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Research paper

Combined effect of oleic acid and propylene glycol on the percutaneous penetration of tenoxicam and its retention in the skin

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Abstract

The influence of oleic acid (OA) on the in vitro percutaneous absorption of tenoxicam (TEN) and its combined effect with propylene glycol (PG) was studied using Franz-type diffusion cells. Furthermore, at defined concentrations of OA, complexes of the drug with cyclodextrins (MβCD and γCD) were added because their combined use may be an interesting approach to raise TEN flux. In addition, the amount of TEN retained in the skin after topical administration of several formulations was determined.

It was found that OA content markedly increased TEN absorption when compared to the control gel; the highest drug flux was obtained by 15% of OA. The absorption rate of TEN increased in parallel with increasing OA concentration, due to the alteration of the stratum corneum caused by this enhancer. Moreover, the action of OA is likely to be strongly dependent on the vehicle used since drug penetration tended to increase with increasing PG content in the vehicle, especially at the high OA concentrations. Contrary to our expectations, addition of CD complexes did not produce a significant further enhancement. Skin pretreatment with OA, independently of the vehicle used to dissolve the fatty acid, dramatically improved TEN percutaneous penetration. The amount of TEN retained in the skin was related to the flux values obtained with each formulation.

Keywords: Tenoxicam; Percutaneous penetration; Oleic acid; Propylene glycol; Cyclodextrins; Pretreatment; Skin retention

1. Introduction

Tenoxicam (TEN), a non-steroidal anti-inflammatory drug (NSAID) of the oxicam family with potent anti-inflammatory and analgesic actions, is widely accepted in the treatment of chronic rheumatic disorders. Until now, the percutaneous administration of TEN has been poorly studied although it seems to have many advantages over other administration routes.

Unfortunately, TEN hardly passes through the skin [1]; therefore, the use of oleic acid (OA) as an absorption enhancer may be a useful tool to lessen the barrier function of the skin. In fact, OA was found to be an effective enhancer for many NSAIDs [2–5]. OA can interact with SC lipids and disrupt their structures, increasing their fluidity and consequently increasing the flux [6–9]. It has also been suggested that the major mechanism of enhancement of OA is an increase in permeation through the non-polar route, as it increases both diffussivity and partitioning. However, it also increases the partitioning parameter in the polar route by increasing hydration of the stratum corneum [10].

Propylene glycol (PG) was selected as a cosolvent not only to solubilise TEN in the vehicle, but also because it can alter the skin structure, thereby modifying the percutaneous absorption [11,12]. PG readily permeates the skin and in so doing may carry the drug molecules across [13]. Furthermore, the use of this cosolvent in combination with a potential penetration enhancer may offer synergistic enhancement [14]. In this way, several authors indicated the convenience of using PG in combination with fatty acids for many compounds [15,16]. One possible explanation for this synergistic effect is the facilitated incorporation of OA into the stratum corneum lipid alkyl domain by the interaction of PG at the polar head group region [17].

On the other hand, pretreating the skin with penetration enhancers before the application of drugs is said to be a good method to promote the percutaneous absorption of drugs. Besides, this method of application enables direct assessment of enhancer activity without the results being affected by the formulation [18]. It has been reported that the use of OA as enhancer to pretreat the skin would influence the structure of the stratum corneum, and increase the permeability of some drugs through the skin [19].

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In a previous study, the enhancing efficiency of the cyclodextrins (CDs) was evaluated and it was found that, among the CDs tested, MβCD and γCD were the best agents, even though TEN flux only increased slightly [20]. Besides, it has been reported that the combined use of CDs with lipophilic enhancers could improve the percutaneous absorption of drugs [21]. Hence, our approach was to study if the combination of disparate enhancers, such as OA and the CDs, could act in a positive way to enhance TEN permeation rates.

The purpose of the present paper was to study the influence of OA on the percutaneous penetration of TEN and its retention in the skin, and to clarify if PG acts as a coenhancer. Pretreatment experiments with OA, PG and mixtures of OA in PG and OA in ethanol (EtOH) were also carried out to study thoroughly the skin permeation-enhancing potential of OA. The feasibility of using OA, alone and combined with some CDs, for enhanced absorption of TEN from carbopol gels was also tested.

2. Materials and methods

2.1. Materials

TEN was kindly provided by Products Roche S.A. (Madrid, Spain). Carbopol 940 (carboxypolymethylene), triethanolamine 99% (TEA) and PG USP were supplied by Roig Pharma S.A. (Barcelona, Spain) while OA, of extra pure reagent grade, was purchased from Merck S.A. (Darmstadt, Germany). The CDs (MβCD and γCD) were kindly supplied by Wacker S.A. (Barcelona, Spain). Absolute ethanol (EtOH) and potassium carbonate were purchased from Panreac Química S.A. (Barcelona, Spain) and tetrahydrofuran (THF) from BDH Laboratory Supplies (Poole, England).

2.2. Preparation of TEN gels

The composition of TEN gels used in this study is shown in Table 1. In general, gels were prepared by dispersing 1% of Carbopol 940 in mixtures of water and PG with TEN (1%) and kept under magnetic stirring for 12 h. The dispersion was then neutralised (pH 7.4) and made viscous by adding TEA. OA was added to the mixture just before suspending the carbopol. The resulting gel was stored at room temperature for 24 h prior to its use.

When including TEN–CDs, gels were prepared as described previously and the amount of complex introduced, obtained by the coprecipitation method, was adjusted in order to maintain the drug concentration at 1% in the vehicle. Complex formation assessment and characterisation was studied elsewhere [20]. Hereafter, the gel that contained 1% TEN and 20% PG will be referred to as the control gel.

Table 1
Composition of TEN gels (% w/w)

TEN/TEN–CD[a]	1 g
Carbopol 940	1 g
Oleic acid	0–15 g
Propylene glycol USP	0–40 g
Distilled water	ad 100 g

[a] Amount of complex required in order to have a 1% drug concentration.

2.3. In vitro release studies

TEN release rates from the different gels were measured through 0.2 μm cellulose nitrate membranes (Sartorious AG, Goettingen, Germany) using a Franz-type diffusion cell with a diffusional area of 1.76 cm^2 (FDC-400, Crown Glass Company, Somerville, NJ). 0.5 g of the gel was placed on the membrane surface in the donor compartment while the receptor was filled with 11 ml of phosphate buffer solution (pH 7.4). During the experiments, the receptor solution was stirred at 600 rpm and kept at 37 ± 1°C. At designated time intervals, 300 μl aliquot samples were removed from the receptor phase and replaced with the same volume of buffer solution. TEN was assayed spectrophotometrically at 368 nm and its release rates (k) were calculated using the Higuchi equation [22].

2.4. In vitro permeation studies

All animal procedures were carried out under approved institutional protocols. The abdominal hair of male Wistar rats (230–240 g) was carefully removed using electric razors. After the animals were sacrificed, the abdominal skin was excised and the adhering fat eliminated. The skin was equilibrated in a buffer solution for 1 h prior to the beginning of each experiment. This membrane was mounted on the Franz-type diffusion cell with the dermis facing the receptor compartment, while the donor side was charged with the vehicle (1 g). Samples of receptor fluid (400 μl) were withdrawn every hour, up to 9 h, and replenished with fresh buffer solution.

The experiments were carried out under the same conditions as those used in the release studies and the amount of TEN in the receptor phase was also determined spectrophotometrically at 368 nm. TEN steady-state flux (J) was estimated from the slope of the straight line portion of the cumulative amount of drug absorbed against time profiles, and the determination of lag time (t_L) was made by extrapolating this straight line to the point at which it crosses the x-axis. In order to normalise TEN permeation parameters among different formulations, the enhancing ratio (ER) was defined as the relationship between the flux from a certain gel and that from the control gel.

2.5. Analytical method for the in vitro release and permeation studies

The amount of TEN in the receptor phase was always assayed spectrophotometrically (Diode Array HP 8452 A

spectrophotometer) at 368 nm. Assay performance of the present method was assessed by the following criteria: linearity, accuracy, precision and applicability in percutaneous permeation studies. The assays exhibited linearity between the response (y) and the corresponding concentration of TEN (x), over the 0.30–24 mg l^{-1} range in the samples (typical equation: $y = 0.046x + 0.003$). Least-square linear regression analysis showed that correlation coefficients of all standard curves were >0.999.

2.6. Pretreatment studies

OA was applied to skin samples as solutions at 5 or 10% (w/v) in PG–phosphate buffer (pH 7.4) mixtures (20:80 w/v), at 5, 15 and 50% (w/v) in PG, at 15% (w/v) in EtOH and as undiluted oil.

After equilibrating the skin for 1 h in the diffusion cell at 37°C, enhancer solution (1 ml) was applied to the skin and the donor chamber was covered with parafilm. After 14 h of pretreatment, the skin was carefully washed with distilled water and 1 g of a certain gel was spread evenly on the skin surface. In this case, the ER was the relationship between the flux from a certain enhancer solution and that from the buffer pretreatment.

2.7. Extraction of TEN from skin samples

After the end of the permeation studies and the removal of the formulation, the circular area of diffusion was cut out and weighed. Then, the skin samples were homogenised in an Ultraturrax (Euro Turrax T 20 basic) in buffer solution for 2 min. To 1 ml of skin homogenate, 700 mg of potassium carbonate, 1 ml of THF and 0.5 ml of EtOH were added. The tubes were vortex-mixed for 1 min, then centrifuged for 12 min at 2550 g and 20°C. The supernatant was placed in a second test-tube and evaporated to dryness at 60°C in an evaporator. The residue was then reconstituted in 1 ml of THF, vortex-mixed for 10 s, and filtered with a 0.45 μm filter (Albet-JCR-045-15) prior to TEN determination by spectrophotometry at 382 nm.

The linearity interval established was 6–50 mg l^{-1} ($r > 0.999$). The accuracy for the method was always within 5% for all concentrations. The efficiency and precision of the extraction method of TEN from skin was previously determined, obtaining percentages of recovery higher than 97.53%. For this purpose, blank samples of skin homogenate were spiked with a known amount of TEN and extracted as described above.

3. Results and discussion

3.1. In vitro release studies

Because the absorption process of a drug is conditioned by the nature of the vehicle and other components of the formulation by affecting the release rate or by influencing the partitioning behaviour of the drug between the vehicle and the skin, TEN diffusion through the different gels was examined using cellulose nitrate membranes.

From the release profiles, the apparent release rates, k, were calculated and listed in Table 2. When the amount of drug released was plotted against the square root of time, a linear relationship was obtained for each vehicle ($r > 0.997$), showing that the release of TEN from the gels was well described by the Higuchi model, where the rate-controlling step is the diffusion process through the gel matrix.

At low concentrations of OA and without PG, TEN diffusion increased slightly compared with that of the control gel,

Table 2
TEN release rates (k)[a] and skin permeation parameters (J, t_L and ER)[a]

Gel	$k \times 10^2$ (mg cm^{-2} min$^{-1/2}$)	J (μg cm^{-2} h^{-1})	t_L (h)	ER
Control (0% OA, 20% PG)	10.44 ± 0.48	1.74 ± 0.17	1.50 ± 0.16	1
3% OA, 0% PG	11.46 ± 0.50	4.37 ± 0.75	2.14 ± 0.29	2.5
3% OA, 20% PG	11.04 ± 0.23	4.62 ± 0.83	1.53 ± 0.52	2.6
3% OA, 40% PG	7.61 ± 0.16	6.52 ± 0.51	2.56 ± 0.11	3.7
5% OA, 0% PG	12.03 ± 0.28	4.70 ± 0.90	1.49 ± 0.31	2.7
5% OA, 20% PG	10.83 ± 0.10	7.32 ± 1.07	2.61 ± 0.35	4.2
5% OA, 40% PG	7.76 ± 0.23	6.57 ± 1.20	2.13 ± 0.40	3.8
10% OA, 0% PG	9.13 ± 0.20	11.42 ± 1.01	3.52 ± 0.18	6.6
10% OA, 20% PG	8.74 ± 0.14	14.07 ± 2.60	1.88 ± 0.61	8.1
10% OA, 40% PG	7.13 ± 0.23	16.69 ± 0.96	2.78 ± 0.14	9.6
15% OA, 0% PG	8.07 ± 0.28	10.49 ± 0.88	3.51 ± 0.40	6.0
15% OA, 20% PG	8.96 ± 0.05	35.39 ± 2.69	2.47 ± 0.18	20.3
15% OA, 40% PG	7.08 ± 0.14	33.75 ± 1.36	3.12 ± 0.30	19.4
MβCD, 0% OA, 20% PG	9.94 ± 0.29	2.67 ± 0.42	1.34 ± 0.21	1.5
MβCD, 5% OA, 20% PG	7.95 ± 0.21	6.79 ± 1.43	4.58 ± 0.45	3.9
MβCD, 10% OA, 20% PG	6.97 ± 0.28	13.98 ± 2.43	3.32 ± 0.29	8.0
γCD, 0% OA, 20% PG	7.53 ± 0.37	2.15 ± 0.35	1.40 ± 0.22	1.2
γCD, 5% OA, 20% PG	7.97 ± 0.49	4.07 ± 0.83	4.32 ± 1.67	2.3

[a] Values are the mean ± SE of four to six determinations at 37°C.

although there were no significant differences. Thus, the higher release rates were achieved with gels without PG and containing 3 or 5% OA. In general, as PG content in the vehicle increased, TEN diffusion decreased, which could be due to the reduction of the TEN partition coefficient between the vehicle and the skin, and to the lower thermodynamic activity. With 40% PG the release of TEN decreased to the same extent, regardless of the concentration of OA used. Besides, when the amount of fatty acid present was increased, especially at the highest concentration of OA, TEN release rates were diminished, even at low concentrations of PG. Similar results, that is, lower release rates with high concentrations of fatty acids have been reported for piroxicam [23] and were attributed to an increase in lipophilicity of the vehicle.

On the other hand, in the presence of OA, both complexes with the CDs gave almost the same release profiles, suggesting that TEN release was not affected by CD nature. Drug release rates were lower than for the control gel, which could be explained because when the CDs were included, the amount of free drug decreased as a consequence of complex formation and also because the gel viscosity increased. Besides, if the amount of OA was increased up to 10% in gels formulated with TEN–MβCD complexes, TEN release profiles also decreased.

3.2. In vitro permeation studies

TEN steady-state flux, J, and lag time, t_L, for each of the different formulations under study are also summarised in Table 2. The effect of increasing both OA and PG content in carbopol gels on the penetration of TEN is illustrated in Fig. 1. The experiments showed an increase of drug permeation as a function of OA content in the vehicle. Addition of OA to the control gel sharply increased TEN absorption; drug flux values increased almost proportionally with the enhancer concentration.

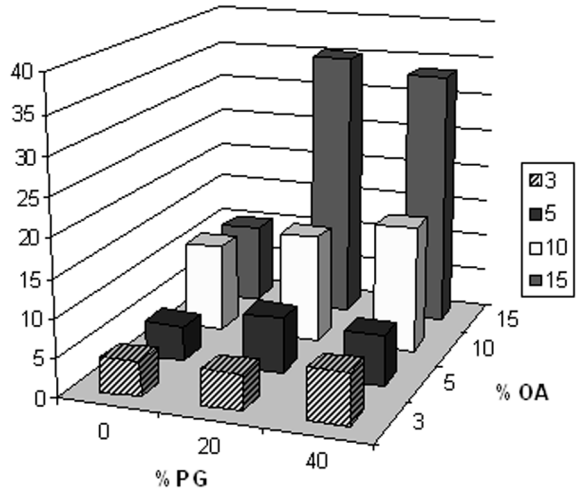

Fig. 1. Effect of different concentrations of OA and PG on TEN flux. Each column represents the mean ± SE of five to eight determinations.

It has been reported that the enhancing effect of OA was dependent on its concentration in the vehicle. Thus, percutaneous absorption of drugs increased with the amount of enhancer until a maximum, after which the penetration decreased [24,25]. However, within the range of concentrations used in the present study, OA always enhanced TEN penetration since a maximum was not reached.

In general, the presence of OA increased the lag time taken to reach the steady-state level (t_L) when compared to the control gel, suggesting that this enhancer requires time to diffuse from the vehicle, reach the skin surface, permeate through the skin and modify its properties. After this period of time, the steady-state level was achieved for the whole experiment length.

Moreover, it was observed that the enhancing effect of OA was strongly affected not only by its concentration, but also by the amount of PG formulated in the gels, as shown in Fig. 1. In most of the experiments, increasing PG content in the vehicle seemed to increase the permeation rate of TEN, although significant differences were only found with 10 and 15% OA. The greatest flux was achieved with 20% PG and 15% OA.

In general, flux values gradually increased with increasing concentration of both OA and PG, suggesting that the effect of OA and PG is additive. Several researchers have also observed a mutual permeation-enhancing effect with OA and PG and considered its combined use a potent approach to enhance the transdermal absorption of many drugs [26,27]. As a matter of fact, systems consisting of PG and fatty acids were more effective enhancers as compared to either PG or fatty acid alone [15,19,28]. Such an enhancement was considered to be the result of two different mechanisms in which PG enhanced intracellular drug mobility by solvating alphakeratin in corneocytes, allowing OA to act on the lipid barrier [29,30].

Taking into account the good results obtained with some enhancers and the CDs [21,31], our next approach was to study the possible promoting effect of the CDs in combination with OA. Earlier studies showed that, among the complexes with the CDs tested, MβCD and γCD were the best absorption enhancers for TEN. The flux data for these CDs were shown in a previous work [20] but they are included here for the sake of comparison.

As shown in Table 2, the combination of OA and the CD complexes improved TEN percutaneous absorption as compared to the control gel, but not as expected. The steady-state flux values obtained were similar to those for gels formed with OA and without the CDs; no significant differences were found between gels containing TEN as free form or as inclusion complexes with the CDs, suggesting that the promoting effect of OA could mask that of the CDs. However, as shown in Fig. 2, the permeation profiles were completely different since the lag times were increased when incorporating TEN–CD complexes to OA formulations. This could be due to the fact that the CDs could decrease the amount of free fatty acid present in the gels,

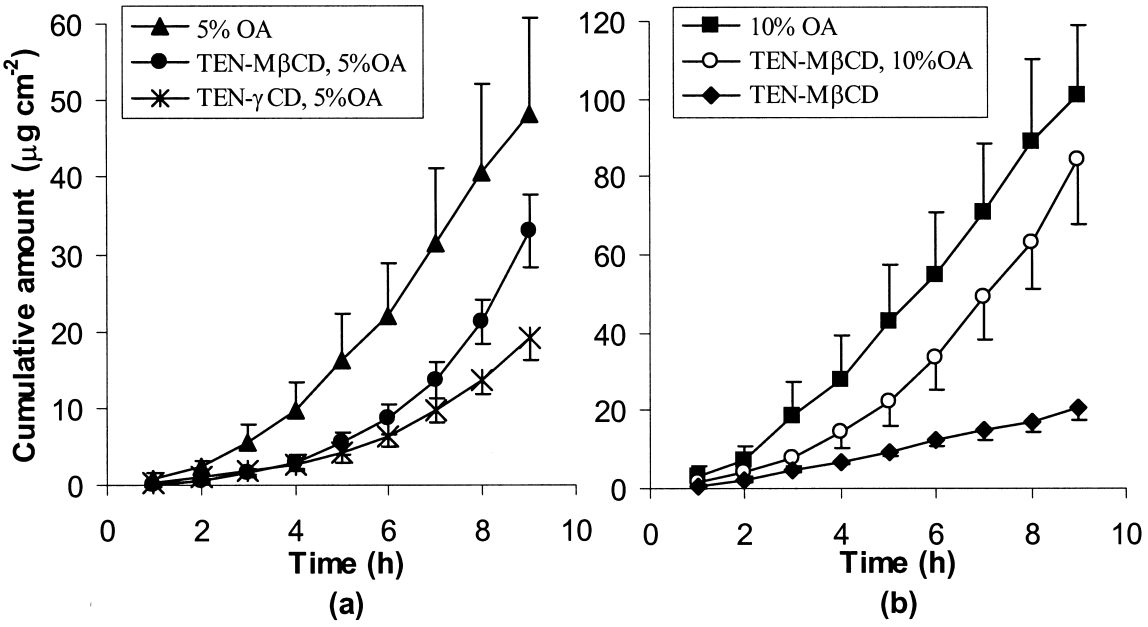

Fig. 2. Permeation profiles of TEN from carbopol gels: (a) 5% OA and TEN–CD complexes; (b) 10% OA and TEN–MβCD complexes. Each point represents the mean ± SE of six experiments.

as a result of OA–CD complex formation, and, thus, increase the time required for OA to exert its enhancing effect in the skin.

3.3. Pretreatment experiments

The effectiveness of a permeation enhancer is often measured after pretreating the skin with the enhancer before applying the formulation. Therefore, in order to study the enhancing effect of OA, pretreatment experiments with solutions of OA in different vehicles were carried out (Table 3).

Application of buffer solutions plus control gel to the skin provided a twofold increase in TEN percutaneous absorption as compared to the non-pretreatment values, which is likely to be due to the hydration of the skin. When compared to buffer pretreatment, mixtures of PG–buffer (20:80 w/w) slightly increased the absorption, but PG and EtOH almost doubled TEN flux values, suggesting that both substances act as penetration enhancers for TEN.

In general, OA sharply increased TEN percutaneous absorption independently of the vehicle in which it had been dissolved. The enhancement ratios achieved with OA pretreatment in several solvents and as an undiluted oil were between 14 and 23. Pretreatment experiments with solutions of OA (5 or 10%) in mixtures of PG–buffer (20:80 w/w),

Table 3
Effect of pretreatment solutions on TEN permeation parameters (J, t_L and ER)[a]

Pretreatment solution +	Gel	J (μg cm^{-2} h^{-1})	t_L (h)	ER
Buffer	Control	3.39 ± 0.49	1.45 ± 0.20	1
PG–buffer (20:80)	Control	4.35 ± 1.83	1.20 ± 0.45	1.3
5% OA (PG–buffer)	Control	51.76 ± 1.16	1.67 ± 0.24	15.3
10% OA (PG–buffer)	Control	49.29 ± 3.78	1.89 ± 0.44	14.5
PG	Control	5.93 ± 1.22	1.24 ± 0.37	1.7
5% OA (PG)	Control	62.53 ± 5.31	1.04 ± 0.29	18.4
15% OA (PG)	Control	67.85 ± 1.34	0.47 ± 0.06	20.5
50% OA (PG)	Control	70.67 ± 1.06	0.90 ± 0.06	20.8
EtOH	Control	7.96 ± 2.74	1.22 ± 0.61	2.3
15% OA (EtOH)	Control	45.02 ± 1.53	1.65 ± 0.31	13.3
100% OA	Control	53.70 ± 4.32	2.45 ± 0.30	15.8
5% OA (PG)	15% OA, 20% PG	70.52 ± 4.66	0.78 ± 0.11	20.8
15% OA (PG)	5% OA, 20% PG	73.09 ± 3.49	0.21 ± 0.09	21.6
15% OA (PG)	15% OA, 20% PG	76.72 ± 6.05	0.23 ± 0.04	22.6
15% OA (EtOH)	15% OA, 20% PG	45.32 ± 4.10	0.72 ± 0.20	13.4

[a] Values are the mean ± SE of four to six determinations at 37°C.

trying to mimic the composition of the different gels under study, were also carried out and the permeation parameters obtained were similar to those for pure OA.

However, the combination of OA with PG gave the best absorption profiles and a significant reduction in lag time was observed, too. These results may suggest that OA and PG act in combination to help either the partitioning of the drug into the skin or its penetration through the skin. Likewise, pretreatment of the skin with OA dissolved in PG improved the percutaneous absorption of piroxicam [32] and naproxen [18] and lag times were diminished too.

In contrast, solutions of OA in EtOH provided a smaller enhancing effect than in the previous solvent and the decrease in the lag times was not as strong as it was for PG. Therefore, it can be said that TEN penetration after OA pretreatment depends on the cosolvent used and its concentration, which could be ascribed to the reduction in the partition and distribution into the lipophilic strata of the skin and to the different solubility of the fatty acid in these vehicles. Besides, EtOH may not facilitate so easily the drug cotransport through the skin.

On the other hand, subsequent application of gels with OA slightly increased TEN flux in comparison with control gel after the same solution pretreatment, although the differences were not statistically significant. However, lag times were shortened even further.

All these findings suggest that OA pretreatment, regardless of the vehicle used to dissolve this fatty acid, could affect the dense barrier structure of the skin. The lack of significant differences between the flux at the different concentrations of OA suggested that the percutaneous penetration did not depend on the amount of OA used. Besides, taking into account that OA causes skin irritation, it can be said that 5% OA in PG was the most appropriate pretreatment solution to increase TEN percutaneous penetration since the application of 5% OA in PG to the skin resulted in minor irritation [33] and the irritant effect increased as a function of OA concentration [34].

Table 4
Amount of tenoxicam retained in the skin after different experiments

Pretreatment solution	Gel	Concentration found (μg/mg of skin) $\times 10^2$
(–)	Control	7.9 ± 0.3
(–)	3% OA, 20% PG	17.5 ± 0.5
(–)	5% OA, 20% PG	20.0 ± 1.0
(–)	10% OA, 20% PG	22.7 ± 1.4
(–)	15% OA, 20% PG	34.1 ± 3.3
Buffer	Control	19.4 ± 0.8
PG	Control	22.2 ± 2.2
Oleic acid	Control	42.2 ± 2.1
5% AO (PG)	Control	111.2 ± 3.7
15% OA (PG)	Control	107.5 ± 3.6
15% OA (PG)	15% OA, 20% PG	83.6 ± 2.5
50% AO (PG)	Control	172.0 ± 6.4

3.4. TEN retention in the skin

Some formulations and pretreatment solutions were chosen to study the amount of TEN retained in the skin after their topical application. The values obtained with their standard error are shown in Table 4 and are the mean ± SE of more than eight experiments.

The low penetration of TEN after administration of the control gel and its optimum release would suggest that the drug either has difficulty in penetrating across the skin or interacts with potential binding sites in the epidermis and possibly forms a reservoir. TEN retention in the skin was not as high as would be expected if the reservoir were formed.

Since the amount of TEN present in the tissue increased as TEN percutaneous absorption increased, it can be said that the accumulation of the drug in the skin was related to the flux values obtained in each case. Thus, the higher the flux values attained, the more the retention of TEN in the skin, as shown in Table 4. Our findings are in good agreement with those reported by Singh and Roberts [35], in which the skin concentration of several NSAIDS was correlated to their flux across the epidermis.

4. Conclusions

In conclusion, it can be said that OA is a suitable enhancer in TEN formulations, resulting in an effective delivery of the drug to the skin surface and an improved percutaneous penetration. The combination of OA with PG led to a greater absorption of the drug. An additive effect of OA and PG on TEN percutaneous absorption was observed since TEN flux values gradually increased with increasing concentrations of both compounds. Besides, this combination in the pretreatment solutions provided the highest fluxes and the shortest lag times. The amount of TEN retained in the skin was found to be related to the flux values obtained in each permeation study.

Therefore, carbopol gels containing OA and PG would be useful in the topical administration of TEN. Incorporation of complexes of the drug with CDs could be interesting when a prolonged absorption in time is desired, as they act as a depot.

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