Drug Development and Industrial Pharmacy, 2009; 35(7): 877–886

informa
healthcare

ORIGINAL ARTICLE

Etodolac/cyclodextrin formulations: physicochemical characterization and in vivo pharmacological studies

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Abstract

Background: The formulation of nonsteroidal anti-inflammatory drugs with cyclodextrins (CDs) has demonstrated to be a suitable strategy to increase drug aqueous solubility, dissolution rate, and gastric tolerance. *Aim*: We investigated the effects of the CDs on the physicochemical and pharmacological properties of Etodolac (ET), a practically water-insoluble nonsteroidal anti-inflammatory drug, to individuate a drug formulation with optimized pharmacokinetics and pharmacodynamics. *Methods*: The interactions in solution of ET with β-CD, hydroxypropyl-β-CD (HP-β-CD), and γ-CD were studied by ^{13}C-NMR spectroscopy and phase solubility method. Solid binary systems, prepared by physical mixing and freeze-drying, were characterized by differential scanning calorimetry, X-ray analysis and Fourier transform infrared spectroscopy, and dissolution studies. An in vivo pharmacological investigation (analgesic activity and gastric tolerance studies) was performed on freeze-dried ET/CD formulations. *Results*: ^{13}C-NMR and phase solubility studies demonstrated the ability of CDs to complex with ET and increase drug solubility. ET/CD interactions at the solid state occurred at the molecular level only for freezed-dried samples. All binary systems, mainly those containing HP-β-CD and γ-CD, showed a significantly improved dissolution profile of ET. In vivo pharmacological studies evidenced an improvement of analgesic activity and a reduction of gastrolesivity of ET/CD-tested formulations with respect to ET alone. *Conclusions*: The formulation of ET with CDs demonstrates relevant pharmaceutical potential in view of decreasing dose and side effects of ET. For industrial applications, HP-β-CD appears to be the best partner for ET, as it is less expensive than γ-CD and gives rise to higher drug solubilization than β-CD.

Key words: *β-Cyclodextrin*; *γ-cyclodextrin; analgesic activity; dissolution; Etodolac; gastric tolerance; hydroxypropyl-β-cyclodextrin; inclusion complexes*

Introduction

The coformulation of drugs and cyclodextrins (CDs) has demonstrated to be a powerful tool in the pharmaceutical field in improving drug dissolution rate[1–5]. This strategy is important in terms of cost-effectiveness of a drug, because such a formulation can enhance the bioavailability, reduce the dosage needed, and, consequently, decrease the untoward side effects of the drug[6,7]. However, this strategy can be successful when the rate-limiting step in drug adsorption is its dissolution rate, not its adsorption across the gastrointestinal mucosa. On the basis of this assumption, nonsteroidal anti-inflammatory drugs (NSAIDs) are a very interesting class of drugs to be coformulated with CDs.

Etodolac (ET), 1,8-diethyl-1,3,4,9-tetrahydropyrano-[3,4-b]indole-1-acetic acid, is a NSAID prescribed for the treatment of acute pain, osteoarthritis and rheumatoid arthritis at relatively high dosage, up to a maximum of 1200 mg/day[8,9]. The drug shows high therapeutic index between gastric irritation and anti-inflammatory effects. ET is recognized as a selective COX-2 inhibitor by Regulatory Authorities since 1997[10] (10-fold selectivity over the COX-1 enzyme[11]).

The most common manifestations of ET toxicity are gastrointestinal irritation and ulceration. Although these side effects occur less frequently with ET than

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(Received 1 Jul 2008; accepted 10 Dec 2008)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.1080/03639040802680263

with some other NSAIDs[12], in the long-term administration of ET required in chronic diseases, the gastrolesivity constitutes a limitation to its usage.

It is worth noting that the drug has gained renewed interest since recent studies have demonstrated its antitumor effect on different human cancer cells[13,14]. We considered this drug as a very interesting candidate for formulation with CDs. The main reason for this choice is that ET is practically water insoluble[15] and the gastric damage it causes is mainly due to local effects (systemic effects play only a marginal role)[16]. Studies on complexation of ET with CDs have been reported in literature and have showed a positive effect of the carrier on physicochemical properties of the drug[17–19].

The aim of this work was to individuate an optimized ET/CD formulation, with enhanced pharmacokinetic and pharmacodynamic properties. On purpose, binary systems of ET with β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), and γ-cyclodextrin (γ-CD) were prepared and characterized. The interactions in solutions between ET and the selected CDs were studied by ^{13}C-NMR spectroscopy and phase solubility method. Solid binary systems, prepared by physical mixing and freeze-drying, were characterized by DSC, X-ray analysis and FTIR spectroscopy, and dissolution studies. The distinctive mark of this study was an in vivo pharmacological investigation (analgesic activity and gastric tolerance studies) performed to verify whether ET/CD formulations provided therapeutic advantages.

Materials and methods

Materials

Etodolac was kindly supplied by C.F.M. S.p.A. (Milano, Italy); β-cyclodextrin (Kleptose®) and hydroxypropyl-β-cyclodextrin (MS = 0.40) by Roquette Freres (Lestrem, France), whereas γ-cyclodextrin was a commercial sample from Fluka (Buchs, Switzerland). All substances were used as received, without further purification. All chemicals were of analytical reagent grade. Double-distilled water was used throughout the study.

^{13}C-NMR studies

^{13}C-NMR spectra were recorded on a Bruker AMX-500 spectrometer at 20.0 ± 0.1°C. Solutions containing 2×10^{-2} M of ET or equivalent amount of ET/CD (1:1 mol/mol) were prepared in 0.1 M NaOD. Spinning tubes of 4 mm containing 0.5 mL of solution were used. Tetramethylsilane was used as external reference, and no correction was made for the susceptibility of the capillary. Chemical shifts were calibrated with an accuracy of 0.01 ppm.

Phase solubility studies

Phase solubility studies were performed in unbuffered water, according to the Higuchi and Connors method[20]. An excess amount of ET (50 mg) was added to 25 mL of water or CD aqueous solutions (from 1×10^{-3} to 1.4×10^{-2} M of β-CD; from 1×10^{-3} to 2×10^{-2} M of HP-β-CD and γ-CD) in screw-capped glass vial; the samples were mechanically shaken (SS40-D Grant shaking bath) at 25°C until equilibrium was achieved (4 days at least). Aliquots were withdrawn, filtered (filter HA-0.45 μm, Millipore, Billerica, MA, USA), and spectrophotometrically analyzed for ET content (Philips PU 8740 spectrophotometer) at 276 nm. The presence of CDs did not interfere with the spectrophotometric assay of the drug. Each experiment was performed in triplicate; the coefficient of variation associated with each measure was never greater than 3%.

Preparation of solid systems

Drug/CD solid systems were prepared by physical mixing and colyophilization. The latter method is generally useful for obtaining inclusion complexes in the solid state[21]. ET and CDs were sieved (IG3/WET/MS, Giuliani, Torino, Italy) and the corresponding 75–150 μm granulometric fractions collected. The ET/CD mixtures in stoichiometric ratio 1:1 (mol/mol) were prepared as follows:

- for the physical mixtures (PMs), ET and each CD were blended in mortar until an homogenous mixture was obtained;
- for the colyophilized samples (CSs), the PMs of ET and each CD were dissolved in the smallest possible amount of aqueous ammonium hydroxide solution (0.06 g/L) at room temperature. The solutions were dried under vacuum after freezing at –60°C (Modulyo Edwards);
- for the physical mixtures of separately lyophilized components (PMSLs), ET was dissolved in the smallest possible amount of aqueous ammonium hydroxide solution (0.06 g/L) at room temperature and the solution was dried after freezing. Each CD was dissolved in water, and the solution was dried after freezing. Lyophilized ET and each lyophilized CD were blended in mortar.

No residual ammonia (Nessler's test) was detected in any of the CSs and PMSLs.

Differential scanning calorimetry

DSC measurements were carried out using a Mettler DSC 30 apparatus equipped with a TC II probe. Samples

were weighed (10–15 mg) (Mettler M3 microbalance) in Al pans pierced with a perforated lid and scanned at 10°C/min in the 30–300°C temperature range. Dry nitrogen was used as purge gas.

X-ray analysis

The X-ray diffraction powder patterns were collected on a Philips PW 3710 diffractometer in the 2–50° 2θ range (scan rate 1°/min). Kα radiation of Cu was generated at 40 kV and 30 mA.

Infrared spectroscopy

FTIR spectra were obtained as KBr disk (0.5 mg of ET or an equivalent amount of the binary systems) on a Bruker IFS-48 apparatus applying Fourier transformation of eight scans.

Dissolution studies

The dissolution profiles of ET and of the different ET/CD solid systems were obtained according to the disperse amount method. One hundred milligrams of ET or equivalent amount of ET/CD blends were added to 1 L of water (nonsink conditions) at 37.0 ± 0.5°C in a Sotax AT7 apparatus. Suitable aliquots were removed at scheduled times, filtered, and spectrophotometrically analyzed for ET content (see 'phase solubility studies'). A correction was calculated for the sampling. Each test was performed in triplicate (coefficient of variation <3%). The percent of drug dissolved after 5 minutes (DP) and the dissolution efficiency (DE)—that is, the area under the dissolution curve at 60 minutes (calculated using the trapezoidal rule and expressed as percentage of the area of the rectangle described by 100% dissolution at the same time[22])—were assumed as indexes of the dissolution process. The relative dissolution rates (RDRs) of the drug/CD systems were also calculated by the ratio of the amount of drug dissolved at 5 minutes to that obtained with the pure drug.

Pharmacological studies

The experimental procedures used for this study have been carried out in compliance with the Italian laws for the care and use of laboratory animals (D.L. 116/92) as well as with EEC regulations (OJ of EC L 3581, December 18, 1996).

Analgesic activity

The acetic acid writhing test was used to evaluate the analgesic activity. ET at a dose of 3.5 and 35 mg/kg and its CSs with β-CD, HP-β-CD, and γ-CD (containing an equivalent drug amount) were orally administered in freshly prepared water suspensions to groups of six male Swiss mice weighing 25–30 g (Harlan Nossan, Correzzana, Italy). Indomethacin was used as a reference compound. A 0.06% acetic acid solution in saline (0.5 mL/mouse) was injected intraperitoneally 60 minutes after oral administration of the samples. The writhing movements of each animal were recorded 20 minutes after the irritant injection. The analgesic effect of the compounds was expressed as number of contractions and percentage of inhibition compared with the control group.

Gastric tolerance

The experiments were performed in male Wistar rats (Harlan Nossan), 120–150 g. ET alone (35 mg/kg) and its CSs at equivalent doses were tested for gastric tolerance by evaluating their attitude to cause ulceration. Indomethacin was used as reference compound, HP-β-CD as blank. The compounds were orally administered to groups of six rats after a 24-hour fast; the treatment was repeated after 2 hours. Six hours after the first dose, the animals were killed by carbon dioxide inhalation, their stomachs removed and examined with a dissecting microscope. The severity of mucosal damage (ulcerogenic index) was scored from 0 to 4. The following arbitrary scale was used to evaluate the ulcers: 0, normal; 1, subepithelial vasocongestion; 2, patches of superficial necrosis; 3, mucosal vasocongestion and focal necrosis; 4, extensive vasocongestion and necrosis involving the full thickness of the mucosa.

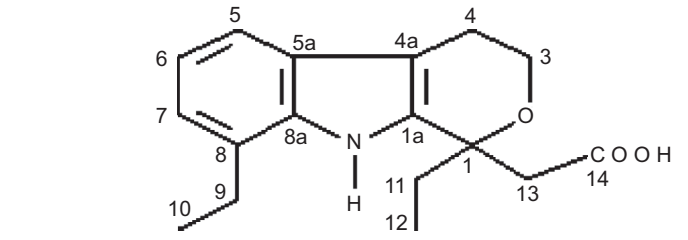
Statistics

Numerical results are expressed as means ± SEM, and statistical significance was evaluated by Student's *t*-test. $P < 0.05$ was considered to indicate a significant difference.

Results and discussion

Solution studies

^{13}C-NMR studies on ET in the absence and in the presence of the CDs were performed to gain insight into the type of interactions between the drug and the CDs considered in the solution. The differences in chemical shift values between ET in the free and in complexed state are presented in Figure 1. The atoms showing the highest variations in chemical shift are only reported; the negative sign of $\Delta\delta$ (i.e., the difference in ET chemical shifts in the presence and in the absence of CDs) refers to an upfield shift, whereas the positive sign indicates a



Structure of Etodolac
(Arbitrary numbers were assigned to the atoms in the structure for ease in presentation of data)

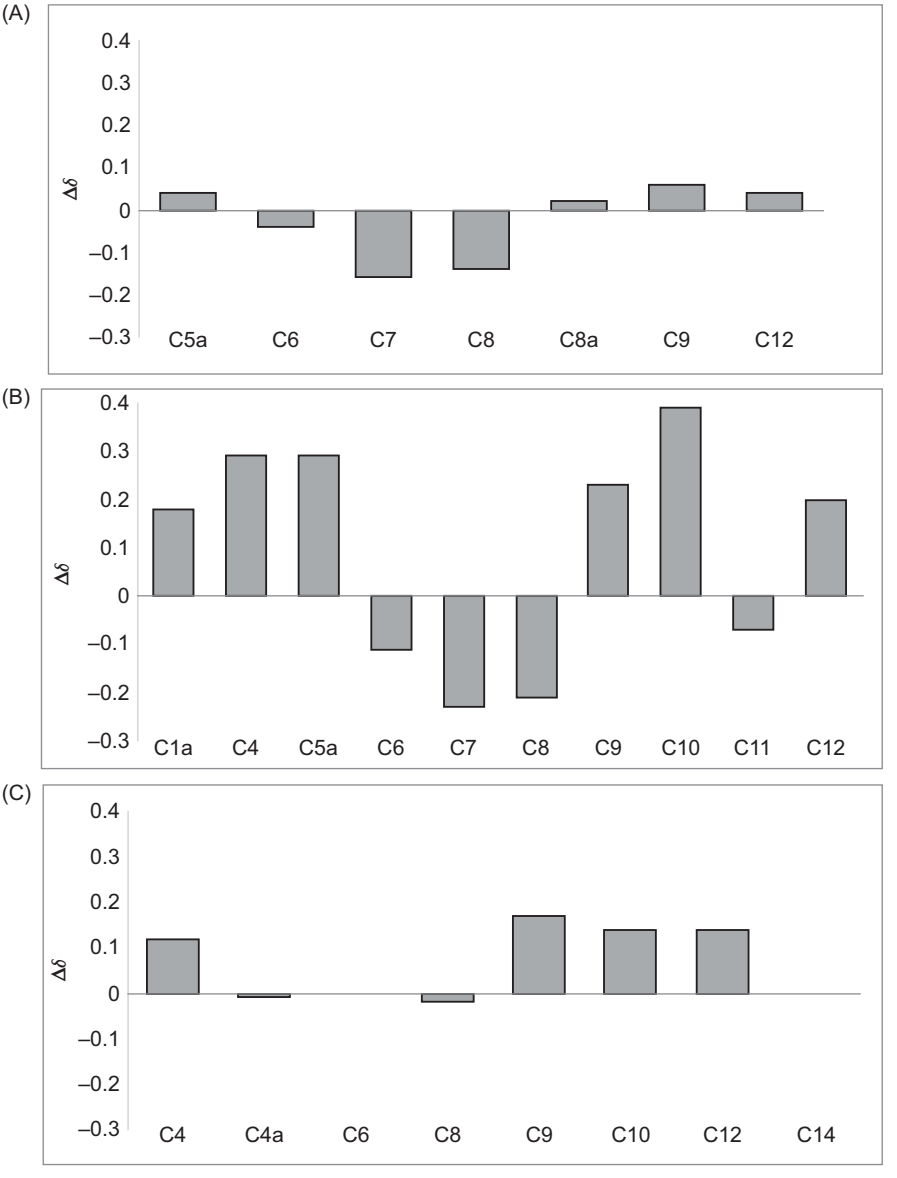


Figure 1. Chemical shift changes of carbons of ET in the presence of β-CD (A), HP-β-CD (B), and γ-CD (C) (1:1 molar ratio); $\Delta\delta = \delta_{complex} - \delta_{free}$.

downfield shift. As can be seen in Figure 1A, in the presence of β-CD, the upfield shifts of C6, C7, and C8 atoms could suggest that the ET benzene moiety is included in the β-CD cavity. In fact, according to Inoue model[23–25], carbon atoms deeply inserted in the CD cavity (from the secondary hydroxyl group side) experience a negative shift. On the other hand, the downfield shifts of the C5a, C8a, C9, and C12 ET atoms suggest that these carbons are externally close to the wider rim of the β-CD hollow cone[26]. In the presence of HP-β-CD (Figure 1B), the

same ET carbons display upfield shifts, but their values are higher than in the presence of β-CD. In addition, the carbon atoms showing downfield shifts are in larger number (C5a, C9, C12 and C1a, C4, C10), and their values are higher than in the presence of the parent CD. This demonstrates the occurrence of stronger interactions of the drug with the external surface of HP-β-CD, involving hydroxypropyl groups of this CD. In the presence of γ-CD (Figure 1C), chemical shift variations are generally similar to those registered with the β-CDs, apart from the upfield shifts that appear reduced, probably for the larger cavity of this CD.

Phase solubility studies have been carried out to investigate the ability of the CDs to complex with and solubilize ET as well as to determine the apparent stability constants of the complexes. The phase solubility plots, reported in Figure 2, show that the CDs tested were able to increase drug solubility. At low CD concentrations, γ-CD was the most effective in improving ET water solubility, with a 1.5-fold enhancement at the concentration 3×10^{-3} M. At high CD concentrations, drug solubility gradually decreased in the presence of γ-CD; the best solubilizing effect was obtained with HP-β-CD, which enhanced drug solubility by 1.5-fold at 7.5×10^{-3} M and by 2.1-fold at 2×10^{-2} M. According to the Higuchi and Connors classification[20], the diagrams for β-CD and HP-β-CD (Figure 2) were of A_L type, as they were characterized by a straight line pattern. The A_L-type diagram indicates the formation of a soluble complex and, in the absence of additional information, is considered as indicative of the 1:1 complex formation[20]. The diagram obtained in the presence of γ-CD was of B_S type, which indicates the formation of a poorly soluble complex precipitating at high γ-CD concentrations.

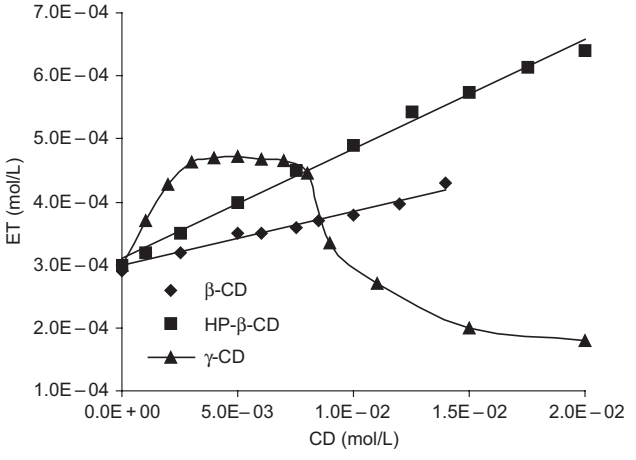


Figure 2. Phase solubility diagrams of ET at increasing amounts of the different cyclodextrins: β-CD (♦), HP-β-CD (■), and γ-CD (▲) (mean of three experiments, CV < 3%, error bars omitted for the sake of clarity).

Assuming a 1:1 stoichiometry, the apparent stability constants ($K_{1:1}$) of the complexes were calculated from the straight line portion of the diagrams, according to the Higuchi and Connors Equation (1)

$$K_{1:1} = \frac{\text{slope}}{S_0\,(1-\text{slope})}, \tag{1}$$

where S_0 is the intrinsic solubility of the drug. The calculated $K_{1:1}$ values for the ET/CD complexes were 22.9 ± 0.5, 58.2 ± 1.5, and 170.7 ± 3.0 M^{-1} for β-CD, HP-β-CD, and γ-CD, respectively. The lower value for the constant of ET/β-CD complex indicated that interactions between the drug and the β-CD were weaker than with the other CDs, whereas the stability constant of ET/γ-CD complex was the highest one; the most effective CD in enhancing drug solubility was HP-β-CD, as γ-CD formed with ET a poorly soluble complex.

Indication about the process of solubilization of ET in the presence of the CDs was obtained from the values of Gibbs free energy change. The Gibbs free energy values of transfer ($\Delta G°_{tr}$) of ET from pure water to the aqueous solutions of β-CD, HP-β-CD, and γ-CD systems were calculated from phase solubility data using Equation (2)[27]:

$$\Delta G°_{tr} = -2.303RT \log \frac{S_S}{S_0}, \tag{2}$$

where S_S/S_0 is the ratio of molar solubility of the drug in aqueous carrier solutions to that in pure water. The obtained values of Gibbs free energy are presented in Table 1. Data provide the information whether the reaction condition is favorable or unfavorable for drug solubilization in the aqueous carrier solutions, negative Gibbs free energy values indicating favorable conditions. $\Delta G°_{tr}$ values were all negative for β-CD and HP-β-CD solutions at various concentrations, indicating the spontaneous nature of ET solubilization, and they decreased with an increase in carrier concentration, demonstrating that the process became more favorable as the concentration of the β-CDs increased. On the other hand, $\Delta G°_{tr}$ values for γ-CD were negative only at low concentrations and became positive when the concentration increased, confirming the varying effect of γ-CD on drug solubilization.

Table 1. Thermodynamic parameters of the solubility process of ET in β-CD, HP-β-CD, and γ-CD solutions at 25°C.

	$\Delta G°_{tr}$ (kJ/mol)		
CD (mol/L)	β-CD	HP-β-CD	γ-CD
0.005	−4.60	−7.86	−11.96
0.01	−6.61	−12.82	0.51
0.012	−7.61	−13.80	4.54
0.02	—	−19.35	11.66

Solid-state studies

Figure 3A–C illustrates DSC profiles of pure components and of ET/CD equimolar solid systems. Thermal curve of ET showed an endothermic peak at 154°C, corresponding to the melting point of the drug, whereas the CDs exhibited a very broad endothermic peak corresponding to the dehydration peak. The curves of the PMs can be regarded as the superimposition of those of the pure components, as the temperatures of both the ET melting peak and CD dehydration peaks did not change. On the other hand, in all CSs the melting peak of ET was absent and CD dehydration peaks were broadened. CSs curves were compared to those of lyophilized ET alone and of the PMSLs[28]. The curve of lyophilized ET alone, as compared to crystalline ET, showed only a shift in the endothermic peak (148°C instead of 154°C) (data not shown). Accordingly, in the case of PMSLs, the melting peak of ET was still evident in the DSC spectra. Although these patterns were consistent with the fact that CSs and PMSLs displayed different host/guest interactions at the solid state, they did not allow us to conclude that a real complexation of ET with CDs does take place for CSs. In fact, we cannot exclude the occurrence of a more complete ET amorphization when it is freeze-dried with CDs[29].

An X-ray diffraction study on the powder was performed for ET/β-CD solid systems. As can be seen in Figure 4, the diffraction pattern of PM was the superimposition of each component. In contrast, an increasing amorphization degree was observed, ranging from PM

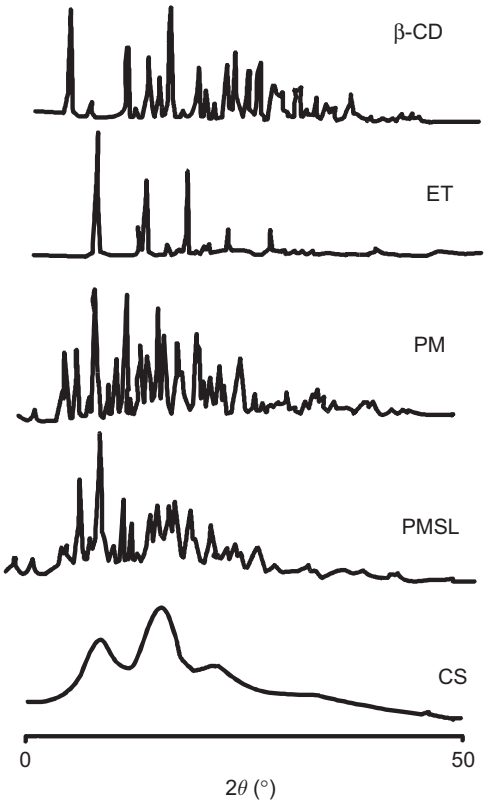


Figure 4. X-ray diffraction patterns of ET, β-CD, and of ET/β-CD 1:1 (mol/mol) systems: physical mixture (PM), physical mixture of separately lyophilized components (PMSL), and colyophilized sample (CS).

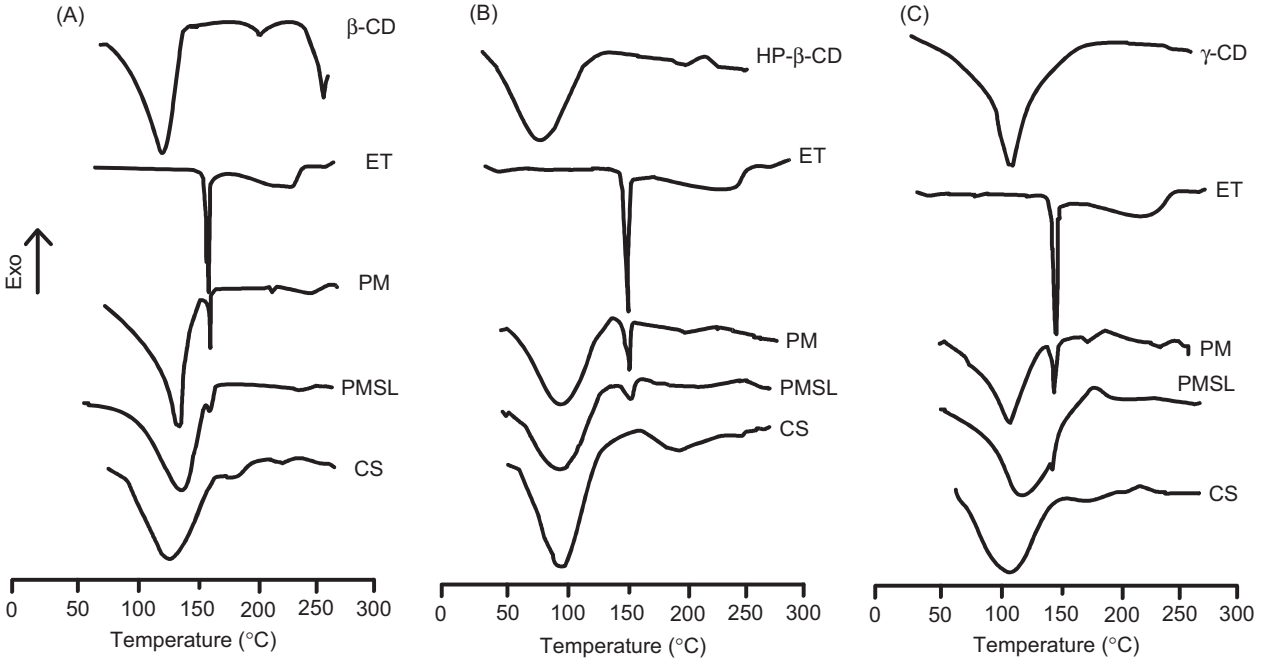


Figure 3. DSC curves of pure β-CD, HP-β-CD, γ-CD, ET, and of ET/β-CD (A), ET/HP-β-CD (B), and ET/γ-CD (C) 1:1 (mol/mol) systems: physical mixture (PM), physical mixture of separately lyophilized components (PMSL), and colyophilized sample (CS).

to PMSL and to CS. These results paralleled those from DSC studies and confirmed that different solid products were obtained as a function of the different preparation methods.

Figure 5A–C shows the IR spectra in the C=O stretching region of ET (carboxyl carbonyl band at 1744 cm^{-1}), β-CD, HP-β-CD, and γ-CD and of their respective equimolar solid systems (PM, PMSL, and CS). The strong carbonyl band, of diagnostic value for the characterization of the systems, was clearly observed in the spectra of all PMs (which were substantially the superimposition of those of CDs and ET) and was still evident for PMSLs. In contrast, for CSs it was noticeably reduced, broadened, and shifted to a lower wave number (around 1716 cm^{-1}). This suggests the existence of drug/CD interactions involving hydrogen bonds in CSs. Therefore, we can assume that the freeze-drying procedure yields to a new solid phase consisting of a monomolecular dispersion of drug and CDs[30]. This conclusion is consistent with the results of phase solubility studies, which indicated the complexation between the drug and the CDs in the solution[31,32]. Indeed, CSs were prepared using a technique widely recognized as saving in the solid state the interaction forms existing in the solution.

Dissolution studies

Figure 6A–C illustrates the dissolution profiles of ET alone and of ET/CD binary systems. As can be seen, all the blends exhibit faster drug dissolution than ET alone. At each time point, the amount of ET dissolved from the samples was higher with respect to ET alone. The results in terms of DP within 5 minutes, DE measured after 60 minutes, and RDR at t = 5 minutes are summarized in Table 2. It is clear that the formulation of ET with the CDs significantly increased DP and RDR (maximum RDR value = 3.7). As expected, DE values, which accounted for the total dissolution process, were also positively affected by the presence of CDs. The enhancement of these indexes, particularly DP, is known to improve drug bioavailability. For every CD considered, the dissolution rate changed in the order PM < PMSL < CS, the latter approaching 100% of drug dissolved after 5 minutes (see DP values in Table 2). These differences, related to

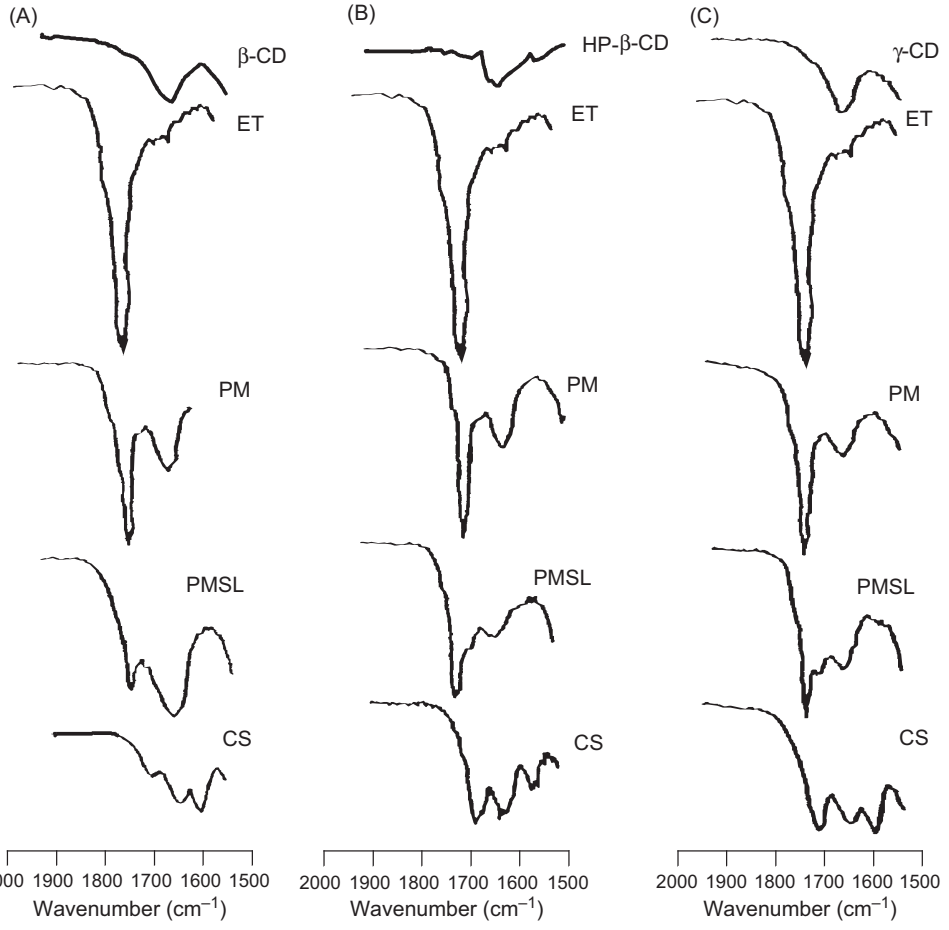


Figure 5. FTIR spectra of pure β-CD, HP-β-CD, γ-CD, ET, and of ET/β-CD (A), ET/HP-β-CD (B), and ET/γ-CD (C) 1:1 (mol/mol) systems: physical mixture (PM), physical mixture of separately lyophilized components (PMSL), and colyophilized sample (CS).

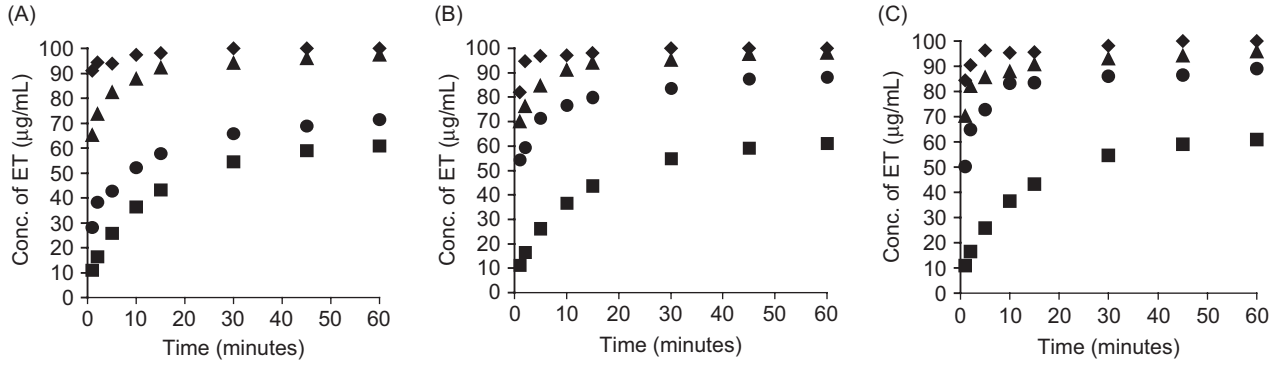


Figure 6. Dissolution curves of ET (■) and of the different equimolar systems ET/β-CD (A), ET/HP-β-CD (B), and ET/γ-CD (C): physical mixture (●), physical mixture of separately lyophilized components (▲), and colyophilized sample (◆) (mean of three experiments, CV < 3%, error bars omitted for the sake of clarity).

Table 2. Percent of active ingredient dissolved after 5 minutes (DP), dissolution efficiency at $t = 60$ minutes (DE) and relative dissolution rate (RDR) at $t = 5$ minutes of ET and ET/CD binary systems: Physical mixture (PM), physical mixture of separately lyophilized components (PMSL), and colyophilized sample (CS).

Sample	DP	DE	RDR
ET	25.9 ± 0.7	43.9	1
PM ET/β-CD	42.7 ± 0.9	57.6	1.6
PM ET/HP-β-CD	71.3 ± 1.4	78.4	2.8
PM ET/γ-CD	72.8 ± 1.2	81.9	2.8
PMSL ET/β-CD	82.3 ± 1.7	88.3	3.2
PMSL ET/HP-β-CD	84.6 ± 1.8	90.7	3.3
PMSL ET/γ-CD	85.5 ± 2.0	88.4	3.3
CS ET/β-CD	94.0 ± 2.1	98.3	3.6
CS ET/HP-β-CD	97.0 ± 2.4	96.8	3.7
CS ET/γ-CD	96.3 ± 1.9	95.7	3.7

the preparation method adopted, are parallel to both the amorphization degree of the binary systems and the occurrence of interactions at the solid state[33], as observed by DSC, X-ray, and IR studies. However, it is important to note that ET dissolution rate (DP = 25.9) was already appreciably improved in PMs with HP-β-CD and γ-CD (DP = 71.3 and 72.8, respectively). In contrast, only a slight improvement in the dissolution profile of ET was observed for PM with β-CD (DP = 42.7), despite this CD was as effective as HP-β-CD and γ-CD in the amorphous mixtures (PMSL and CS). Thus, β-CD gained the greatest advantage in terms of dissolution rate by the amorphization process, and only in its amorphous state showed optimal dissolving properties toward such a poorly water-soluble partner as ET (8.6 mg/100 mL).

Pharmacological studies

The analgesic activity of freeze-dried ET/CD products and of the drug alone was measured to evaluate the effects of the different CDs on the pharmacological activity of ET. Experiments were performed administering to

Table 3. Analgesic activity of ET, its equimolar colyophilized samples (CSs) with β-CD, HP-β-CD, and γ-CD (± indicates SEM of the respective values).

	Dose (mg/kg)	Number of contractions	Inhibition % versus control
Control	—	35 ± 5	—
Indomethacin	5	18 ± 5*	48
ET	3.5	23 ± 3*	34
CS ET/β-CD	3.5[a]	15 ± 4*	57
CS ET/HP-β-CD	3.5[a]	16 ± 3*	54
CS ET/γ-CD	3.5[a]	16 ± 4*	54

The experiments were carried out using groups of six mice; $n = 18$. *$P < 0.05$ compared with control. [a]Expressed as ET content.

mice two different ET doses, namely 3.5 and 35 mg/kg, either as pure drug or as CSs. No noticeable differences were found between CSs and ET alone at the higher dose (data not shown). In contrast, at the lower dose, all CSs were more active than ET alone (Table 3) and even slightly more active than indomethacin at the same molar concentration. These results directly call into play the therapeutic application, as the lowest dose selected is of the same order of magnitude (expressed in mg/kg) as the single doses used in human therapy (200–300 mg). Therefore, CD formulations with reduced drug content could be used to achieve a given pharmacological effect. Moreover, all the samples containing CDs showed a substantial equivalent analgesic effect, indicating that the improvement in the pharmacological activity of ET was dependent on the greater water solubility of the mixtures, regardless of the kind of CD employed.

The experiments of gastric tolerance in rats were performed considering the dosage (35 mg/kg) of ET able to enhance the response. This was evaluated not only by taking into account the number of ulcerations but also their extent, following the arbitrary scale described in 'Material and Methods'. These results are summarized in Table 4. As can be seen, ET alone induced a mean

Table 4. Ulcerogenic activity of ET and its equimolar colyophilized samples (CSs) with β-CD, HP-β-CD, and γ-CD (± indicates SEM of the respective values).

Sample	Dose (mg/kg)	Number of ulcerations	Inhibition % versus ET	Ulcerogenic index	Inhibition % versus ET
HP-β-CD	165	0	—	0	—
Indomethacin	15	52 ± 3	—	2.61 ± 0.13	—
ET	35	33 ± 4	—	1.62 ± 0.09	—
CS ET/β-CD	35[a]	12 ± 2**	64	0.91 ± 0.06*	44
CS ET/HP-β-CD	35[a]	15 ± 1**	54	0.75 ± 0.04**	54
CS ET/γ-CD	35[a]	17 ± 2*	48	0.78 ± 0.05**	52

The experiments were carried out using groups of six rats; $n = 18$. *$P < 0.05$ compared with ET. **$P < 0.01$ compared with ET.
[a]Expressed as ET content.

number of ulcerations of 33 ± 4 with an ulcerogenic index of 1.6 ± 0.09. All CSs showed a significantly lower ulcerogenic activity than ET alone as the mean number of ulcerations was reduced by approximately 50% in the presence of the three CDs examined. Moreover, a significantly reduced severity of mucosal damage was observed. As a matter of fact, the mean score of the ulcerogenic indexes for all CSs was rated by values lower than unity, indicating only slight subepithelial vasocongestion. Similarly to the results of pharmacological activity studies, no significant difference was found to arise from the kind of CD employed.

These results suggest that the reduced gastrolesivity of ET in the presence of CDs arises from its enhanced water dissolution rate. In fact, this would hinder high local concentration and/or formation of crystalline drug aggregates in the gastric mucosa, thus minimizing the ulcerative damage of the tissues.

In conclusion, this study demonstrates that for industrial applications, HP-β-CD and γ-CD are better partners than β-CD for ET as they do not require expensive preparations to be effective on drug solubilization (simple physical mixing of drug with HP-β-CD and γ-CD gives rise to a noticeable improvement in dissolution rate, whereas liophilization is required with β-CD). However, HP-β-CD is to be considered the CD of choice because it is less expensive than γ-CD. Pharmacological studies gave evidences that ET/CD formulations, mainly ET/HP-β-CD, can be a valid therapeutic option allowing a reduction of drug content, without decreasing the analgesic activity and showing a gastrolesivity reduced by more than 50% in comparison to the drug alone.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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