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RESEARCH PAPER

Interaction of Tenoxicam with Cyclodextrins and Its Influence on the In Vitro Percutaneous Penetration of the Drug

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ABSTRACT

Solid complexes of tenoxicam (TEN) with cyclodextrins (CDs), in a 1:1 molar ratio, were obtained by the coprecipitation method and characterized by x-ray diffractometry, infrared spectroscopy, and differential scanning calorimetry. The binding capacity of the CDs with TEN was also demonstrated in aqueous solution and in water-propylene glycol mixtures. The purpose of this study was to determine the effect of CDs on the in vitro percutaneous penetration of TEN from carbopol gels, taking into account the role of the CD cavity size and the nature of the substituents. The effect of pretreatment was studied too. In vitro permeation experiments were carried out on Franz diffusion cells using cellulose nitrate membranes and abdominal rat skin. In these results, the release rates of the drug scarcely decreased when the CDs were added, probably because of a lower concentration of the free drug and an increased gel viscosity. However, it was also found that CDs, particularly γ-CD and M-β-CD, can improve slightly TEN absorption through the skin. Pretreatment studies with CDs, however, provided no effects on TEN permeation, but lag time was markedly reduced, suggesting a faster partitioning of TEN into the skin. Therefore, the use of pretreatment with CDs would be interesting when a quick action of the drug is desired.

KEY WORDS: Percutaneous penetration; Tenoxicam; Cyclodextrins; Inclusion complexes; Carbopol gels.

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INTRODUCTION

Tenoxicam (TEN), a nonsteroidal 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. Although a practically complete absorption is achieved orally (1), like other oral NSAIDs, TEN also causes some form of gastrointestinal ulceration and bleeding (2,3). Therefore, a possible percutaneous administration would be interesting. However, the stratum corneum (SC) is the rate-limiting barrier to percutaneous delivery for most drugs, and investigators have attempted to increase its permeability.

The cyclodextrins (CDs), which are a group of structurally related cyclic nonreducing oligosaccharides, are typical host molecules, forming inclusion complexes in both solid and solution states. In the last few years, CD derivatives such as hydroxypropyl-CDs (HP-CDs) and methyl-CDs (M-CDs) have been developed to overcome the limitations of solubility and to improve the safety and subsequent pharmaceutical usefulness of the parent CDs. These substituents, which are more hydrophobic than the hydroxyl groups, can modify the drug-CD interaction and binding ability.

Recently, CDs have attracted interest for topical use as penetration enhancers (4–12). They can act on drug absorption by means of two mechanisms, indirectly, by influencing physicochemical properties of the drugs, and/or directly, by influencing the biomembrane permeability (13).

Nevertheless, CDs have been poorly studied on transdermal absorption, and their mechanisms of action still have not been clarified. It is obvious that the solubilizing properties of the CDs can improve drug release, absorption, and bioavailability, but their enhancing effects on the percutaneous absorption of drugs by altering the skin permeability is a controversial issue. On the one hand, CDs may affect the permeation of drugs via the interaction with some SC components (10), by inclusion of phospholipids and cholesterol (14), by extraction of proteins (15), by removal and disorganization of the lipids matrix resulting from complexation (9,16,17), and by interactions with keratin with an increased order in the lipid lamella (12). On the other hand, intuitively, it would appear that such large, relatively hydrophilic molecules as the CDs would not readily permeate into the skin, and in fact it has been reported that CDs permeate lipophilic biological membranes with considerable difficulty and at a very slow rate (18,19). In this regard, Williams et al. also suggested that CDs do penetrate into the SC lipid domains to some extent (12), and the distribution of HP-β-CD throughout the skin layers was reported by Vollmer et al. (20).

The purpose of this study was to analyze several aspects of the role of the CDs (β-CD, HP-β-CD, M-β-CD, γ-CD, and HP-γ-CD) in the percutaneous absorption of TEN from hydrophilic gels of carbopol.

MATERIALS AND METHODS

Materials

TEN was kindly provided by Products Roche S.A. (Madrid, Spain). Carbopol 940 (carboxypolymethylene), triethanolamine 99% (TEA), and propylene glycol USP (PG) were purchased from Roig Pharma S.A. (Barcelona, Spain). γ-CD, HP-γ-CD (D.S. 0.6), β-CD, and M-β-CD (D.S. 1.8) were generously supplied by Wacker S.A. Barcelona, Spain and HP-β-CD (D.S. 0.9) from Cerestar S.A. (Indiana, USA.)

Preparation of TEN Gels

Gels were prepared by dispersing 1% of Carbopol 940 in a mixture of water and PG (80:20, w/w) with TEN (1%) and kept under magnetic stirring for 12 hr. The dispersion was then neutralized (pH 7.4) and made viscous by adding TEA. The resulting gel was stored at room temperature for 24 hr before use.

When including the CDs, gels were prepared as described previously, and the amount of complex introduced was adjusted to maintain the drug concentration at 1% in the vehicle.

Preparation and Characterization of the Complexes

The inclusion complexes of TEN with the different CDs, in a 1:1 molar ratio, were prepared by the coprecipitation method. The procedure consisted in stirring an aqueous solution (pH 12) of CD at 60°C with an appropriate amount of drug. Immediately after the complete dissolution of TEN, the solvent was removed under reduced pressure at 70°C. Then, the coprecipitate was dried at 60°C in an oven for 24 hr. Physical mixtures were also prepared by mixing the corresponding substances in a mortar. Inclusion complex formation in solid state was assessed by x-ray diffractometry, infrared spectroscopy (IR), and differential scanning calorimetry (DSC). X-ray powder diffractograms were obtained using a Siemens Kristalloflex 810 x-ray diffractometer, CuKα radiation, Ni-Filter (40 kV, 20 mA). Studies of the IR spectra of TEN and its complexes were conducted with a Perkin-Elmer 1600 FTIR infrared spectrophotometer with KBr disks. Thermal analyses were carried out using a Setaram

DSC 92 differential scanning calorimeter. Each sample (inclusion complexes, physical mixtures in the same molar ratio, and pure components) was scanned at a speed of 5°C/min, in a temperature range between 25 and 250°C using nitrogen as purging gas. The interaction between TEN and the CDs in aqueous solution and in water-PG mixtures (80:20, w/w) was studied spectrophotometrically using a Diode Array Hewlett Packard 8452 A spectrophotometer.

In Vitro Release Studies

TEN release rates from the different gels were measured through 0.2 μm of 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). The gel (0.5 g) was placed on the membrane surface in the donor compartment while the receptor one 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, and its release rates (k) were calculated using the Higuchi equation (21).

In Vitro Permeation Studies

The abdominal hair of male Wistar rats (200 to 250 g) was removed carefully using electric razors. After the animals were sacrified, the abdominal skin was excised and the adhering fat eliminated. The whole skin was equilibrated in a buffer solution for 1 hr before 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 hr, and replenished with fresh buffer solution.

The experiments were carried out under the same conditions as those used in the release studies. 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 lag time (t_L) from the x-intercept.

For the pretreatment studies, the skin mounted on the diffusion cell was pretreated with an aqueous solution of CD (1, 5, or 20%, w/v) for 12 hr before topical application of TEN control gel.

Analytical Method

The amount of TEN in the receptor phase was assayed spectrophotometrically (Diode Array Hewlett Packard 8452 A spectrophotometer) at 368 nm. The linearity interval established was 0.3 to 24 $\mu g/mL^{-1}$ ($r > 0.999$).

RESULTS AND DISCUSSION

Characterization of the Complexes

Based on the methods used, it was verified that TEN can form inclusion complexes in solid phase with all the CD tested. Remarkable differences between the coprecipitated products and the physical mixtures were found by powder x-ray diffractometry (Fig. 1). The diffraction pattern of the physical mixtures was simply the sum of those of the components, whereas the reduction in crystallinity is complete in the complexes with β-CDs and other CD derivatives, which are entirely amorphous, indicating the inclusion complex formation with these CDs. Only the coprecipitated products with γ-CD were apparently different, showing other diffraction peaks attributable to a new solid phase with low crystallinity.

In the same manner, the IR spectra of TEN-CD complexes show considerable differences when compared with those of the physical mixtures. Thus, there is a broadening in the IR signals corresponding to the carbonyl group (1637 cm^{-1}). The OH and NH stretching modes of the secondary amide (2700 to 3000 cm^{-1}) exhibit a broadening and increase of intensity when the inclusion complex is formed, as well as in the 1043 cm^{-1} band, which is related to the OH deformation. The CNH deformation (1557 cm^{-1}) is practically undetectable in the spectra of the complexes, but it is important to note that over 1520 cm^{-1}, there is a band that only appears when the TEN-CD complexes are formed. All these modifications can be observed with all the different CDs studied and clearly indicate the presence of host-guest interactions (Fig. 2). The formation of stable hydrogen bonds between TEN and the CDs is a possible reason for these spectral changes shown.

In addition, several differences were also found between the DSC curves of the complexes with each CD and their corresponding physical mixtures (Fig. 3). The thermal behavior of TEN is illustrated in Figure 3(A). An endothermic peak at approximately 217°C was observed, followed by a strong exothermic, which corresponds to the drug melting and decomposition peaks, respectively. DSC profiles of the different CDs show broad endothermic peaks representing a loss of water molecules, as

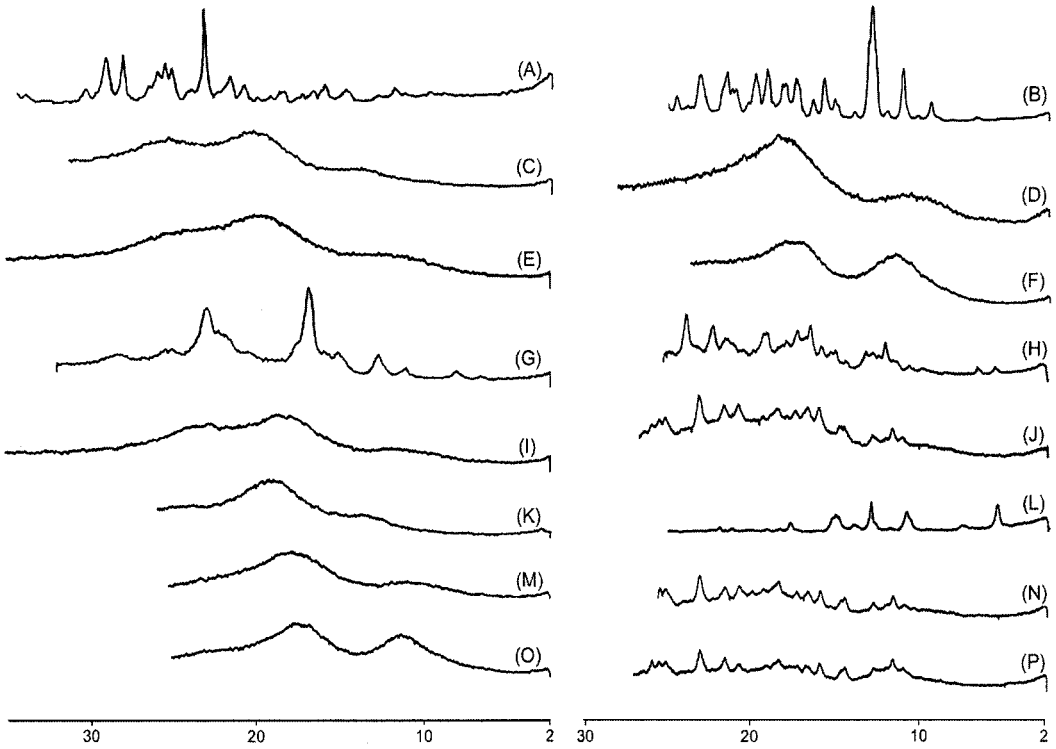

Figure 1. Powder x-ray diffraction patterns of TEN-CD systems: (A) TEN; (B) β-CD; (C) γ-CD; (D) HP-β-CD; (E) HP-γ-CD; (F) M-β-CD; (G) γ-CD complex; (H) γ-CD physical mixture; (I) HP-γ-CD complex; (J) HP-γ-CD physical mixture; (K) β-CD complex; (L) β-CD physical mixture; (M) HP-β-CD complex; (N) HP-β-CD physical mixture; (O) M-β-CD complex; (P) M-β-CD physical mixture.

described in the bibliography. The main difference observed between solid complexes and physical mixtures remains in the exothermic peak attributable to TEN decomposition. This peak can be detected clearly in the case of the physical mixtures, whereas it is not present when inclusion complexes are formed. Therefore, it is likely that complex formation can have stabilizing effects on TEN, protecting it from further decompositions over time.

UV spectrophotometric studies support the interaction between drug and CDs in aqueous solution and in water-PG mixtures (pH 7.4) at a defined concentration of TEN ($3.55 \cdot 10^{-5}$ M). Under the concentration ranges of M-β-CD studied ($2 \cdot 10^{-3}$ to 10^{-2} M), the absorbance at 204, 258, and 286 nm increased as a function of host concentration, whereas at 368 nm it remained unchanged (Table 1). The complex was formed even in the presence of 20% w/w

Table 1.

Changes of TEN Absorbance at Different Wavelengths as a Function of M-β-CD Concentration

	Water λ (nm)			Water-PG (80:20 w/w) λ (nm)		
[CD] $\cdot 10^3$ M	286	258	204	286	258	204
0	0.3877	0.4390	0.4419	0.4109	0.4791	0.4460
2	0.3965	0.4591	0.5042	0.4198	0.4930	0.4594
4	0.4044	0.4665	0.5596	0.4299	0.5112	0.4912
6	0.4126	0.4880	0.5966	0.4327	0.5238	0.5262
8	0.4252	0.5075	0.6412	0.4423	0.5406	0.5581
10	0.4374	0.5241	0.6939	0.4488	0.5512	0.5828

PG, although the cosolvent system made the interaction with M-β-CD weaker because of the competitive inclusion. In a similar manner, when studying the other CDs, such interactions were also observed.

In Vitro Release Studies

With regard to drug permeation through the skin from vehicles, a drug should first diffuse out from the vehicle to the skin. To evaluate changes in TEN diffusion through carbopol gels, the in vitro release of the drug from these vehicles was studied as a function of CD nature. Cellulose nitrate membranes were used because they do not offer resistance to drug permeation.

The release profiles from gels containing TEN or the inclusion complexes are shown in Figure 4, in which some SD bars are omitted to avoid overlapping. When the amounts of TEN released were plotted against the square root of time, a linear relationship was obtained for each vehicle ($r > 0.992$), showing that the permeation of TEN across the synthetic membrane is well described by the Higuchi model, in which the rate-controlling step is the diffusion process through the gel matrix. The apparent release rates, k, were calculated and are listed in Table 2.

Only small modifications were detected in the release rates of TEN when the CDs were incorporated in the vehicle. The greatest release rate was achieved with the control gel, which contained all the drug in its free form, whereas TEN release was decreased by complexation with CDs. These results can be explained because when the CDs are included, the amount of the free drug decreases and because the gel viscosity increases. Among the CDs, γ-CD and HP-β-CD showed the least release, which may be attributable to three different factors: the stability constants of the complexes, drug solubility, and gel viscosity.

In a similar manner, Orienti et al. reported that β-CD and HP-β-CD changed the diffusion profiles of ketoprofen from hydrophilic gels of carbopol (22). The

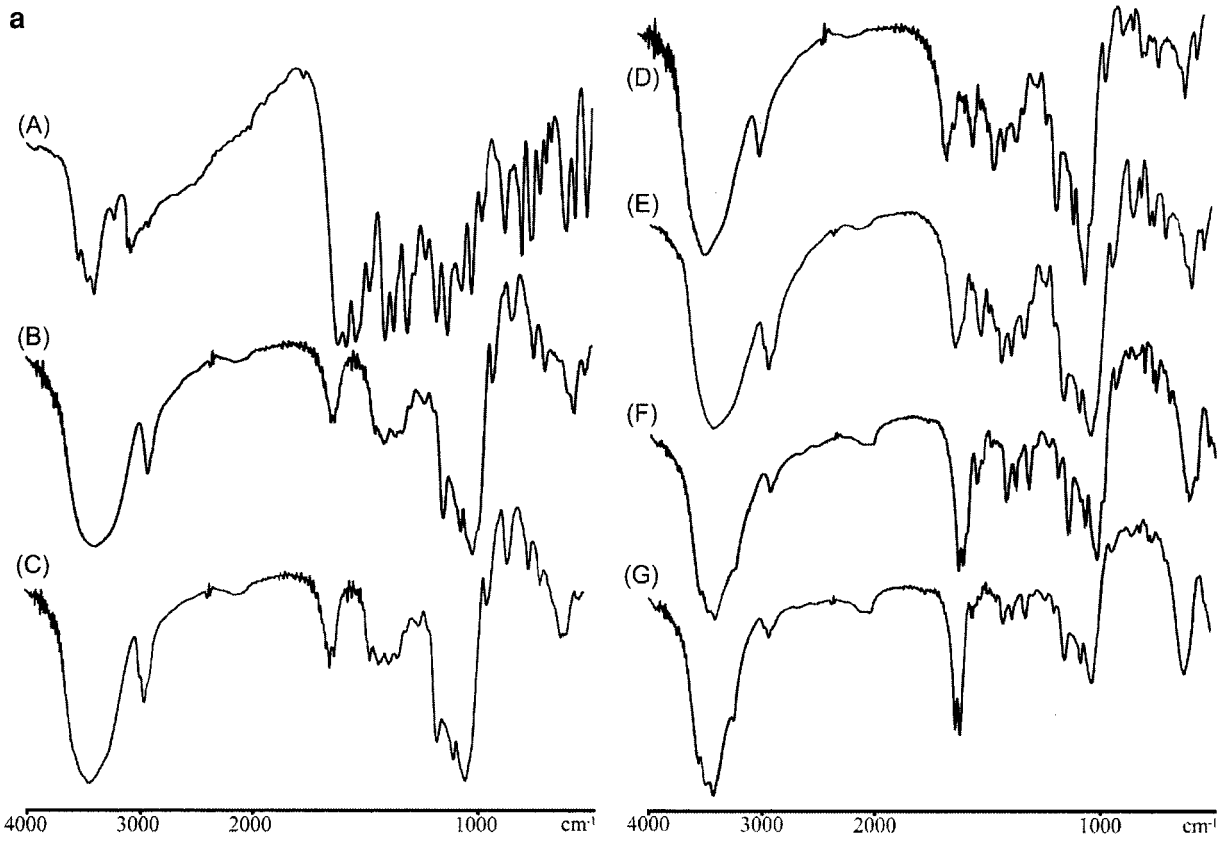

Figure 2. IR spectra of TEN-CD systems: (a) TEN-γ-CD systems: (A) TEN; (B) γ-CD; (C) HP-γ-CD; (D) γ-CD complex; (E) HP-γ-CD complex; (F) γ-CD physical mixture; (G) HP-γ-CD physical mixture. (b) TEN-β-CD systems: (A) TEN; (B) HP-β-CD; (C) β-CD; (D) M-β-CD; (E) β-CD complex; (F) β-CD physical mixture; (G) HP-β-CD complex; (H) HP-β-CD physical mixture; (I) M-β-CD complex; (J) M-β-CD physical mixture.

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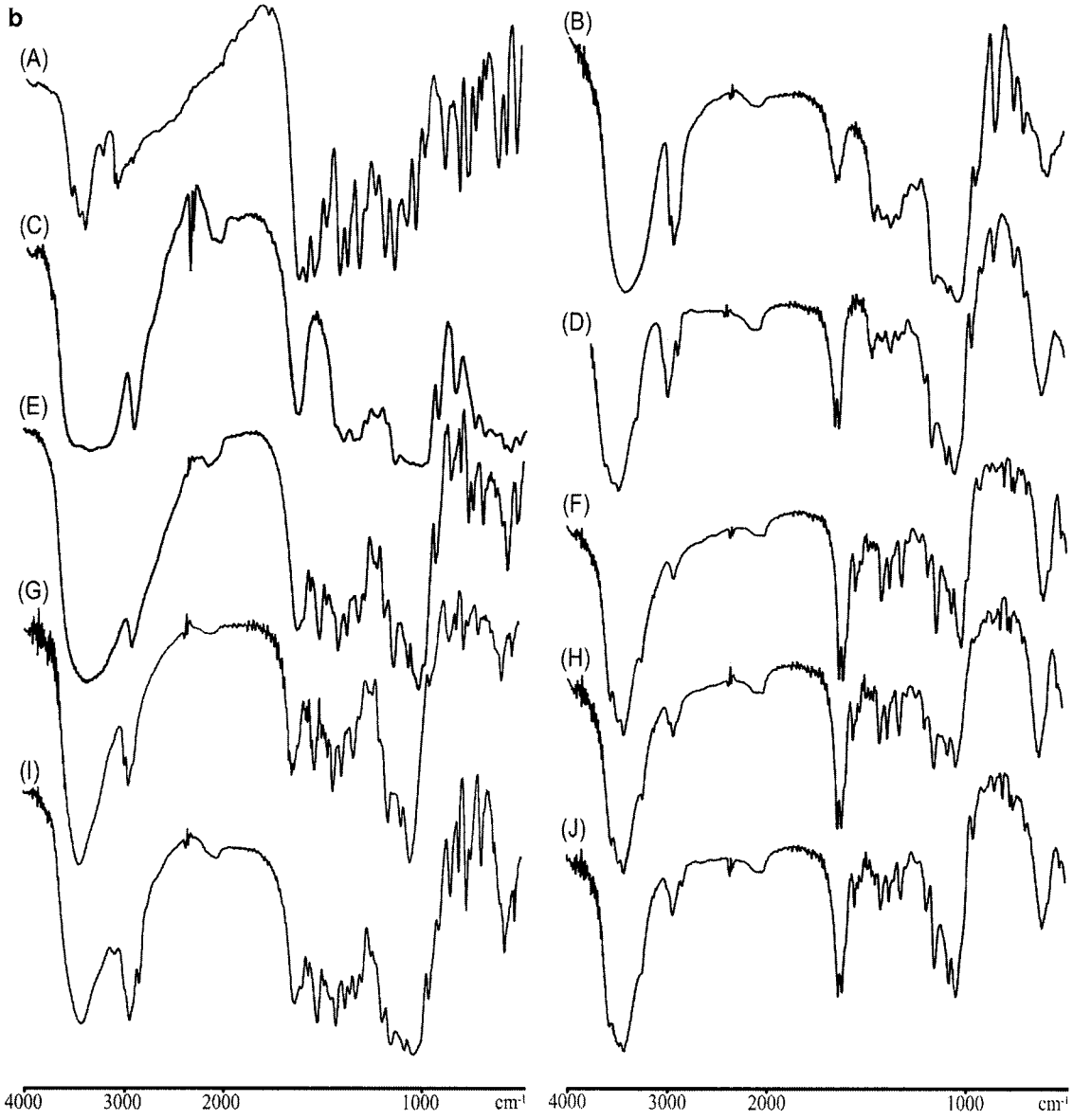

Figure 2. *Continued.*

presence of CDs lowered not only the free drug concentration, but also the diffusivity during the first hours, and then they act as a reservoir. Samy and Safwat attributed the decrease in flurbiprofen release with β-CD to the reduction in the thermodynamic activity and the increased microviscosity of the gel/water phase (23). In contrast, complexation of indomethacin with DE-β-CD resulted in an increased release rate from carbopol gels (24), and the release of diclofenac sodium was improved by β-CD complexation because of the lower affinity of the complex in the gel bases (23). The enhanced release rate of piroxicam by β-CD from hydrophilic gels resulted from an increase in the hydrophilia of the vehicle. However, TEN is more hydrophilic than is piroxicam as their partitioning coefficients, 0.4 and 5.3, respectively, make evident (25).

In Vitro Permeation Studies

Figure 5 shows the permeation profiles of TEN and its complexes, in a molar ratio of 1:1, across rat skin. The

Table 2.

TEN Release Rate Across Cellulose Membranes and Skin Permeation Parameters[a]

Gel	$k \cdot 10^{2}$ [b] (mg cm^{-2} min$^{-1/2}$)	J[c] (μg cm^{-2} hr^{-1})	t_L[c] (hr)
Control	10.59 ± 0.58	1.74 ± 0.17	1.50 ± 0.16
β-CD complex	9.34 ± 0.19	1.96 ± 0.57	0.97 ± 0.38
HP-β-CD complex	7.89 ± 0.20	1.90 ± 0.41	1.47 ± 0.14
M-β-CD complex	9.94 ± 0.29	2.67 ± 0.42	1.34 ± 0.21
γ-CD complex	7.53 ± 0.37	2.15 ± 0.35	1.40 ± 0.22
HP-γ-CD complex	9.14 ± 0.31	1.83 ± 0.27	1.08 ± 0.21

[a] Values are the mean $\pm$ SE of 5 to 8 determinations at 37°C.
[b] TEN release rate values across cellulose nitrate membranes.
[c] Skin permeation parameters: TEN steady-state flux (J) and lag time (t_L).

steady-state flux, J, and lag time, t_L, for each of these gels are also summarized in Table 1.

Complexation of TEN with CDs slightly increased the drug flux compared with control gel, whereas lag times were found to be shorter. Although all the CDs seem to behave similarly, the difference in flux observed among the parent CDs can be explained depending on the magnitude of the stability constants of the inclusion complexes. The complex stability constant determined by the solubility method, $K_{1:1}$, of β-CD (26) was 61.3 ± 2.3 M^{-1}, slightly higher than that of γ-CD (51.7 ± 1.9 M^{-1}) (27) because the size of the CD cavity to hold TEN is smaller and more appropriate in the case of β-CD. Although the conditions used in these studies were not the same as in our own, in all probability, the differences observed in the apparent stability constants will remain. Thus, the β-CD-TEN interactions are stronger, which leads to less drug in its free form ready to be absorbed through the skin.

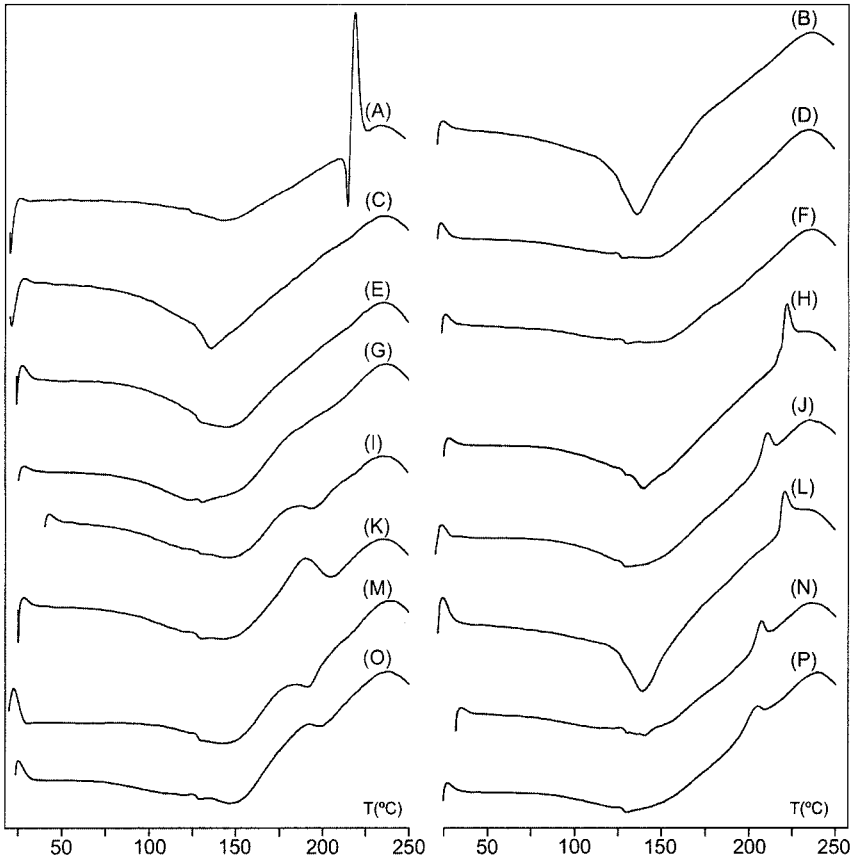

Figure 3. DSC thermograms of TEN-CD systems: (A) TEN; (B) β-CD; (C) γ-CD; (D) HP-β-CD; (E) HP-γ-CD; (F) M-β-CD; (G) γ-CD complex; (H) γ-CD physical mixture; (I) HP-γ-CD complex; (J) HP-γ-CD physical mixture; (K) β-CD complex; (L) β-CD physical mixture; (M) HP-β-CD complex; (N) HP-β-CD physical mixture; (O) M-β-CD complex; (P) M-β-CD physical mixture.

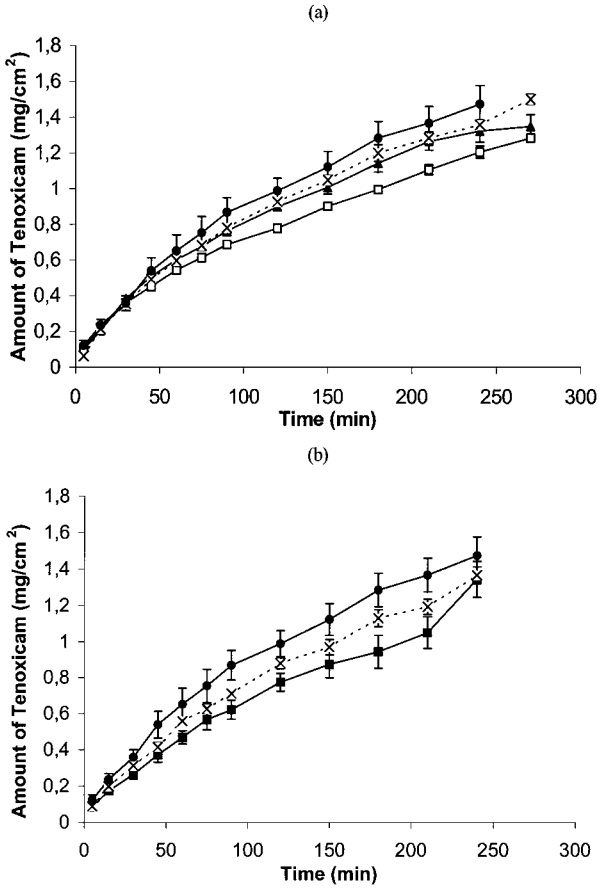

Figure 4. Release profiles of TEN from carbopol gels containing TEN or its CD complexes through cellulose membranes. (a) Effect of β-CD complexes: (●) control; (▲) β-CD; (□) HP-β-CD; (×) M-β-CD. (b) Effect of γ-CD complexes: (●) control; (■) γ-CD, (×) HP-γ-CD. Each point represents the mean ± SE of five to six experiments.

It is well known that less polar substituents on the parent CD rings may either prevent or enhance the inclusion of guest molecules. M-β-CD shows the highest flux, suggesting that size and lipophilicity of the methyl group can play a more important role in the percutaneous absorption of TEN than the other CDs tested.

It has been reported that M-β-CD can increase drug absorption through interaction with skin components (10) because M-β-CD has been attributed to be able to include cholesterol and tryglicerides, altering the barrier permeability and hence influencing the flux of various drugs (28). Biphenylylacetic acid permeation through hairless mouse skin was also enhanced using M-β-CD and HP-β-CD as complexing agents from a hydrophilic ointment (29).

In this study, TEN percutaneous absorption was slightly increased in the presence of the CDs. In all cases in which the CDs were used, lag time seem to be shorter. However, the enhancing effect of CDs on TEN permeation could not be explained by the diffusion process through the vehicle because release rates were diminished with the complexes. In general, many investigators believe that CDs might function as effective penetration enhancers by influencing the distribution and partitioning of the drug in the skin or by changing the structure of the impermeable barrier (16,30). In contrast, other investigators have reported no enhancement activities for CDs through interaction with skin components, but have ascribed increases in drug delivery to a solubilizing effect of the CD on drugs in donor solutions (9,29,31). In this research, because TEN

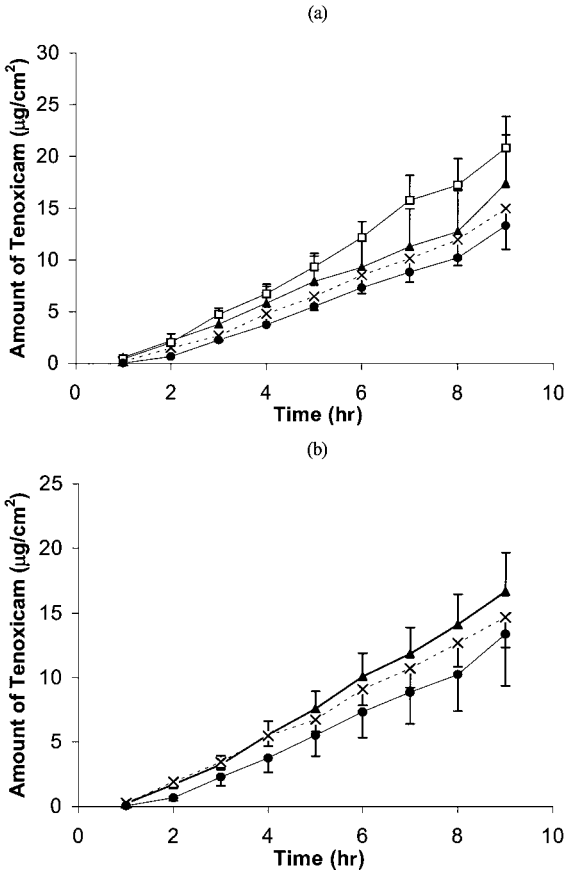

Figure 5. Permeation profiles of TEN through abdominal rat skin from carbopol gels containing TEN or its CD complexes. (a) Effect of β-CD complexes: (●) control; (▲) β-CD; (×) HP-β-CD; (□) M-β-CD. (b) Effect of γ-CD complexes: (●) control, (▲) γ-CD, (×) HP-γ-CD. Each point represents the mean ± SE of six to eight experiments.

Table 3.

Effect of Pretreatments on TEN Permeation Paramenters[a]

Pretreatment Solution	J[b] (μg cm^{-2} hr^{-1})	t_L[b] (hr)
Buffer	4.11 ± 0.59	1.17 ± 0.15
γ-CD (1%)	4.28 ± 1.33	0.34 ± 0.23
M-β-CD (1%)	4.54 ± 1.22	0.43 ± 0.10
M-β-CD (5%)	4.34 ± 0.92	0.62 ± 0.33
M-β-CD (20%)	4.36 ± 0.77	0.52 ± 0.20

[a] Values are the mean $\pm$ SE of 5 to 8 determinations at 37°C.
[b] Skin permeation parameters: TEN steady-state flux (J) and lag time (t_L).

has a rather high solubility in water, CDs presence only has a slight solubilizing effect on the drug. Hence, our results suggest that CDs may either interact in some manner with the SC components or influence the partitioning of the drug into the skin. Therefore, to gain insight into the mechanism of action, pretreatment experiments were carried out.

Pretreatment of skin by M-β-CD and γ-CD provided an almost 2-fold increase in TEN percutaneous absorption compared with the nonpretreatment values, but this enhancement is likely to be attributable to the hydration of the skin because the control pretreatment with buffer solution gave similar values (Table 3). In fact, aqueous solutions of γ-CD and M-β-CD provided no effects on the penetration of TEN, regardless of the concentration of CDs used. According to Loftsson et al. pretreatment with CDs does not usually enhance the permeability of drugs (19). In this regard, Arima et al. reported an insufficient pretreatment effect for M-β-CD (29). In addition, Williams et al. pretreatment permeation studies clearly showed that β-CD and HP-γ-CD did not enhance human transdermal permeation of a lipophilic drug, estradiol, and a hydrophilic drug, 5-fluorouracil (12), whereas Okamoto et al. found an increased absorption with M-β-CD pretreatment and a decreased lag time for sulfanilic acid (28).

Under our experimental conditions, an important reduction in lag time was observed and may suggest that these compounds help the partitioning of the drug into the skin to be faster.

CONCLUSION

It can be said that the different CDs essayed in this work have a low effect on TEN flux through rat skin. Although CD cavity size and substituents nature slightly affect the permeability of the drug across the skin, the greatest enhancement was achieved with M-β-CD and γ-CD. The increased dermal absorption found by pretreatment with M-β-CD and γ-CD was attributed to hydration of the skin. However, lag times always decreased when using the CDs, which could suggest that CDs affect the partitioning rate of TEN into the skin because it cannot be ascribed either to solubility modifications or to skin disturbance. Moreover, binding forces between TEN and CDs were found in solution and in solid state, suggesting a protective effect on TEN decomposition during its melting. Thus, the formulation of CDs in topical vehicles would be useful not only to alleviate skin irritation and to increase the stability of drugs, but also to allow TEN to reach the target site more quickly. Likewise, incorporation of CDs in carbopol hydrogels would allow the inclusion of more drug in solution with a subsequent improvement in delivery.

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