Drug Development and Industrial Pharmacy, 33:311–326, 2007

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040600901978

informa
healthcare

Ternary Complexes of Flurbiprofen with HP-ß-CD and Ethanolamines Characterization and Transdermal Delivery

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ABSTRACT Binary and multicomponent systems complexes prepared with HP-ß-CD and/or with monoethanolamine (MEA), diethanolamine (DEA) or triethanolamine (TEA) were obtained.

The results of solid-state studies indicated the presence of strong interactions between the components in the binary and the ternary systems. Drug solubility and dissolution rate in water were notably improved by employing the HP-ß-CD and the alkanolamines. The combined use of cosolvency and complexation with MEA in the presence of HP-ß-CD on the permeation of flurbiprofen through the human skin was evaluated. The combination of IPM, PG, and HP-ß-CD yield the highest permeation for the flurbiprofen–MEA complex.

KEYWORDS Flurbiprofen, Cyclodextrin, Ethanolamines, Inclusion complex, Dissolution, Human skin permeation

INTRODUCTION

Flurbiprofen is a non-steroidal anti-inflammatory drug that is slightly water-soluble (Martindale, 1989). It is one of the most potent inhibitors of platelet aggregation currently available; it is used to treat gout, osteoarthritis, rheumatoid arthritis, and sunburn (Poul et al., 1993).

However, because flurbiprofen is administered orally, gastrointestinal disorders become an issue as far as side effects are concerned. For this reason, we are studying the percutaneous administration of flurbiprofen as a way to minimize these gastrointestinal side effects.

Cyclodextrins (CDs) are cyclic oligosaccharides, containing six (α-CD), seven (β-CD), or eight (γ-CD) α-(1,4)-linked glucose units, formed from the enzymatic degradation of starch by bacteria. The most important structural feature of these compounds is their toroid shape, with hydrophobic interior cavity and hydrophilic faces. It is well known that they are capable of forming inclusion compounds both in solution and in solid state with a variety of guest molecules, which are placed in their hydrophobic interior cavity (Nakai et al., 1987).

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CDs are widely used in the pharmaceutical field to improve the chemical stability, absorption, bioavailability, the controlled release of some drugs, and the dissolution and solubility of nonpolar compounds (Duchêne & Wouessidjewe, 1990; Antoniadou et al., 1997; Scalia et al., 1998; Williams & Liu, 1999).

Recently, researchers have investigated the effects of CDs on the transdermal permeation of drugs (Loftsson et al., 1998; Lopez et al., 2000; Loftsson et al., 2001). These oligosaccharides are known to solubilize lipophilic therapeutic entities through molecular encapsulation and deliver the drugs to the skin in a solubilized form from which the drug partitions into and through the skin (Loftsson & Masson, 2001). The solubilization potential of these agents allows a greater amount of the drug to be loaded in the donor phase and at unit thermodynamic activity they are hypothesized to increase the drug transport through a kinetic barrier that exists at the skin-vehicle interface (Szejtli, 1994).

Although β-cyclodextrin (β-CD) is the most useful one of natural CDs for pharmaceutical applications since its central cavity has good affinity for the hydrophobic structures of many compounds, it is not always ideal for drug formulations because of its relatively low aqueous solubility (i.e., 1.8% at 25°C), renal toxicity and membrane destabilizing properties after parenteral administration. Recently, a number of chemically modified CDs such as hydroxyalkylated, methylated or branched β-CDs have been prepared to improve the inclusion capacity and physicochemical properties of natural CDs, and widely studied in various pharmaceutical preparations.

In particular, Hydroxypropyl-β-cyclodextrin (HP-β-CD), a chemical derivative of β-CD, has been extensively investigated on account of its superior water-solubility and safety profile by the parenteral route as well as higher complexation potential relative to the parent β-CD (Szente & Szejtli, 1999).

The efficiency of complexation is not frequently very high and, consequently, relatively large amounts of cyclodextrins must be used to complex small amounts of the drug.

The total solubility of a drug in the presence of CDs can be highly improved by pH adjustment or use of a proper third component. Likewise, the combined effect of inclusion complexation has been studied to improve the solubility of base-type drugs (Faucci et al., 2000).

A recent study in our laboratory has demonstrated that the solubility capacity of the HP-β-CD significantly enhances when a basic compound, triethanolamine (TEA), is incorporated as a ternary component in the complexes of the acid drug sulfisoxazole (Granero et al., 2003).

Likewise, it was reported that the formation of a salt with monoethanolamine (MEA) or diethanolamine (DEA) improved the percutaneous absorption of piroxicam (Cheong and Choi, 2002).

No studies, however, have addressed the influence of joining cyclodextrin complexes of lipophilic drugs with ethanolamines by simultaneously exploiting the cyclodextrins and ethanolamines solubilizing power towards the drug, to improve their dermal absorption favoring their delivery to the skin surface.

This investigation was conducted to prepare ternary inclusion compounds of the low soluble drug flurbiprofen with HP-ß-CD and some alkanolamines (MEA, DEA, and TEA). It seemed interesting to deeply investigate the role of alkanolamines in improving the pharmaceutical properties of flurbiprofen. Understanding the physical properties of each complex is important so as to select the complex to be developed for transdermal applications.

Formation of complexes was confirmed using infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and phase solubility diagrams. The pharmaceutical properties were characterized by measuring the dissolution performance.

Taking into account the results of the first part of these previous studies, we have developed a ternary complex system of flurbiprofen to investigate the effects of joining HP-ß-CD with MEA, and its combination with binary cosolvent systems, on the permeation of flurbiprofen across human skin. The vehicles used in the study were isopropyl myristate (IPM), propylene glycol (PG), and ethanol (EtOH).

MATERIALS AND METHODS

Materials

Flurbiprofen was obtained from Sigma Chemical Co. (99%,); MEA and DEA were purchased from Laboratorios Cicarelli (>99%, Buenos Aires, Argentina); TEA was obtained from Aldrich® (98%,); HP-β-CD (Mw = 1326–1400, degree of molar substitution 7.0) was kindly supplied by Roquette (Lestrem, France). Isopropyl myristate (IPM) was obtained from Parafarm (Droguería Saporiti S.A.C.I.F.I.A, Argentina). Propylene

glycol (PG) was purchased from Laboratorios Cicarelli (Argentina). The phosphate buffer solution (PBS), adjusted to pH 7.4, was prepared by dissolving KH_2PO_4 (14 mmol/L), Na_2HPO_4 (57 mmol/L), and NaCl (70 mmol/L) in Milli-Q water. All the others materials and solvents were of analytical reagent grade. A Millipore Milli-Q water purification system generated the water used in these studies.

Phase solubility studies

Solubility diagrams were obtained according to Higuchi & Connors (1965). Excess of flurbiprofen was added to vials containing various concentrations of HP-β-CD (0–0.23 M) or alkanolamines (MEA, 0–0.011 M; DEA, 0–0.008 M; or TEA, 0–0.013 M). In addition, the effects of the HP-β-CD on the solubility of flurbiprofen were studied with the presence of each alkanolamine. All the suspensions were sonicated in an ultrasonic bath for 1 hr and then, placed in a 25.0 ± 0.1°C constant- temperature water bath until equilibrium was reached (72 hr). The content of each vial was filtered through a 0.45 μm membrane filter (Millipore, Massachusetts, USA) and the concentration of flurbiprofen in the filtered solutions was measured by UV spectrop hoto metry (Shimadzu UV 260 UV-Vis spectrophotometer) at 246 nm. The presence of HP-ß-CD, MEA, or DEA did not interfere with the spectrophotometric assay of the drug. When TEA was used, the quantifications were done obtaining calibration curves at each TEA concentration. The equilibrium pH of each solution was measured (ORION SA 520 pH-meter). Each experiment was repeated at least three times and the results reported were the mean values.

The apparent stability constant (K_c) of 1:1 (guest–host) complex was calculated from the slope of the phase-solubility diagrams and the solubility of flurbiprofen in water (S_0):

$$K_c + slope / S_o(1 - slope) \quad (1)$$

Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectra of flurbiprofen and the complexes obtained were measured as potassium bromide discs on a Nicolet 5 SXC FT-IR Spectrophotometer. The FT-IR spectra of binary or ternary complexes were compared with their physical mixtures, and with pure HP-β-CD or each alkanolamine.

Differential scanning calorimetry (DSC) and termogravimetric analysis (TGA)

The DSC curves of the different samples were recorded on a DSC TA 2920 and the TGA curves on a TG TA 2920, at heating rates of 10°C min^{-1}. The thermal behavior was studied by heating 1–3 mg of samples in aluminum-crimped pans under nitrogen gas flow, over the temperature range of 25–400°C.

Solid samples preparation

The preparation of the solid complex of flurbiprofen–HP-β-CD in 1:1 molar ratios or the multicomponent complexes of flurbiprofen–HP-β-CD–alkano lamine (MEA, DEA, or TEA) in 1:1:1 molar ratios were performed by the freeze-drying method (Funk et al., 1969). The flurbiprofen complexes with MEA, DEA, or TEA were prepared by dissolving equimolar amounts of flurbiprofen and the amines in EtOH by mixing, and EtOH was removed in vacuo after treatment with ultrasound for 1 hr (Fang et al., 2004).

Dissolution studies

Dissolution studies were performed in triplicate with an USP XXIV rotating paddle apparatus (Hanson SR11 6 Flask Dissolution Test Station, Hanson Research Corporation, Chatsworth, California, USA) at 37°C using the paddle method at a rotation of 50 rev/min for flurbiprofen alone and for physical mixtures and complexes of binary or ternary systems. Suitable quantities of each powder containing 200 mg of flurbiprofen were compressed using a hydraulic press at an appropriate force of 2 tons to obtain discs, which should not be disintegrated under the test conditions. The discs were placed in the dissolution medium (400 mL of monopotassium phosphate, pH 7.2). The samples (3 mL) were withdrawn at prearranged time intervals and analyzed spectrophotometrically at 246 nm.

The fit factor f_2 was calculated for each profile and was used to compare the dissolution curves in relation

to the pure drug, using the following equation (Shah et al., 1999):

$$f_2 = 50\log\left\{\left(1+\frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right)^{-0.5} x100\right\} \quad (2)$$

In vitro drug skin absorption studies

Skin samples

Excised human skin from healthy patients, who had undergone abdominal plastic surgery, was used to get full-thickness skin. Immediately after excision the subcutaneous fatty tissue was removed using scissors and scalpel. The skin was cut into 10 × 10 cm pieces, wrapped in aluminium foil and stored in polyethylene bags at –26°C until use. Under these conditions the skin is stable with regard to the penetration of drugs as well as the thickness of the SC over a time period of 3 and 6 months, respectively (Harrison et al., 1984; Schaefer & Loth, 1996).

Skin permeation studies

The full thickness skin was mounted in a Franz diffusion cell and clamped between the donor and the receptor chambers (available diffusion area 1.54 cm^2) with the stratum corneum side in contact with the donor phase. The skin membranes were first hydrated in PBS at 4°C for 24 hr. A 2 mL aliquot of saturated solution of flurbiprofen obtained from the solubility studies was used as reservoir for the permeation studies. The receptor compartment was filled with 10 mL of degassed PBS to prevent the formation of air bubbles at the skin-receptor fluid interface. The temperature of the diffusion cell was maintained at 32 ± 1°C with a circulating water bath. A magnetic bar was used to stir the receptor phase to ensure uniform mixing. The solution in the receptor compartment was replenished after each withdrawal with an equal volume of fresh solution, allowed maintaining sink conditions in the experiments at scheduled intervals for a period of 12 hr. The drug concentration in the aliquots was estimated by the HPLC method. The donor vehicle was changed periodically to avoid a reduction in the drug thermodynamic activity and a change in vehicle components in the system throughout the experiment. As a control, a saturated solution of flurbiprofen in PBS was used in the donor compartment. The sampling arms and the donor compartment were occluded to prevent evaporation and therefore changes in the concentration. At the end of the experiment, the drug-exposed skin area was excised from the skin sample in order to measure the tissue drug concentration. The formulation was washed off the skin, and the weighed tissue was placed in methanol at room temperature overnight. The extracted drug samples were analyzed by HPLC.

Determination of flurbiprofen flux and permeability in the skin

The cumulative amount of flurbiprofen permeating across the skin was plotted against time. Drug flux (μg/h/cm^2), J_{ss}, at steady state was calculated by dividing the slope of the linear portion of the curve by the area of the exposed skin surface (1.54 cm^2). The permeabilities, K_p (cm/h), were calculated by dividing the steady-state flurbiprofen fluxes by the saturation solubility of the drug in the vehicle, C_0.

Analytical method

Flurbiprofen was analyzed using a HPLC system (Spectra SYSTEM, Providence, Rhode Island, USA), consisting of a UV detector (Spectra 100 variable wavelenght detector), a pump (Spectra SYSTEM P2000). A reverse phase column (150.0 × 4.00 mm MicroPak MCH-5-n-cap LC 18 5-μm HPLC column (VARIAN)) was used. The injection volume was 20 μL. The mobile phase was acetonitrile/0.057% phosphoric acid (60:40, v/v) at a flow rate of 1 mL/min; the detector was set at 250 nm. Under these conditions retention of approximately 3.5 min was obtained. Standard solutions of flurbiprofen were prepared in the range 2–15 μg mL^{-1} and analyzed to obtain a calibration curve.

Solubility test

The solubility of flurbiprofen and its complex with MEA in a series of vehicles was determined by saturating the vehicles with the drug. Excess amounts of flurbiprofen or flurbiprofen–MEA complex were added to vehicles, and they were equilibrated at 32 ± 1°C for 24 hr in a water bath, then filtered through a membrane filter with a pore size of 0.45 μm. Each experiment was performed in triplicate. The amounts of

flurbiprofen and the flurbiprofen–MEA complex in the filtrates were determined by UV spectrophotometry (Shimadzu UV 260 UV-Vis spectrophotometer) at 250 nm.

RESULTS AND DISCUSSION

Phase-solubility analysis

Flurbiprofen is a weak acid with an apparent pKa of 4.2 and it is practically insoluble in water (i.e., 0.14 mM; Tavornvipas et al., 2002). The aqueous solubility (S_0) of flurbiprofen at 25°C was 0.10 mM, which is in good agreement with the reported literature data. Solubility studies showed that the concentration of flurbiprofen at 25°C is notably affected by the presence of the HP-ß-CD or alkanolamines. Thus, a 0.23 M HP-ß-CD solution or 0.011 M MEA, 0.012 M DEA, and 0.013 M TEA solutions provided for a 99.1 mM, 12.0 mM, 11.7 mM, and 7.7 mM content of flurbiprofen corresponding to a 991-, 120-, 120-, and 77-fold increase in the concentration of flurbiprofeno in comparison with the drug aqueous solubility, respectively (Table 1). The solubility of flurbiprofen increases linearly along with the ethanolamine concentrations. Although, the pH-ethanolamine solutions trended slightly to increase when the MEA, DEA, or TEA concentration rise, it is clear from Table 1, that the flurbiprofen solubility-enhancement obtained with the binary systems containing the alkanolamines, is not only because of a pH effect. For 0.011 M MEA (pH 6.51) the drug solubility is 12 mM, the flubiprofen solubility alone from the literature at pH 6.5 is approximately 9.2 mM; for 0.012 M DEA (pH 6.52), the drug solubility is 11.7 mM and for 0.013 M TEA (pH 6.17), the flurbiprofen solubility is 7.7 mM, at the pH of 6.2, the solubility of the drug alone from the literature is 6.8 mM (Li & Zhano, 2003).

Solubility studies of flurbiprofen in ternary systems with 0.08 M HP-ß-CD and each alkanolamine in water a 25°C showed that the DEA in a concentration of 0.0012 M was the most effective of the examined alkanolamines in improving flurbiprofen solubility, since a clear synergistic effect on flurbiprofen solubility enhancement was found (Table 2). In fact, by dividing the drug solubility obtained in the examined FB-0.008 M HP-ß-CD-0.0012 M DEA ternary system, by the solubility of the drug in the FB-0.008 M HP-ß-CD binary system, a 2.6-fold increase was found. Moreover, the drug solubility in the ternary system was higher than the one calculated by adding the solubilities in the presence of CD and DEA separately. This phenomenon may be attributed to the combined effects of salt formation and inclusion in cyclodextrin. However, when the DEA concentrations were higher than 0.0012 M, a decrease of the drug solubility was observed. This effect could be attributed to a salting out effect as the concentration of the counterion was increased beyond the solubility product.

A synergistic effect on flurbiprofen solubility enhancement was found in ternary systems with TEA, the drug solubility increase with the enhancement in the TEA concentration, i.e., the solubility of flurbiprofen increased ~1.9-fold in the presence of 0.008M HP-ß-CD and 0.013 M TEA in comparison with the FB-HP-ß-CD binary one.

On the contrary, ternary systems with MEA and HP-ß-CD did not improve the flurbiprofen solubility

TABLE 1 Solubility Data of Flurbiprofen in the Presence of HP-ß-CD or Alkanolamines (MEA, DEA, and TEA)

	Additive concentration (M)	Final pH	Solubility of FB (mM) ± SD
HP-ßCD	0	4.52	0.10 ± 0.01
	0.02	3.82	7.79 ± 0.05
	0.08	2.86	27 ± 2
	0.11	2.81	47 ± 4
	0.15	2.68	67 ± 4
	0.19	2.67	75 ± 6
	0.23	2.64	99 ± 8
MEA	0.0014	6.32	1.8 ± 0.1
	0.0028	6.40	3.4 ± 0.1
	0.0056	6.54	6.4 ± 0.2
	0.0084	6.44	9.49 ± 0.04
	0.011	6.51	12.0 ± 0.1
DEA	0.0012	5.77	1.35 ± 0.02
	0.002	6.02	2.13 ± 0.05
	0.004	6.18	4.08 ± 0.04
	0.006	6.34	6.07 ± 0.07
	0.008	6.39	7.9 ± 0.3
	0.01	6.44	9.91 ± 0.01
	0.012	6.52	11.7 ± 0.2
TEA	0.0013	5.18	0.767 ± 0.006
	0.0022	5.30	0.93 ± 0.03
	0.005	5.50	2.60 ± 0.01
	0.007	6.34	3.32 ± 0.05
	0.009	6.59	5.60 ± 0.01
	0.011	6.07	6.7 ± 0.1
	0.013	6.17	7.7 ± 0.1

TABLE 2 **Solubility Data of Flurbiprofen in the Presence of 0.08 M HP-ß-CD at Different Concentrations of Alkanolamines (MEA, DEA, or TEA)**

	Alkanolamine concentration (M)	Final pH	Solubility of FB (mM) ± SD
MEA	0	2.85	25 ± 1
	0.0017	3.23	22 ± 5
	0.0033	3.41	22 ± 2
	0.0067	3.61	22 ± 4
	0.010	3.79	33 ± 5
	0.013	3.90	28 ± 6
DEA	0.0012	3.39	74 ± 1
	0.002	3.50	54 ± 7
	0.004	3.71	56 ± 5
	0.006	4.22	26 ± 2
	0.008	3.91	31 ± 4
	0.01	4.19	21 ± 5
	0.012	4.31	20 ± 2
TEA	0.0013	3.25	28.1 ± 0.2
	0.0022	3.43	44 ± 1
	0.005	3.62	31 ± 3
	0.007	3.72	37 ± 1
	0.009	3.76	29 ± 1
	0.011	3.83	34 ± 2
	0.013	3.89	54 ± 2

with respect to binary systems between flurbiprofen and HP-ß-CD.

The solubility method is useful for investigating the inclusion complexation of poorly water-soluble drugs with CDs in water, because it gives not only the solubililizing ability of host molecules but also the stability constant of complexes by analyzing solubility curve. Fig. 1 shows the phase solubility diagram of flurbiprofen for the HP-ß-CD complex in water. The diagram shows that the solubility increased in an approximately linear manner as a function of HP-ß-CD concentration. The curve obtained was of A_L type, as defined by Higuchi & Connors (1965), this profile is characterized by a slope of less than 1 (i.e., 0.427), and thus it was assumed that the solubility increase is caused by the formation of a 1:1 complex. The stability constant for the complex calculated from the slope of the plot of the solubility diagram was determined to be 7452 M^{-1}.

The solubility diagrams of the flurbiprofen with the alkanolamines in water are shown in Fig. 2. These diagrams demonstrate that the solubility increased in an approximately linear manner as a function of each alkanolamine concentration. The curves obtained were of A_L-type. The performance of the flurbiprofen–alkanolamine binary systems was in the order flurbiprofen–MEA > flurbiprofen–DEA > flurbiprofen–TEA.

Solid-state studies

Fig. 3 shows the DSC and TGA thermograms of pure components. The DSC profile of flurbiprofen shows a characteristic sharp endothermic peak of the drug at 115°C ($\Delta H = 209.6$ J/g) corresponding to its melting point, following by a decomposition phenomenon. This value is in good agreement with that in the literature (Gamisans et al., 1999). The DSC curve of HP-ß-CD was typical of an amorphous substance which exhibited a typical broad endothermic peak between 50 and 160°C assigned to its dehydration as it was determined by the weight loss registered by TGA.

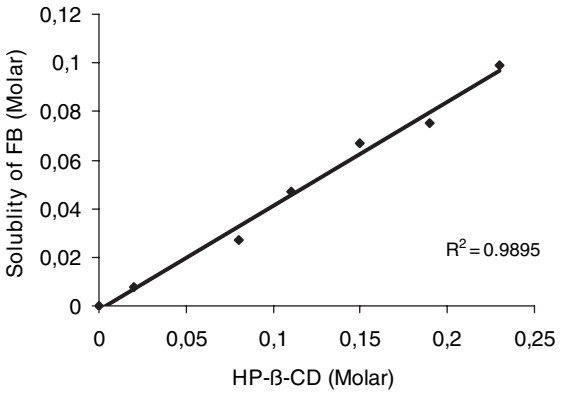

FIGURE 1 **Phase solubility diagram of flurbiprofen in the presence of HP-β-CD in water.**

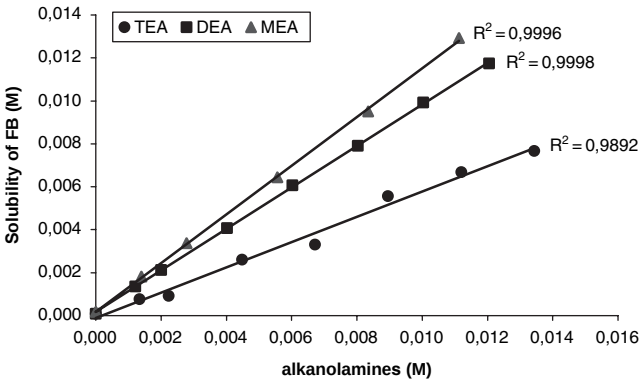

FIGURE 2 **Phase solubility diagrams of flurbiprofen with increasing concentrations of each alkanolamines (MEA, DEA, or TEA).**

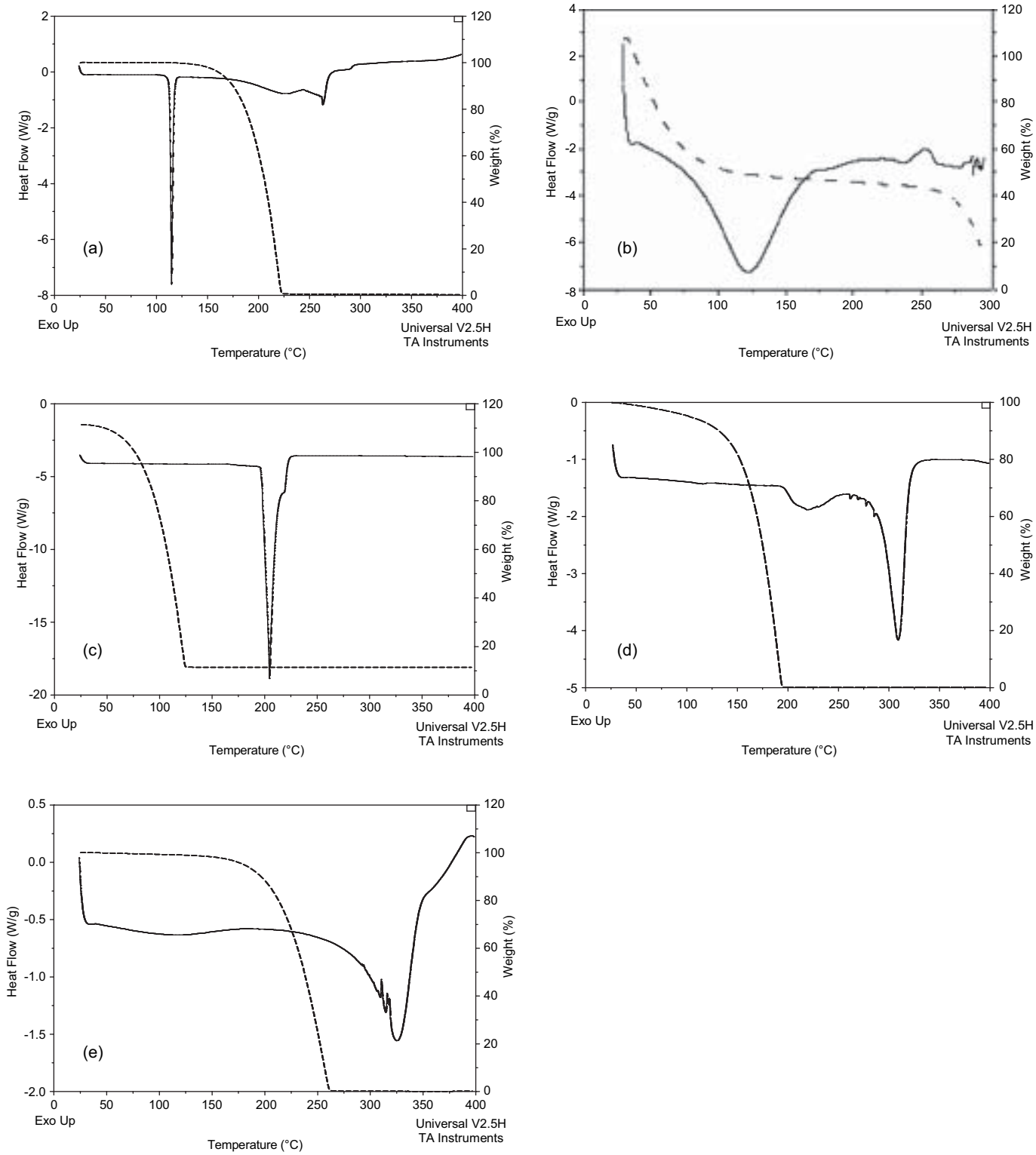

FIGURE 3 **DSC ( ) and TGA ( ) curves of pure components: flurbiprofen (a); HP-ß-CD (b); MEA (c); DEA (d); and TEA (e).**

The physical mixture flurbiprofen–HP-ß-CD (Fig. 4a) shows the melting endothermic peak assigned to flurbiprofen, although this peak was decreased compared with pure flurbiprofen (108°C, $\Delta H = 71.48$ J/g). However, this peak disappeared in the case of the solid binary prepared by freeze-drying (Fig. 4b). These results can be explained on the basis of a better interaction between the drug and the cyclodextrin, indicating the complexation of flurbiprofen with HP-ß-CD. In ternary products prepared by freeze-drying, the

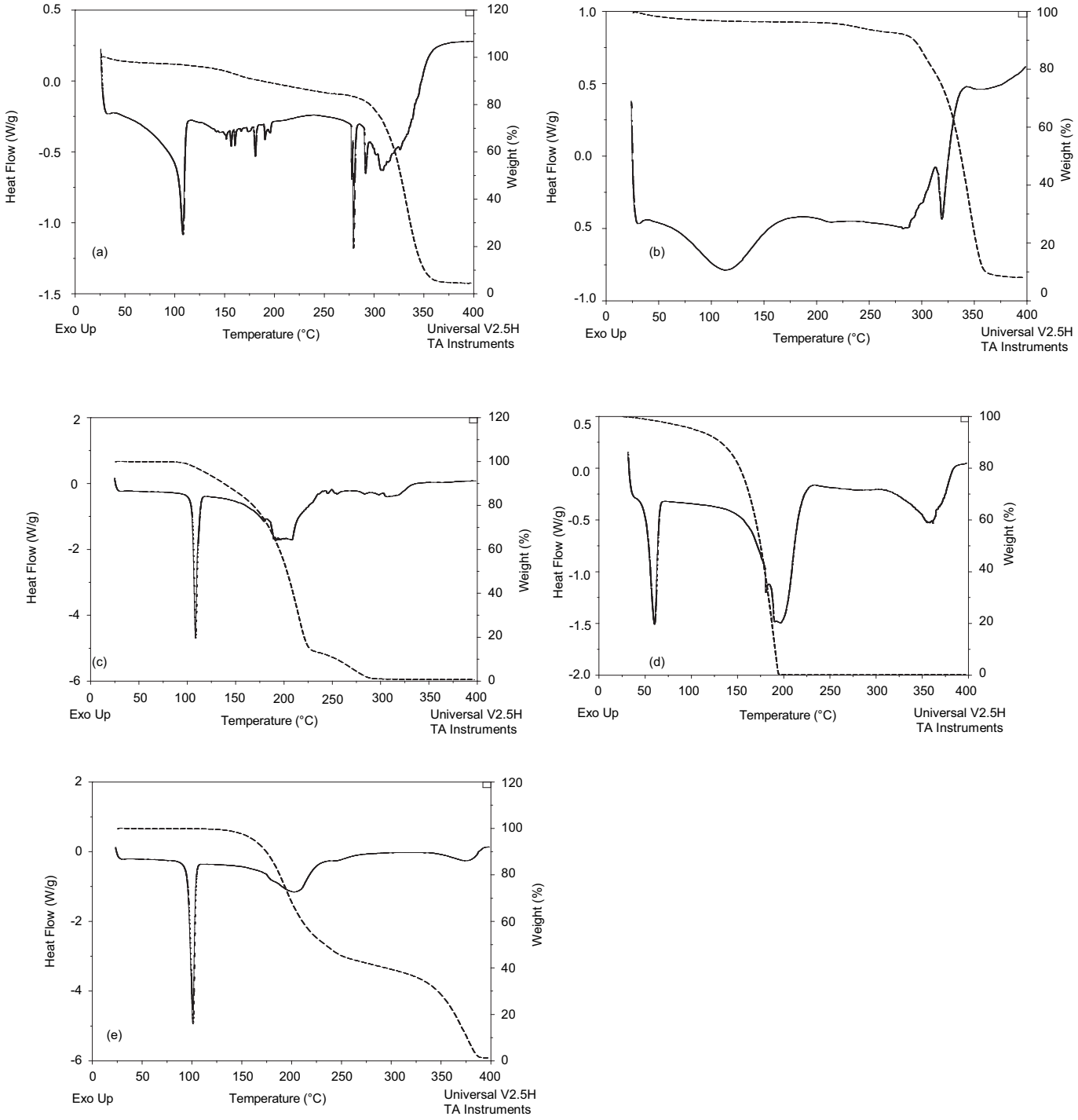

FIGURE 4 **DSC ( ) and TGA ( ) curves of: physical mixture flurbiprofen–HP-ß-CD (a); and of binary complexes of: flurbiprofen–HP-ß-CD (b); flurbiprofen–MEA (c); flurbiprofen–DEA (d); and flurbiprofen–TEA (e).**

melting point of flurbiprofen disappeared and only the endothermic effects caused by dehydration of the HP-ß-CD and decomposition were observed, indicating complete interaction between the components (Fig.5a–c). Melting points of flurbiprofen–MEA (108°C), flurbiprofen–DEA (60°C), and flurbiprofen–TEA (101°C) complexes were decreased compared with pure flurbiprofen (fig. 4c–e). The broad endothermic peaks at about 200°C were caused by the complex decomposition. The rank order for molar enthalpy (ΔH) of fusion is flurbiprofen–TEA (ΔH = 136.1 J/g) > flurbiprofen–MEA (ΔH = 98.48 J/g) > flurbiprofen–DEA (ΔH = 66.09 J/g).

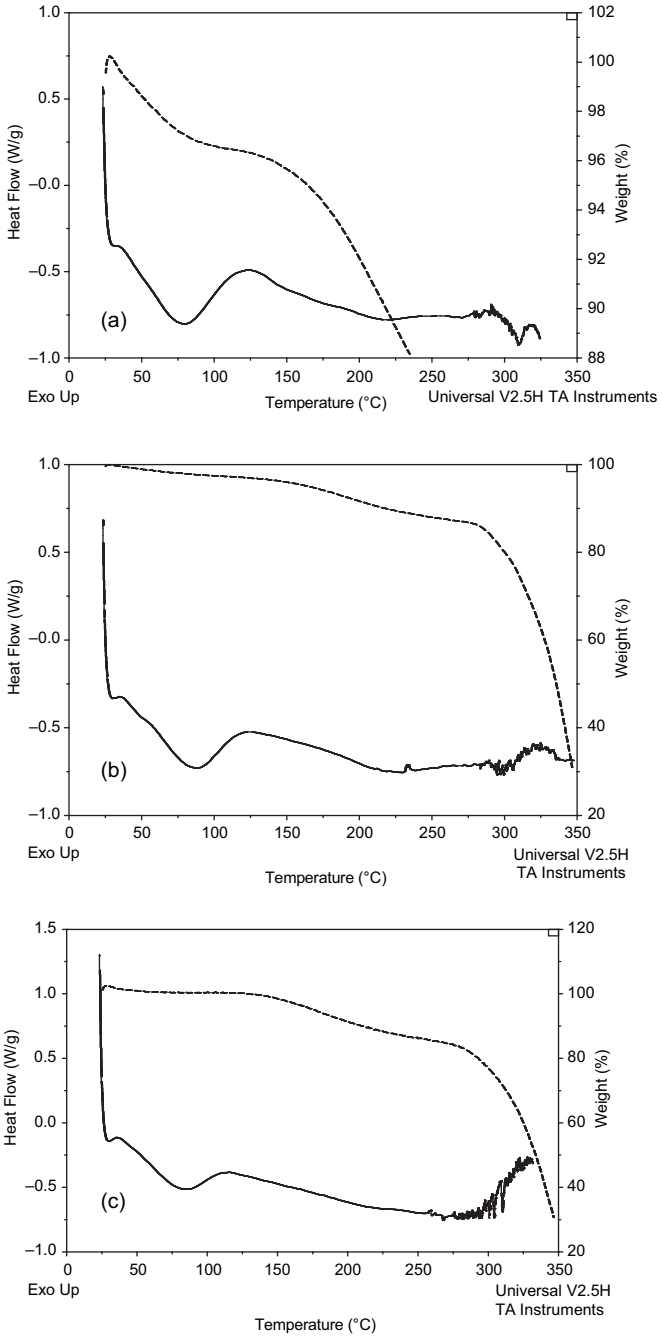

FIGURE 5 **DSC ( ) and TGA ( ) curves of ternary complexes of: flurbiprofen–HP-ß-CD–MEA (a); flurbiprofen–HP-ß-CD–DEA (b); and flurbiprofen–HP-ß-CD–TEA (c).**

The shift in melting point and the change in the molar enthalpy of fusion are the result of flurbiprofen complexation with alkanolamine.

To investigate the possibility of an interaction between flurbiprofen and HP-ß-CD or alkanolamines, more information was gathered from infrared spectroscopy. The FT-IR spectra of flurbiprofen and its complexes are shown in Figs. 6–8. As it is shown in Fig. 6a, we found the characteristic strong band at 1703.5 cm^{-1} in the infrared spectrum of flurbiprofen because of the ν (C=O) stretching band of the carbonyl group. The FT-IR of the physical mixture between flurbiprofen and HP-ß-CD (Fig. 7a) did not differ significantly from those of the single components. However, in Fig. 7b the IR spectrum of the complex prepared by freeze-drying shows no similar features to pure flurbiprofen. The band located at 1703.5 cm^{-1} had totally disappeared. This can be probably attributed to the inclusion complexation of flurbiprofen into the HP-ß-CD cavity. The infrared of the flurbiprofen–alkanolamine complexes are shown in Fig. 7c–e. A sign of interaction would be reflected by shifts in the C=O vibrations. The carbonyl peak of the flurbiprofen in the complexes was shifted to a lower wavenumber (flurbiprofen–MEA 1657.7, flurbiprofen–DEA 1667.9, and flurbiprofen–TEA 1627.2 cm^{-1}). These are assigned to the ν (COO^-) vibrations in the infrared spectra of alkanolamine complexes of flurbiprofen, indicating that the carbonyl group of flurbiprofen has been converted to a carboxylate anion. Also the IR spectrum of the sodium flurbiprofen was registered for comparison purposes. A shift of the flurbiprofen carbonyl peak to a lower wave number (~1655.8 cm^{-1}) caused by the salt formation was observed. This result corroborates the salt formation of flurbiprofen by complexation with alkanolamines.

Dissolution rate studies

The results of these studies are shown in Fig. 8. As it can be seen from the reported data, the aqueous dissolution rate of pure flurbiprofen is very slow with 31.6% dissolving at the end of 60 min. The Q10, Q30, and Q60 values (Angiano-Ogea et al., 1996; i.e., percentage of dissolved flurbiprofen at 10, 30 and 60 min) were 5.6; 15.8, and 31.6%, respectively, for the pure drug. The dissolution of the binary complexes is very fast in the initial stage and reaches more than 90% within about 20 min. The corresponding Q10, Q30, and Q60 values, indeed, were 76.9 and 100% for flurbiprofen–HP-ß-CD; 66.5; 97.3 and 100% for flurbiprofen–MEA; and 66.2 and 100% for flurbiprofen–TEA, respectively. Thus, although the dissolution rate of the three complexes behaves quite similarly, flurbiprofen–HP-ß-CD and flurbiprofen–MEA exhibit higher flurbiprofen fractions dissolved with respect to flurbiprofen–TEA. The flurbiprofen–DEA

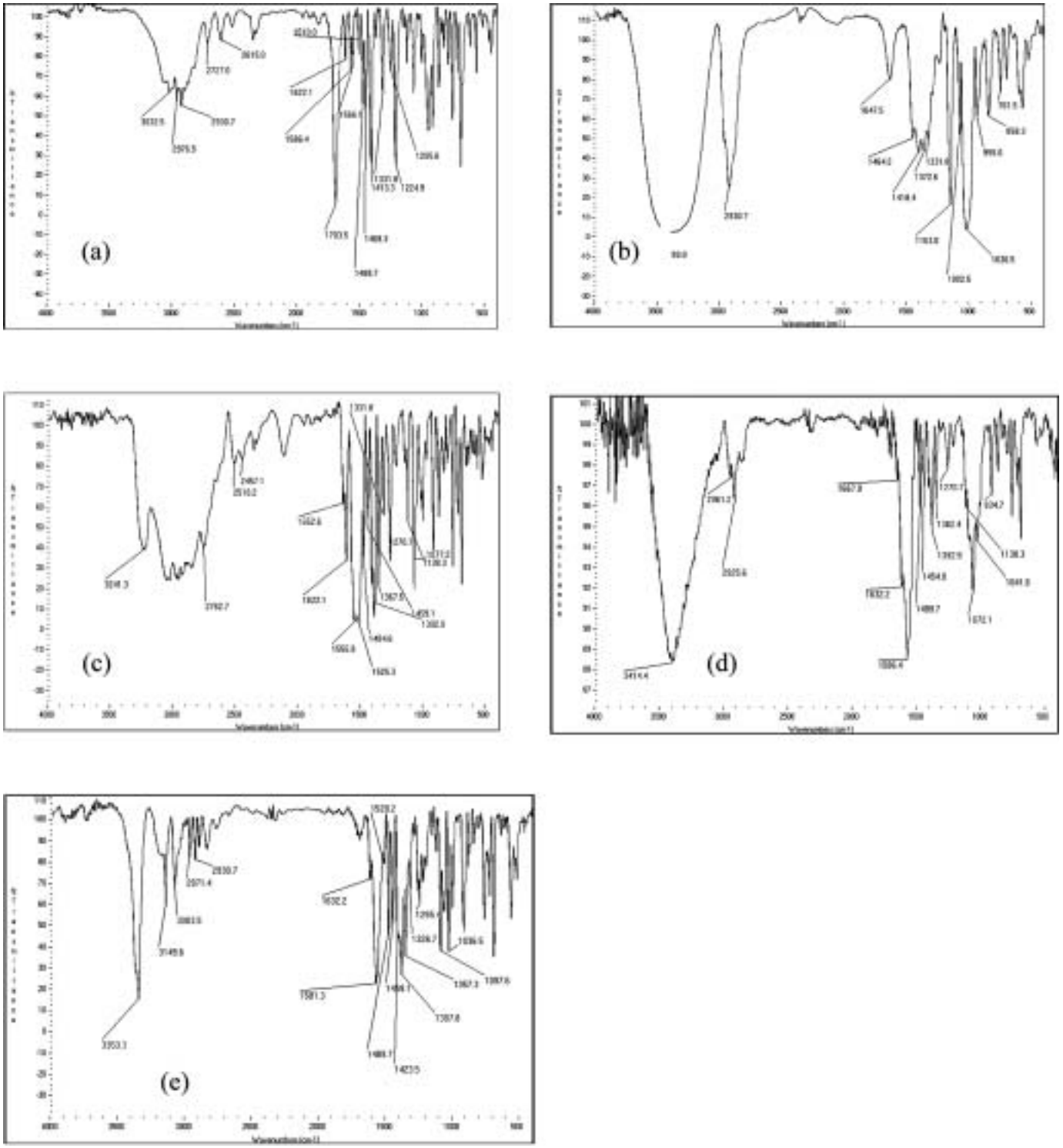

FIGURE 6 **FT-IR spectra of pure components: flurbiprofen (a); HP-ß-CD (b); MEA (c); DEA (d); and TEA (e).**

complex was not solid. The physical mixture of flurbiprofen with HP-ß-CD significantly enhanced flurbiprofen dissolution rate (33.1, 81.1, and 96.1%), even though it showed slower drug dissolution than the complexes. The dissolution rate increase reached for the physical mixture is only because of the wetting effect of the HP-ß-CD. With the ternary systems, a strong increase of the dissolution profile is observed, despite the slightly slower dissolution rate obtained by these formulations in relation to the binary systems. The corresponding Q10, Q30, and Q60 values were 75.7, 96.8, and 100% for flurbiprofen–HP-ß-CD–MEA; 68.8, 96.6, and 100% for

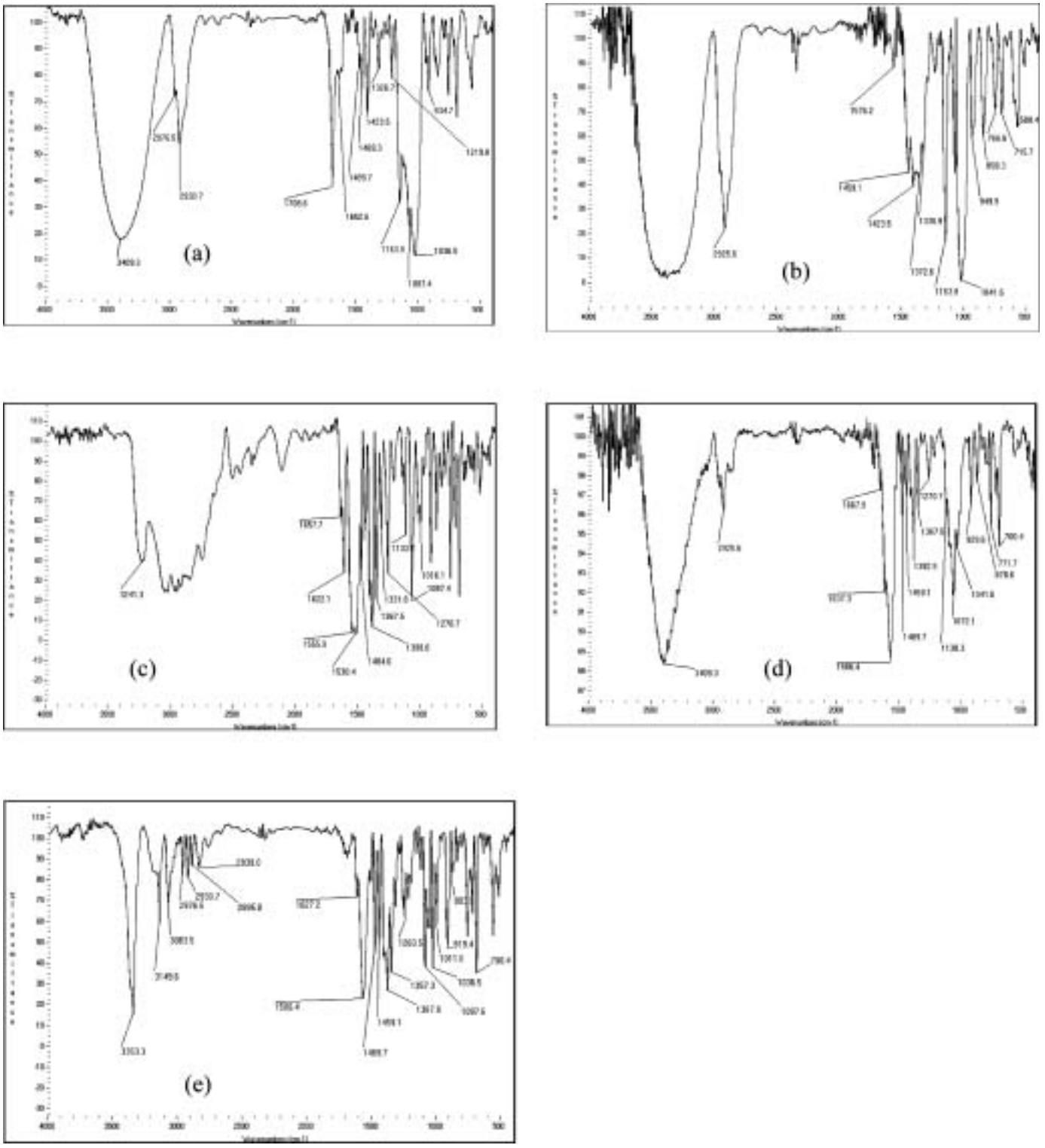

FIGURE 7 **FT-IR spectra of: physical mixture flurbiprofen–HP-ß-CD (a); and of binary complexes of: flurbiprofen–HP-ß-CD (b); flurbiprofen–MEA (c); flurbiprofen–DEA (d); and flurbiprofen–TEA (e).**

flurbiprofen–HP-ß-CD; and 54.4, 92.5, and 100% for flurbiprofen–HP-ß-CD–TEA.

The increase of the dissolution rate in the complexes with HP-ß-CD is clear in relation to the formation of the inclusion complex with HP-ß-CD, in agreement with the results obtained by DSC, TGA, and FT-IR techniques. The effect of complexation with HP-ß-CD on the solubility of flurbiprofen can be

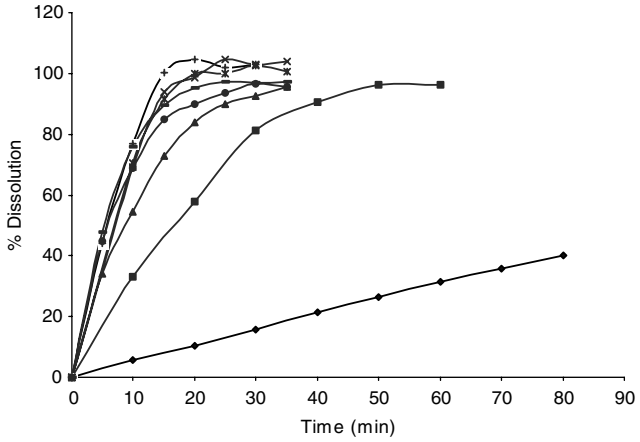

FIGURE 8 Dissolution rate profiles of flurbiprofen alone (♦), physical mixture with HP-ß-CD (■), and freeze-dried complex 1:1 with HP-ß-CD (+), MEA (×), TEA (*), HP-ß-CD and MEA (–), HP-ß-CD and DEA (•), and HP-ß-CD and TEA (▲).

explained in terms of the reduction in the crystallinity of the drug caused by the freeze-drying process and the inclusion into the hydrophobic cavity of the HP-ß-CD.

A model-independent for dissolution profile comparison that uses the dissolution data in their native form was used in this study. The f_1 (difference factor) is proportional to the average difference between the two profiles, whereas f_2 (similarity factor) is inversely proportional to the average squared difference between the two profiles, with emphasis on the larger difference among all the time points. The use of these factors was also recommended for dissolution profile comparison in the FDA's guided for industry (1997), and in this study, f_2 was calculated and used. According to these guides, f_2 values are greater than 50 (50–100), which indicate an equivalence of the two curves. The f_2 factor for each flurbiprofen formulation versus the pure drug were calculated by using Eq. 2 and are listed in Table 3.

The lowest f_2 values indicate the largest difference between dissolution profiles in relation to the pure drug. f_2 values were lower than 50, indicating that all dissolution profiles were different from the pure drug. Results showed that the smallest f_2 values for the formulation were achieved with the binary systems.

TABLE 3 Dissolution Parameters and the Similarity Factor, f_2, Between the Binary and the Ternary Systems and the Pure Drug Dissolution Profiles

	Dissolution profile parameters			
Formulations	Q_{10}	Q_{30}	Q_{60}	f_2
Flurbiprofen–HP-ß-CD physical mixture	33.1	81.1	96.1	11.34
Flurbiprofen–HP-ß-CD complex	76.9	100	–	3.58
Flurbiprofen–MEA complex	66.5	97.3	100	4.73
Flurbiprofen–TEA complex	66.2	100	–	4.52
Flurbiprofen–MEA–HP-ß-CD complex	75.3	96.8	100	5.15
Flurbiprofen–DEA–HP-ß-CD complex	68.8	96.6	100	6.26
Flurbiprofen–TEA–HP-ß-CD complex	54.4	92.5	100	8.53

In vitro drug skin absorption studies

Effect of HP-ß-CD and cosolvents on skin permeation of flurbiprofen

Taking in consideration that it has been reported good results with the combined use of cyclodextrins and lipophilic enhancers on the percutaneous absorption of drugs (Uekama et al., 1992; Sigurdardóttir & Loftsson, 1995), our next approach was to study the possible promoting effect of HP-ß-CD in combination with some cosolvents on the skin permeation of flurbiprofen.

By comparing the behavior of flurbiprofen in the two binary cosolvent mixtures, IPM/PG and IPM/EtOH, with or without HP-ß-CD, it was clear that the addition of the cyclodextrin to these binary systems resulted in entirely different permeation profiles and trends and is shown in Fig. 9 and presented in Table 4.

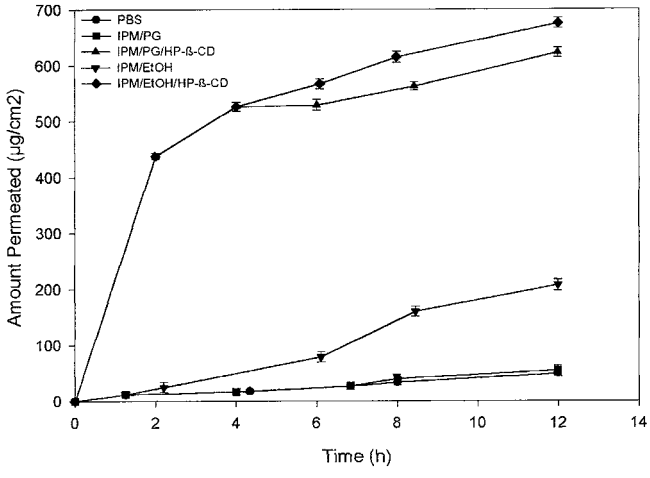

FIGURE 9 Effect of HP-ß-CD and cosolvents on the permeation of flurbiprofen through excised human skin. Each point represents an average of three measurements.

TABLE 4 **Permeability coefficient, Maximum Flux, Permeated Amount of Flurbiprofen at 12 hr and Residual Drug Remaining in the Skin for the Vehicles Assayed. Results are Shown as Mean and Standard Deviation (n = 3)**

Vehicle	K_p (cm h^{-1}) 10^3	Flux (μg cm^2 hr^{-1})	Permeated amount at 12 hr (μg/cm^2)	Residual drug per miligram of skin (μg/mg)	Solubility (mg/mL)
PBS	0.45	4.2 ± 0.2	45 ± 1	0.7 ± 0.3	9.4 ± 0.2
(9:1) IPM/PG	0.12	5 ± 0.8	55 ± 2	2.0 ± 0.1	40.78 ± 0.01
(9:1) IPM/EtOH	0.16	18 ± 2	208 ± 4	4.3 ± 0.1	112 ± 2
(9:1) IPM/PG/2.5% (w/v) HP-ß-CD	0.38	15.7 ± 0.9	623 ± 3	1.8 ± 0.1	41.36 ± 0.01
(9:1) IPM/ EtOH /2.5% (w/v) HP-ß-CD	0.25	29 ± 4	676 ± 2	2.0 ± 0.1	116.5 ± 0.8

The permeation curves with the binary systems containing HP-ß-CD do not show a classic profile with a steady state phase. The curves initially provided very high permeation rate followed by gradual decrease.

On the other hand, after two days, crystals were observed in the cosolvent mixtures without HP-ß-CD. But, the crystallization process was retarded when cyclodextrin was added to these systems.

It was observed that the flux of flurbiprofen in the systems IPM/PG and IPM/EtOH containing HP-ß-CD (concent. 2.5% (w/v)) was improved as compared to that obtained for these binary mixtures without the cyclodextrin (Table 5). It could be proposed that the HP-ß-CD prevents the formation of flurbiprofen crystals. Although the saturated solubilities of flurbiprofen in systems containing HP-ß-CD were slightly higher than those obtained in the systems without HP-ß-CD (Table 4), it appears that this only partly explains the mechanism of action and it is likely that other mechanisms also contribute to the stabilizing effect.

The fluxes of flurbiprofen obtained in the group systems, with and without HP-ß-CD, containing EtOH (29 ± 4 μg cm^{-2} hr^{-1} and 18 ± 2 μg cm^{-2} hr^{-1}, respectively) were higher than those obtained in the group systems, with and without HP-ß-CD, containing PG (15.7 ± 0.9 μg cm^{-2} hr^{-1} and 5.0 ± 0.8 μg cm^{-2} hr^{-1}, respectively).

The permeability coefficients were quite similar in the binary cosolvent mixtures without HP-ß-CD (0.12×10^{-3} cm h^{-1} and 0.16×10^{-3} cm h^{-1}, respectively). Whereas, in the systems with HP-ß-CD, the ternary system containing EtOH yield a slightly lower drug permeability coefficient (0.25×10^{-3} cm h^{-1}) than that obtained with the ternary systems containing HP-ß-CD/IPM/EtOH.

These results suggest that the large solubilizing power of the EtOH group systems, as shown by the highest drug solubilities, which leads to larger concentration gradients towards the skin and thus driving force for drug permeation through the skin, appears to be the main factor in the transdermal flux enhancement for flurbiprofen.

Effect of HP-ß-CD and cosolvents on skin permeation of flurbiprofen-MEA complex

Considering the results obtained in the early stage of this work, we decided to study the possible promoting effect of the increase of the aqueous solubility of flurbiprofen through its complexation with MEA in combination with the addition of HP-ß-CD and the binary cosolvent mixtures IPM/EtOH and IPM/PG.

Permeation profiles of flurbiprofen–MEA complex from different formulations are shown in Fig. 10.

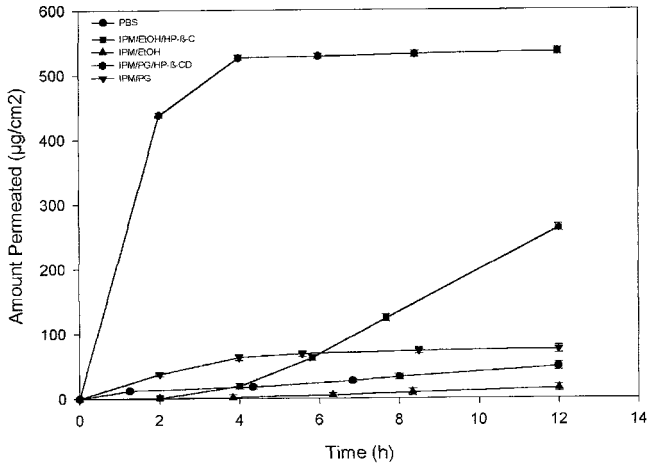

FIGURE 10 **Effect of HP-ß-CD and cosolvents on the permeation of flurbiprofen–MEA complex through excised human skin. Each point represents an average of three measurements.**

TABLE 5 Permeability Coefficient (K_p), Maximum Flux, Permeated Amount of Flurbiprofen–MEA Complex at 12 hr and Residual Drug Remaining in the Skin for the Vehicles Assayed. Results are Shown as Mean and Standard Deviation (n = 3)

Vehicle	K_p (cm h^{-1}) 10^3	Flux (μg cm^2 hr^{-1})	Permeated amount at 12 hr (μg)	Residual drug per miligram of skin (μg mg^{-1})	Solubility (mg mL^{-1})
PBS	0.04	1.3 ± 0.7	14.4 ± 0.7	1.6 ± 0.2	33 ± 1
(9:1) IPM/EtOH	0.22	1.7 ± 0.8	15 ± 2	1.9 ± 0.3	7.9 ± 0.4
(9:1) IPM/PG	1.20	1.0 ± 0.3	76 ± 2	0.40 ± 0.04	0.85 ± 0.04
(9:1) IPM/EtOH/ 2.5% (w/v) HP-ß-CD	4.02	31 ± 1	263 ± 2	4.3 ± 0.1	7.72 ± 0.06
(9:1) IPM/PG/2.5% (w/v) HP-ß-CD	0.71	1.1 ± 0.7	536 ± 3	0.7 ± 0.1	1.56 ± 0.07

Table 5 summarizes the permeability parameters of the flurbiprofen–MEA complex from each system.

In spite of the fact that the complexation of flurbiprofen with MEA increased 3.5-fold the solubility of flurbiprofen in PBS, this complex formation was found to have a negative effect on the percutaneous permeation of flurbiprofen as reflected from its permeation profile (Fig. 10) and its permeation parameters (Table 5). This decrease in skin permeation is probably a result from the stronger hydrophilic nature and molecular size of the drug complex with respect to the drug molecule itself (Knutson et al., 1990; Potts & Guy, 1995; Williams et al., 1998).

The mixture of HP-ß-CD with the binary cosolvent system consisting in IPM and PG significantly enhanced the skin permeated amount of the flurbiprofen–MEA complex after 12 hr of application. This combination gave the best permeation profile for this complex among all formulations tested. Whereas, the combination of HP-ß-CD with the binary system consisting of IPM and EtOH provided a smaller enhancing effect than that obtained in the previous ternary system.

PG-based formulations do not show a classic profile with a steady state phase. The curves initially provided very high permeation rate followed by gradual decrease. This may be attributed to the PG ability to permeate the skin readily and, in doing so, may carry the drug molecules across. Thus the initial higher permeation rate of the drug complex may be attributed to the solvent drag mechanism. On the other hand, it was observed that the solubility of the flurbiprofen–MEA complex, in this cosolvent system, was increased 1.8 times by the addition of 2.5% (w/w) HP-ß-CD. Here, it would be favor the complexation, which may increase the partitioning of this drug complex from the formulation into the stratum □ corneum. In this system, HP-ß-CD appears to exert a cooperative effect in enhancing the drug complex permeation. It is interesting to note that a little reduction in the solubility of the flurbiprofen–MEA complex, was observed in comparison with the solubility values in the cosolvent systems, IPM/EtOH, with and without HP-ß-CD (Table 5).

The presence of 2.5% (w/w) HP-ß-CD in the cosolvent system IPM/EtOH increased significantly the binary complex flux (~18 times) and the skin accumulation after 12 hr of application (2.3-fold) compared with the same cosolvent system without HP-ß-CD. These findings may indicate that the HP-ß-CD do not increase the binary complex permeation by solubility mechanism. An explanation for these facts can be offered based on the thermodynamic activity of the binary complex. The flux is proportional to the gradient of thermodynamic activity. Hence the solubility of flurbiprofen–MEA complex in this vehicle was lower than that without HP-ß-CD. The thermodynamic activity of the binary complex was thus thought to have already attained a maximum level in this condition.

CONCLUSION

Complexation with HP-ß-CD and salt formation with alkanolamines was successfully applied to improve the solubility and dissolution properties of flurbiprofen. All binary systems always showed better dissolution performances than the corresponding ternary systems. The positive effect of binary complexation was particularly evident in the first phase of the dissolution process, with a percentage of drug dissolved at 10 min about 10 times higher than that dissolved from the pure

drug. The complexation was proved in the solid state by DSC, TGA, IR, and dissolution studies, and in solution by the phase-solubility analysis.

The data collected from the in vitro permeation studies indicate that different enhancement-promoting effects would be involved in the skin permeation for the binary flurbiprofen–MEA complex and for the free drug flurbiprofen.

The contribution of the HP-ß-CD to the flurbiprofen delivery from the cosolvent systems IPM/PG and IPM/EtOH is dependent on the complexation of this drug with MEA and on the nature of the added solvent to IPM.

While for the free flurbiprofen, the solubilizing effect of HP-ß-CD could be involved in the nucleation inhibition process, which results in an enhancement of the drug permeation, for the binary complex between flurbiprofen with MEA, the main contribution of HP-ß-CD dependent on the nature of the added cosolvent, EtOH, or PG, to the system. In the formulation that contained EtOH, the HP-ß-CD appears to increase the thermodynamic activity of the binary complex enhancing the transdermal transport of this complex. In the case of the formulation containing PG, the enhancement on the permeation of the binary complex might be attributed to the PG drag mechanism together with changes of the hydrophobic driving force for formation of drug complex.

Based on the results of this investigation, incorporation of HP-ß-CD to flurbiprofen–MEA complex combined with the binary cosolvent system IPM and PG, could be interesting for the development of a novel, transdermal therapeutic system for flurbiprofen.

ACKNOWLEDGMENT

The authors thank the Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT), and the Consejo Nacional de Investigaciones Científicas y Tecnológicas de la Nación (CONICET) for financial support. We also thank the Ferromet S. A. (agent of Roquette in Argentina) for their donation of hydroxypropyl-ß-cyclodextrin. Supply of human skin by The Beauty Center, Dr. Mottura, was greatly appreciated.

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