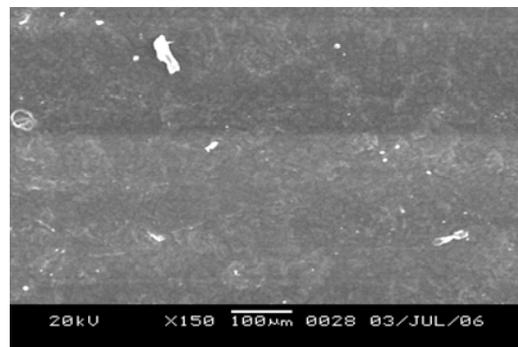
Skin Retention

The amount of rofecoxib retained in the skin at different pre-treatment concentrations of ascorbic acid and TEC was higher than for the control (untreated skin) (Table 1). Increasing pre-treatment concentrations of ascorbic acid and TEC increased the flux values across the skin. This resulted in an increase in the quantity of rofecoxib retained in the skin, indicating that the tissue concentration could be related to the flux value across the skin. This might be due to an increase in tissue binding up to the saturation value.

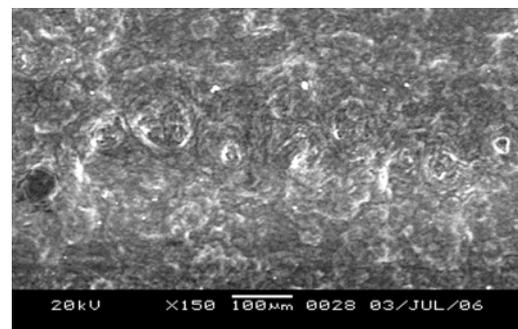
A close observation of the SEM photomicrographs of the skin after the permeation study revealed that there were no histological changes in the skin. This confirms that the integrity of the skin was maintained throughout the permeation experiment. The SEM photomicrographs of fresh skin, after permeation studies across untreated skin, ascorbic acid pre-treated skin and TEC pre-treated skins are presented in Figure 4.



(a)



(b)



(c)

Fig. 4: SEM photomicrographs of (a) rat epidermis before the permeation study; (b) rat epidermis pre-treated with 30% w/w ascorbic acid after the permeation study; and (c) rat epidermis pre-treated with 20% v/v TEC after the permeation study.

CONCLUSION

The permeation of rofecoxib through rat epidermis was enhanced by ascorbic acid and TEC. Skin pre-treatment with ascorbic acid and TEC at different concentrations followed by rofecoxib gel was found to increase rofecoxib retention in the skin. These pre-treatment experiments did not show any significant change in lag time as compared to control. These enhancers act by interacting with the lipid alone or both lipid and protein of the SC in a dose-dependent manner. The present study suggests that a rapid percutaneous absorption at effective therapeutic level is possible when using ascorbic acid and TEC as permeation enhancers for faster anti-inflammatory activity.

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