Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 987–994
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Interaction of naproxen with ionic cyclodextrins in aqueous solution and in the solid state

P. Mura*, S. Furlanetto, M. Cirri, F. Maestrelli, G. Corti, S. Pinzauti

Dipartimento di Scienze Farmaceutiche, Università di Firenze, Polo Scientifico di Sesto Fiorentino, 50019 Sesto Fiorentino, Firenze, Italy

Received 27 April 2004; received in revised form 4 June 2004; accepted 7 June 2004
Available online 9 August 2004

Abstract

The possible role of the cyclodextrin charge in the interaction with an acidic drug such as naproxen (pK_a 4.8) has been evaluated. Sulfobutylether-ß-cyclodextrin (SBE-ßCyd) and trimethylammonium-ß-cyclodextrin (TMA-ßCyd) were selected as, respectively, anionically and cationically charged carriers and their performance was compared with that of the parent ß-cyclodextrin (ßCyd) and of its methyl-derivative (MeßCyd) previously found as the best partner for the drug. Interactions in solution were investigated by phase-solubility, fluorescence and circular dichroism analyses. Equimolar drug–carrier products prepared by different techniques (blending, cogrinding, sealed-heating, colyophilization) were characterized by differential scanning calorimetry and X-ray powder diffractometry and tested for drug dissolution properties. Anionic charges of SBE-ßCyd did not negatively influence interactions in unbuffered aqueous solutions (pH ≈5) with the acidic drug. In fact, it was a very effective carrier, exhibiting solubilizing and complexing properties considerably better than the parent ßCyd and comparable to those of MeßCyd. On the contrary, the positive charges of TMA-ßCyd did not favour interactions with the counter-ionic drug (despite the presence of about 60% ionised drug) and it was less efficacious also than native ßCyd. Therefore, the role of the Cyd charge on the complexing and solubilizing properties towards naproxen was not important whereas other factors, such as steric hindrance effects and favourable hydrophobic interactions were significant in determining the drug affinity for the Cyd inclusion. Solid state studies evidenced similar amorphizing properties of both charged Cyds towards naproxen. On the other hand, dissolution tests, in agreement with solution studies, showed that all products with SBE-ßCyd exhibited significantly better dissolution properties than the corresponding ones with TMA-ßCyd. A clear influence of the preparation method of drug–Cyd solid systems on the performance of the end product was also observed. Colyophilization was the most effective technique, followed by the cogrinding one. Colyophilized product with SBE-ßCyd allowed a 10-times increase in drug dissolution efficiency (D.E.) (with respect to the five-times increase obtained with the corresponding coground product) and a reduction of $t_{50\%}$ from about 60 min (for the coground product) to less than 2 min.

Keywords: Naproxen; Trimethylammonium-ß-cyclodextrin; Sulfobutylether-ß-cyclodextrin; Circular dichroism; Fluorescence; Dissolution

1. Introduction

Naproxen ((*S*)-(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid) is a non-steroidal antiinflammatory drug whose very low water solubility (about 27 mg L^{-1} at 25 °C) can be significantly improved by complexation with native ß-cyclodextrin [1] and even more so with alkyl- and hydroxyalkyl-ß-cyclodextrin-derivatives [2–6]. The several studies performed in this regard have made it possible to highlight the important role of the nature of the cyclodextrin derivatives substituent in determining their solubilizing and complexing power towards naproxen and indicated the methyl-ß-derivative as the most efficient partner for this drug [2–8].

In the present work, we thought it worthy of interest to extend the study to some recently available ionic cyclodextrins, with the objective of evaluating the possible role of the cyclodextrin charge in the interaction with an acidic drug such as naproxen (pK_a 4.8 [9]). Sulfobutylether-ß-cyclodextrin

* Corresponding author. Tel.: +39 055 4573672; fax: +39 055 4573671.
E-mail address: mura@unifi.it (P. Mura).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.06.016

and trimethylammonium-ß-cyclodextrin were selected as, respectively, anionically and cationically charged carriers and their performance towards naproxen was compared with that of the parent ß-cyclodextrin and of the methyl-ß-cyclodextrin (the best drug partner among the previously examined neutral ß-derivatives [7]).

Phase-solubility analysis at different temperatures, fluorescence and circular dichroism analyses were used to investigate the interaction of naproxen with the ß-cyclodextrin derivatives in solution.

As for the study of the drug–carrier interaction in the solid state, because there is not a single method or process for obtaining solid inclusion complexes, and the best process must be developed for each guest to be complexed with each cyclodextrin [10–12], equimolar products of the drug with each examined cyclodextrin were prepared by different techniques (blending, cogrinding, sealed-heating, and colyophilization) in order to investigate the influence of the preparation method on the physico-chemical properties of the end product and to select the most effective system for improving the drug dissolution properties. Differential scanning calorimetry (DSC) and X-ray powder diffractometry were used to check and evaluate the crystallinity of naproxen in the different equimolar drug–cyclodextrin systems and to evaluate possible drug–carrier interactions in the solid state, whereas the drug dissolution properties from the various binary combinations were determined according to the dispersed amount method.

2. Materials and methods

2.1. Materials

Naproxen (NAP) and ß-cyclodextrin (ßCyd) were from Sigma Chemical Co. (St. Louis, MO, USA). Trimethylammoniumpropyl-ß-cyclodextrin (TMA-ßCyd) and methyl-ß-cyclodextrin (MeßCyd) were kindly donated by Wacker-Chemie (Munich, D). Sulfobutylether-ß-cyclodextrin (SBE-ßCyd) (Captisol) was a gift from CyDex Inc. (Texas, USA). All other materials and solvents were of analytical reagent grade.

2.2. Phase-solubility studies

Solubility measurements of NAP were carried out by adding 30 mg of drug to 20 mL of water or aqueous solution of ßCyd-derivative in the 5–25 mM concentration range, in a sealed glass container which was electromagnetically stirred (500 rpm) at a constant temperature (25, 37 or 45 °C) until equilibrium (3 days). An aliquot was then withdrawn and filtered (pore size 0.45 μm), and the NAP concentration was determined by a second derivative ultraviolet absorption method at 274 nm [1]. Each experiment was performed in triplicate (coefficient of variation, C.V. <2%). Apparent 1:1 stability constants were calculated from the straight line portion of the phase-solubility diagrams, according to Higuchi and Connors [13].

2.3. Fluorescence spectra

Measurements were carried out using a Perkin-Elmer Mod. 650-10 S spectrofluorimeter. The excitation and emission slits were fixed at 5 and 2.5 nm, respectively. The fluorescence intensities of the 0.015 mM NAP unbuffered (pH ≈5) aqueous solution in the absence and in the presence of either TMA-ßCyd or SBE-ßCyd at concentration values of 1.5 mM were measured at excitation and emission maxima of 330 and 358 nm, respectively. The scan rate was selected at 240 nm/min.

2.4. Circular dichroism spectra

Spectra of unbuffered (pH ≈5) 0.4 mM aqueous solution of NAP in the absence and in the presence of MeßCyd, or TMA-ßCyd or SBE-ßCyd (at a concentration of 0.4 mM) were obtained with a Jasco J-500D recording spectropolarimeter (sensitivity, 0.5 m° cm^{-1}; time constant, 16; chart speed, 10 cm min^{-1}; wavelength expansion, 10 nm cm^{-1}; scan speed, 50 cm min^{-1}; range, 350–230 nm).

2.5. Preparation of drug–Cd solid systems

Four different methods were used for the preparation of equimolar drug–Cyd solid systems. Physical mixtures (P.M.) were obtained by 15 min tumble mixing equimolar amounts of the respective components (75–150 μm sieve granulometric fraction). Coground products (GR) were prepared by 30 min ball-milling physical mixtures in a vibrational mill (Retsch, GmbH, Düsseldorf, Germany). Sealed-heated products (S.H.) were prepared by heating physical mixtures in a sealed container at 90 °C for 24 h. Colyophilized products (COL) were prepared by freeze-drying (Lyovac GT2, Leybold-Heraeus) at −50 °C and 1.3×10^{-2} mmHg equimolar drug–Cyd aqueous solutions on prechilled shelves of 20 cm diameter and 18 mm height. Each solid product was sieved and the 75–150 μm granulometric sieve fraction was used for the following tests.

2.6. Differential scanning calorimetry (DSC)

Temperature and enthalpy measurements were performed with a Mettler TA4000 apparatus equipped with a DSC 25 cell (10 K min^{-1}) on 5–10 mg (Mettler M3 microbalance) samples scanned in pierced Al pans under static air in the 30–200 °C range.

2.7. X-ray powder diffractometry

X-ray diffraction patterns were collected with a computer-controlled Philips PW 1800 apparatus in the 2–40° 2θ interval

(scan rate 1° min^{-1}), using a Cu Kα radiation monochromatized with a graphite crystal.

2.8. Dissolution studies

Dissolution rates of NAP alone (75–150 μm sieve granulometric fraction) or from the different equimolar systems with TMA-ßCyd and SBE-ßCyd (75–150 μm sieve granulometric fraction), were determined in water at 37 ± 0.3 °C according to the dispersed amount method [14]. Experiments were performed under non-sink conditions, by adding 60 mg of NAP or NAP equivalent to 75 mL of water, in a 400 mL beaker. A glass three-blade propeller was centrally immersed in the beaker and rotated at 100 rpm. Suitable aliquots were withdrawn with a filter-syringe (pore size 0.45 μm) at the specified times and assayed for NAP content as in the phase-solubility studies. The same volume of fresh medium was added to the beaker and the correction for the cumulative dilution was calculated [7]. Each test was repeated four-times (coefficient of variation, C.V. <4%). Dissolution efficiency (D.E.) was calculated from the area under the dissolution curve at time *t* and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time [15]. One-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls multiple comparison post test (Graph Pad Prism, Version 3) was used to evaluate the effect of both the binary system preparation method and the carrier type on the drug D.E. and percent dissolved at a given time.

3. Results and discussion

3.1. Interaction in aqueous solution

NAP solubility linearly increased in the presence of increasing concentrations of both TMA-ßCyd and SBE-ßCyd, according to the A_L type phase-solubility diagrams [13] (Fig. 1a), analogously to that previously observed in the presence of both native [1] and randomly alkylated [2,5,6] ßCyds. The slopes of the straight lines were in all cases less than unity, thus indicating the formation of 1:1 complexes. The relative increase in NAP aqueous solubility in the presence of 25 mM of both ßCyd derivatives was more pronounced at the lowest tested temperature (25 °C) (Fig. 1b) and was about 80-times the NAP solubility for the SBE-derivative and about 35-times for the TMA-derivative, in comparison with the about 90-times increase obtained in the same conditions with the methyl-ß-derivative [2]. On the other hand, the drug solubility increase in the presence of 13 mM ßCyd concentration (i.e. its saturation solubility at 25 °C) was about 19-times [1].

The apparent 1:1 formation constants of NAP inclusion complexes with SBE-ßCyd and TMA-ßCyd, calculated from the phase-solubility diagrams according to Higuchi and Connors [13], are shown in Table 1, together with the corresponding data previously obtained for NAP-ßCyd and NAP-MeßCyd inclusion complexes [3], for comparison purposes. In all cases, the stability constant values decreased with increasing the temperature, indicating the exothermal nature of the complexation process.

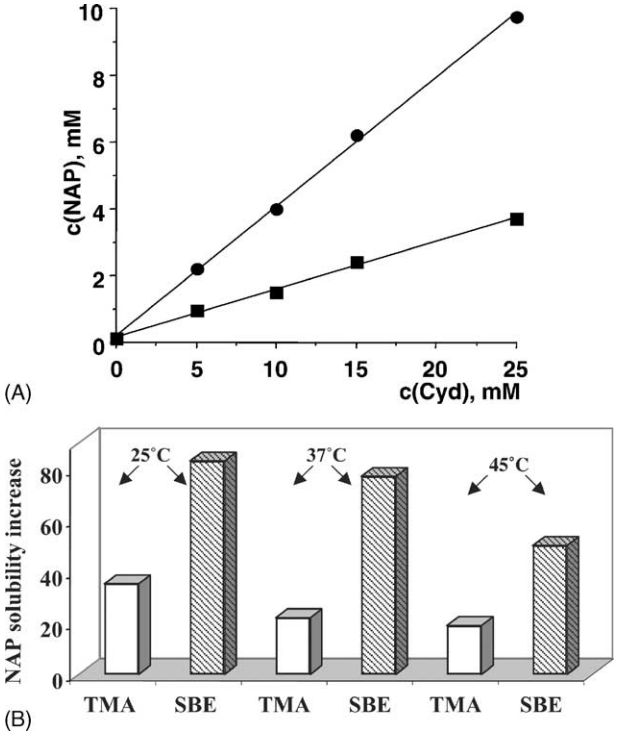

Fig. 1. (A) Phase-solubility diagrams of naproxen (NAP) and SBE-ßCyd (●) or TMA-ßCyd (■) in water at 25 °C; (B) Relative solubility increase of NAP aqueous solubility in the presence of 25 mM SBE-ßCyd (▨) or TMA-ßCyd (□) at different temperatures.

The stability constant of the drug complex with SBE-ßCyd at 25 °C was clearly higher than that with the parent ßCyd and similar to that with MeßCyd, which showed the highest complexing power towards naproxen among all the previously examined ßCyd-derivatives [2–7]. This result indicated the very high affinity between NAP and SBE-ßCyd and showed that its negative charge did not inhibit complexation of the acidic drug in unbuffered aqueous solution (pH ≈5), in spite of the presence of about 60% of the dissociated

Table 1
Stability constants for the interaction of naproxen with native ßCyd, Methyl-ßCyd (MeßCyd), sulfobutylether-ßCyd (SBE-ßCyd), trimethylammoniumpropyl-ßCyd (TMA-ßCyd) in aqueous solutions

Cyclodextrin	Solubilizing efficiency[a]	Apparent stability constant $K_{1:1}$ (L mol^{-1})		
		25 °C	37 °C	45 °C
ßCyd	19	1700	1390	–
MeßCyd	90	6890	5855	5135
SBE-ßCyd	80	5230	3760	3110
TMA-ßCyd	31	1400	940	795

[a] Relative NAP solubility increase at 25 °C (ratio between drug solubilities in 25 mM aqueous solution of Cyd, or 13 mM ßCyd, and in water).

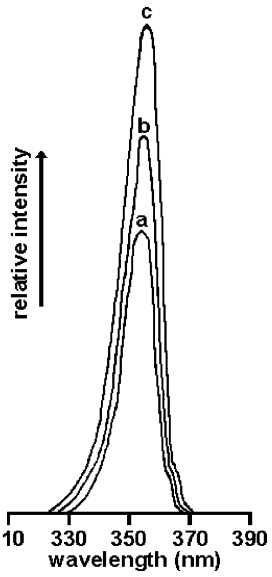

Fig. 2. Fluorescence emission spectra of naproxen (NAP) (*c*(NAP) 0.015 mM) in water in the absence (curve a) or in the presence of 1.5 mM SBE-ßCyd (curve b) or TMA-ßCyd (curve c).

anionic form, as determined from its pK_a value [9]. To explain the better performance of SBE-ßCyd with respect to the native carrier, it has been hypothesized, that, as in the case of the methyl-ß-derivative, the presence of substituents can extend the hydrophobic region of the Cyd cavity, thus favouring and stabilizing inclusion complexation of the hydrophobic guest molecule [3].

As for the positively charged TMA-ßCyd, differently from what was expected, no positive effects on the complex formation as a consequence of possible electrostatic interactions in aqueous solutions with the anionic drug were observed. On the contrary, unexpectedly, the binding constants of NAP with TMA-ßCyd were lower not only than those with all the other ßCyd-derivatives previously examined [2–7], but even than with the natural ßCyd, in spite of the higher water solubility of the chemically modified carrier. Evidently, serious steric hindrance effects, due to the presence of the substituents, hampered the inclusion of the guest molecules, thus reducing the potential attractive effects due to the Cyd positive charge. An analogous effect of steric blocking of the cavity was previously observed with some hydroxyalkyl-ßCyd derivatives, with a consequent (even though less intense) lowering of the binding constant of their complexes with NAP [5]. Moreover,

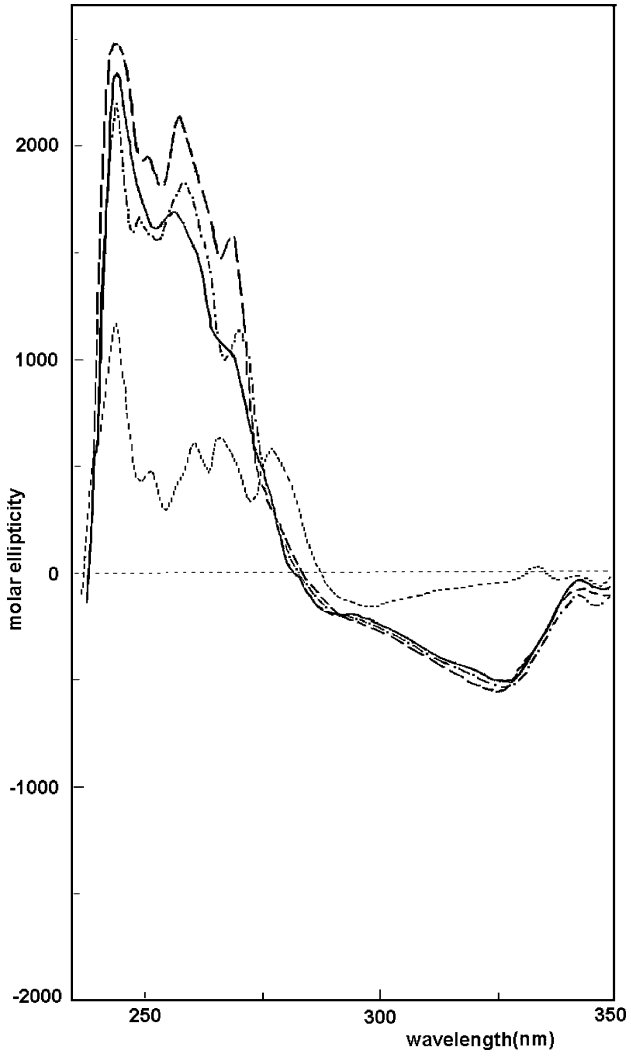

Fig. 3. Circular dichroism spectra of naproxen (NAP) (*c*(NAP) 0.4 mM) in water in the absence (···) and in the presence of 0.4 mM MeßCyd (- - -), SBE-ßCyd (- · -) or TMA-ßCyd (—).

ionic interactions and drug–Cyd interactions within the cavity could be in this case competitive with each other and did not cooperate in stabilizing the complex. In fact, when Cyd and drug molecules carry opposite charges, the drug molecule has to suitably adapt itself within the cavity in order to allow for the establishment of ionic interactions, eventually partly locating its hydrophobic portion outside the cavity; therefore, as a consequence, the interaction forces between

Table 2
Thermodynamic parameters for the interaction of naproxen with native ßCyd, Methyl-ßCyd (MeßCyd), sulfobutylether-ß-Cyd (SBE-ßCyd), trimethylammonium-propyl-ß-Cd (TMA-ßCd) in aqueous solutions

Cyclodextrin	$\Delta G^{\circ}_{25\,^{\circ}C}$ (kJ mol^{-1})	ΔH° (kJ mol^{-1})	$\Delta S^{\circ}_{25\,^{\circ}C}$ (kJ mol^{-1} K^{-1})
ß-Cyd	−18.5	−13.2	17.6
MeßCyd	−21.9	−11.5	35.1
SBE-ßCyd	−21.2	−20.5	2.5
TMA-ßCyd	−17.9	−22.6	−15.5

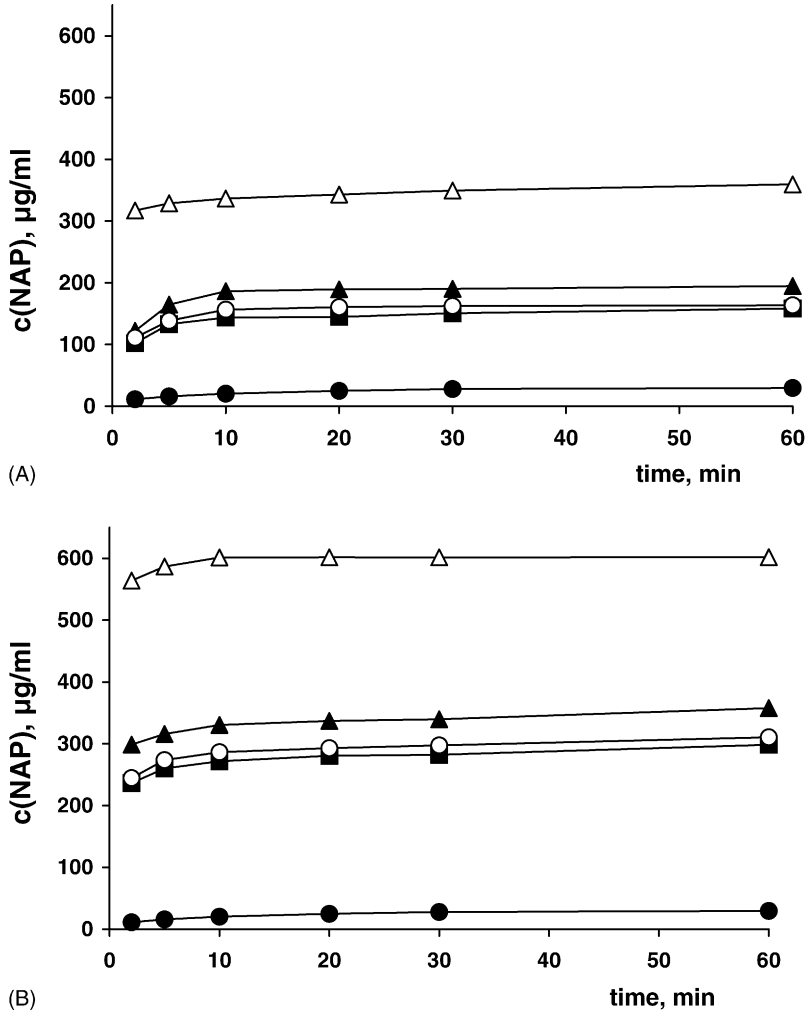

Fig. 4. Dissolution curves of naproxen (NAP) from its equimolar systems with TMA-ßCyd (A) and SBE-ßCyd (B) prepared by different methods. (●) NAP alone; (■) physical mixture; (◯) sealed-heated, (▲) coground and (△) colyophilized products (n = 4; coefficient of variation C.V. <4%).

drug and Cyd molecule within the cavity could be reduced [16].

The thermodynamic parameters, obtained from the temperature dependency of the apparent 1:1 stability constants of the inclusion complexes within the 25–45 °C temperature range in aqueous unbuffered solutions are collected in Table 2 together with the corresponding data previously obtained in the same experimental conditions for NAP-ßCyd and NAP-MeßCyd inclusion complexes [3], for comparison purposes. The obtained values accounted for a specific spontaneous interaction of NAP with Cyds in aqueous medium, as demonstrated by the negative ΔG changes, accompanied by negative enthalpy variations, indicative of a strong involvement of dipole–dipole interactions [17,18]. A contribution of hydrophobic bonding, involving the removal of ordered water molecules surrounding the apolar guest molecule inside the cavity can also be assumed by the positive entropy change observed in the case of both native ßCyd and its methyl- and sulfobutylether-derivatives [19,20]. On the contrary, in the case of TMA-ßCyd, a negative entropic variation was observed, which could be attributed to a different behavior of the solvent (i.e. of the water molecules) during complexation, as well as to a reduction in the rotational freedom of the guest molecule [20] stronger than with the other examined Cyds. These results suggested different host-guest interactions and/or complexation mechanism and inclusion mode of NAP with the cationic ßCyd-derivative. The effectiveness of such an hypothesis was then further investigated by fluorescence and circular dichroism studies.

NAP fluorescence increased in the presence of both examined Cyds, providing further experimental evidence that NAP-Cyd interactions occurred through insertion of a portion of the guest molecule into the host cavity (Fig. 2). A direct relationship was previously found between the intensity of NAP fluorescence change in the presence of a given Cyd and the stability constant of the corresponding drug–Cyd complex [21]. On the contrary, in the present case, the enhancement of fluorescence of NAP in the presence of SBE-ßCyd was lower than that observed with TMA-ßCyd at the same concentration level of SBE-ßCyd, in spite of the highest stability

constant of its complex with the drug. Therefore, this result seems to account for a different inclusion mode of the NAP molecule within these two macrocycles.

In circular dichroism spectra (Fig. 3), both negative and positive maxima of NAP showed a variation in intensity and some shifts in the presence of ßCyd derivatives. The MeßCyd and SBE-ßCyd induced similar modifications of the original NAP spectral pattern, indicating similar complexation mechanisms and inclusion modes of the drug into their cavities; on the contrary, the presence of TMA-ßCyd led to a different spectral pattern, characterized by a loss of resolution of the positive NAP peaks, which appeared as simple shoulders of the main peak at 245 nm. This can be considered the effect of different interactions and/or of a different fit between host and guest molecules in the case of the positively charged ßCyd-derivative.

3.2. Dissolution studies

The mean dissolution curves of NAP alone, and from the different equimolar binary systems with TMA-ßCyd and SBE-ßCyd are presented in Fig. 4. The results in terms of dissolution efficiency at 60 min, percent of active ingredient dissolved at 10 min, time to dissolve 50% drug and relative dissolution rate at 5 min are collected in Table 3. All the systems with Cyds exhibited faster dissolution rates than NAP alone. Statistically significant differences ($P < 0.001$) in terms of both dissolution efficiency and percent of dissolved drug were found between all the NAP-SBE-ßCyd systems and the corresponding ones with TMA-ßCyd, reflecting the stronger interaction of the drug with the SBE-derivative previously found in phase-solubility studies and pointing out the importance of the proper choice of the carrier. In addition, the extent of the dissolution enhancing effect was found to be dependent on the method adopted for the preparation of the solid systems. The same trend was observed with both Cyds:

Table 3
Dissolution efficiency at 60 min (D.E.), % drug dissolved at 10 min (D.P.), time to dissolve 50% drug and relative dissolution rate (RDR) of naproxen (NAP) from physical mixtures (P.M.), sealed-heated (S.H.), coground (GR) and colyophilized (COL) products with sulfobutylether-ßCyd (SBE-ßCyd) and trimethylammoniumpropyl-ßCyd (TMA-ßCyd) (mean of four determinations; coefficient of variation, C.V. <4%)

Sample	D.E.[a]	D.P.	$t_{50\%}$(min)	RDR[b]
NAP-TMA-ßCyd P.M.	18.6	18.0	≫60	8.4
NAP-TMA-ßCyd S.H.	19.9	19.6	≫60	8.7
NAP-TMA-ßCyd GR	23.5	23.3	≫60	10.3
NAP-TMA-ßCyd COL	43.9	42.1	≈60	20.7
NAP-SBE-ßCyd P.M.	35.6	34.0	≫60	16.4
NAP-SBE-ßCyd S.H.	37.3	35.8	≫60	17.2
NAP-SBE-ßCyd GR	43.0	41.3	≈60	19.9
NAP-SBE-ßCyd COL	76.0	75.2	<2	36.9

NAP alone: D.E. 3.8; D.P. = 1.3.
[a] Percentage of the area of the rectangle described by 100% dissolution in the same time.
[b] Ratio between amount of drug dissolved from a NAP-Cyd system and that dissolved from NAP alone at 5 min.

the colyophilization technique exhibited the highest dissolution rate, followed in order by cogrinding, sealed-heating and physical mixing. The improvement of the initial dissolution rate obtained with physical mixtures can be attributed to both improved drug particle wettability, due to the surfactant-like properties of the carrier, and "in situ" formation of readily soluble complexes in the dissolution medium [22,23]. On the other hand, the non significance ($P > 0.05$) of the difference in dissolution efficiency between these systems and the corresponding sealed-heated products seems to indicate the poor effectiveness of the sealed-heating method in promoting drug–Cyd interaction and complexation. The better dissolution properties of coground products ($P < 0.001$) could be due to the increased drug–carrier contact surface and the decrease of drug crystallinity obtained with the sample mechanical treatment, as well as to a phenomenon of at least partial drug inclusion complexation in the solid state. Finally the better performance of colyophilized compared to coground products ($P < 0.001$) can be ascribed to a higher solubility of NAP as a consequence of its in-depth interaction and more effective complexation with Cyd in the solid state, obtained by colyophilization, as well as to the high energetic amorphous state/reduction of drug crystallinity following complexation [22,23]. In particular, colyophilized prod-

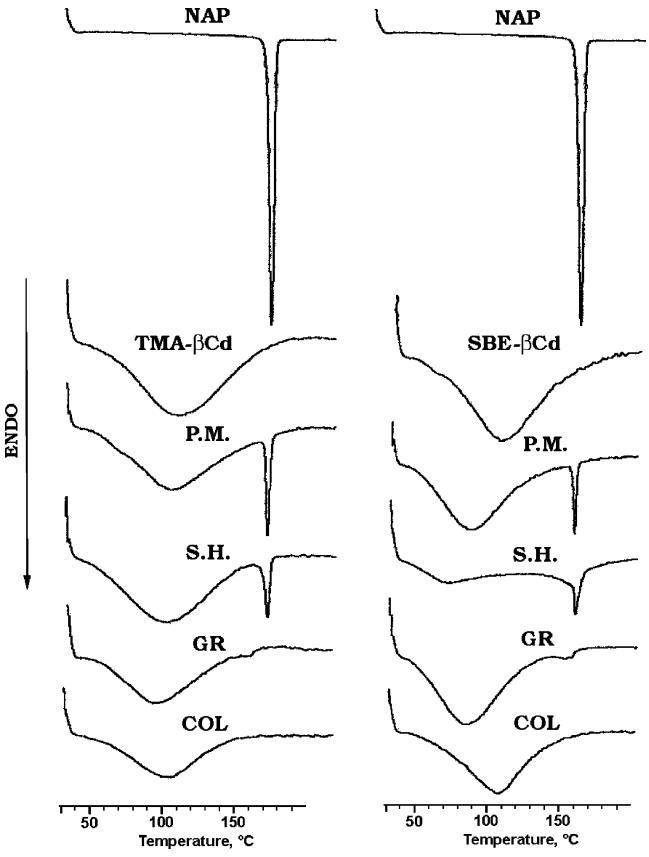

Fig. 5. DSC curves of pure naproxen (NAP), TMA-ßCyd and SBE-ßCyd and equimolar drug–Cyd physical mixtures (P.M.), sealed-heated (S.H.), coground (GR) and colyophilized (COL) products.

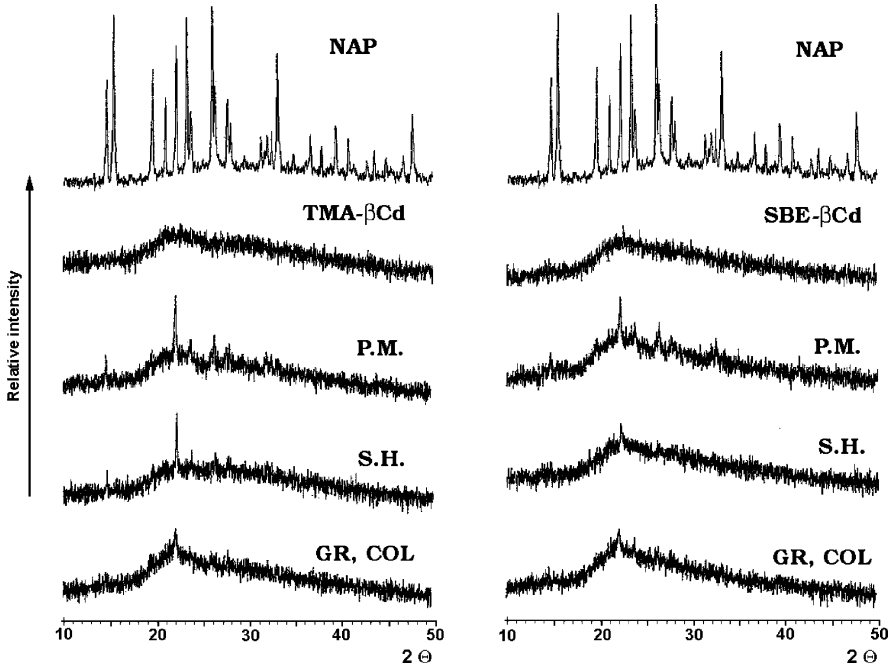

Fig. 6. X-ray diffractograms of pure naproxen (NAP), TMA-ßCyd and SBE-ßCyd alone and equimolar drug–Cyd physical mixtures (P.M.), sealed-heated (S.H.), coground (GR) and colyophilized (COL) products.

ucts with SBE-ßCyd were clearly the best, being immediately dispersed in water and giving more than 50% drug dissolved before 2 min.

3.3. Solid state studies

DSC curves of pure components and of the various drug–carrier binary systems are shown in Fig. 5. The DSC curve of NAP was typical of a crystalline anhydrous substance, showing a sharp endothermic peak (T_{onset} = 155.9 ± 0.14 °C, T_{peak} = 156.8 ± 0.17 °C, ΔH = 142.5 ± 1.3 J g^{-1} (six runs)) due to the drug melting. On the other hand, the DSC traces of both ßCyd-derivatives were indicative of their amorphous nature, only exhibiting a broad endothermic effect, ranged between 50 and 130 °C, due to their dehydration process. The characteristic thermal profile of the drug was present in both physical mixtures and remained well recognizable also in sealed-heated products, even though some size reduction and broadening of endothermic peak of drug was observed, indicative of some drug–Cyd interaction resulting in a loss of NAP crystallinity. On the contrary, all samples obtained by co-grinding and colyophilization showed the complete disappearance of the NAP thermal profile, indicating the formation of an amorphous solid dispersion, or the molecular incapsulation of the drug inside the Cyd cavity, or both the phenomena.

X-ray powder diffraction patterns (Fig. 6) confirmed the results of DSC analysis. An analogous behavior was observed in the different systems with both ßCyd-derivatives, which showed a similar amorphizing power towards the drug. The presence of free crystalline NAP in both the physical mixtures and sealed-heated products was revealed by few and broad peaks of low intensity which emerged on the diffuse background due to the amorphous carriers, indicating a clear loss of crystallinity of the drug. A hollow pattern, was instead observed in products prepared by both cogrinding and colyophilization, indicating that both these techniques were effective for complete drug amorphization and/or complexation.

4. Conclusions

Phase-solubility studies, performed for comparison purposes in the same experimental conditions of previous studies with neutral ßCyd-derivatives [2–7], highlighted very different complexing and solubilizing abilities towards NAP of SBE-ßCyd and TMA-ßCyd. However, differently from what was hypothesized, anionic charges of SBE-ßCyd did not negatively influence the drug–Cyd interactions in aqueous medium and the anionic Cyd was an optimal carrier for the drug, giving a performance notably better than the parent ßCyd and comparable to that of MeßCyd, previously found as the best partner for the drug [7]. On the contrary, the positive charges of TMA-ßCyd did not favour interactions with the counter-ionic drug and it was less effective also than native ßCyd, in spite of its higher water solubility. On the basis of these preliminary results, it can be concluded that the Cyd-derivative charge did not have an important role in favouring or hampering drug–carrier interactions in aqueous unbuffered solution. However, further studies at different pH values will be necessary to evaluate in depth the role of the Cyd charge

as a function of different ratios of ionized-unionized forms of the drug. Evidently, in the present conditions, other factors, such as steric hindrance effects and favourable hydrophobic interactions, were decisive in determining the drug affinity for the Cyd inclusion.

Dissolution test results were in agreement with those of phase-solubility studies. In fact, all products with SBE-ßCyd exhibited significantly better dissolution properties ($P < 0.001$) than the corresponding ones with TMA-ßCyd. The different performance shown by the two carriers was not attributable to stronger solid state interactions in the case of the SBE-derivative, since both Cyds exhibited analogous amorphizing properties towards the drug, as indicated by DSC and X-ray diffraction analysis results. On the contrary, the greater effectiveness of products with SBE-ßCyd was directly related to its stronger solubilizing and complexing properties, as demonstrated by phase-solubility studies. However, a clear influence of the preparation method on the performance of the end product was also observed. Colyophilization was clearly the most effective preparation method of drug–Cyd solid systems, followed by the cogrinding one. In particular, the colyophilized product with SBE-ßCyd allowed reduction of $t_{50\%}$ from about 60 min (for the coground product) to less than 2 min and an about 10-times increase in the initial drug D.E. (with respect to the about 5-times increase obtained with the corresponding coground product). Moreover, it should also be taken into account that the dissolution parameters obtained with colyophilized products with the cationic carrier were about 50% lower than those of the corresponding ones with SBE-ßCyd and not significantly different ($P > 0.05$) from those obtained with the anionic carrier by cogrinding, a much more economical and rapid technique.

Acknowledgment

Financial support from MIUR is gratefully acknowledged.

References

[1] G.P. Bettinetti, P. Mura, A. Liguori, G. Bramanti, Farmaco 2 (1989) 195–213.
[2] G.P. Bettinetti, P. Mura, F. Melani, F. Giordano, in: D. Duchêne (Ed.), Min. 5th International Symposium on Cyclodextrins, Editions de Santé, Paris, 1990, pp. 239–242.
[3] G.P. Bettinetti, F. Melani, P. Mura, M. Monnanni, F. Giordano, J. Pharm. Sci. 80 (1991) 1162–1170.
[4] G.P. Bettinetti, A. Gazzaniga, P. Mura, F. Giordano, M. Setti, Drug Dev. Ind. Pharm. 18 (1992) 39–53.
[5] P. Mura, G.P. Bettinetti, F. Melani, A. Manderioli, Eur. J. Pharm. Sci. 3 (1995) 347–355.
[6] G.P. Bettinetti, P. Mura, M.T. Faucci, M. Sorrenti, M. Setti, Eur. J. Pharm. Sci 15 (2002) 21–29.
[7] P. Mura, G.P. Bettinetti, M.T. Faucci, M. Sorrenti, A. Negri, Supramol. Chem. 11 (2001) 379–391.
[8] P. Mura, M.T. Faucci, F. Maestrelli, S. Furlanetto, S. Pinzauti, J. Pharm. Biomed. Anal. 29 (2002) 1015–1024.
[9] K. Okimoto, R.A. Rajewski, K. Uekama, J.A. Jona, V.J. Stella, Pharm. Res. 13 (1996) 256–264.
[10] D.O. Thompson, Crit. Rev. Ther. Drug Carrier Syst. 14 (1997) 1–104.
[11] P. Mura, M.T. Faucci, P.L. Parrini, S. Furlanetto, S. Pinzauti, Int. J. Pharm. 179 (1999) 117–128.
[12] P. Mura, M.T. Faucci, A. Manderioli, G. Bramanti, Int. J. Pharm. 193 (1999) 85–95.
[13] T. Higuchi, K.A. Connors, Adv. Anal. Chem. Instrum. 4 (1965) 117–150.
[14] H. Nogami, T. Nagai, I. Yotsuyanagi, Chem. Pharm. Bull. 17 (1969) 499–509.
[15] K.A. Khan, J. Pharm. Pharmacol. 27 (1975) 48–49.
[16] M. Màsson, T. Loftsson, S. Jonsdottir, H. Fridriksdottir, D.S. Petersen, Int. J. Pharm. 164 (1998) 45–55.
[17] W.Q. Tong, J.L. Lach, T.F. Chin, J.K. Guillory, J. Pharm. Biomed. Anal. 9 (1991) 1139–1145.
[18] S. Tommasini, D. Ranieri, R. Ficarra, M.L. Calabrò, R. Stancanelli, P. Ficarra, J. Pharm. Biomed. Anal. 35 (2004) 379–387.
[19] H. Ueda, J.H. Perrin, J. Pharm. Biomed. Anal. 4 (1986) 107–110.
[20] C. Ravelet, A. Geze, A. Villet, C. Grosset, A. Ravel, D. Wouessidjewe, E. Peyrin, J. Pharm. Biomed. Anal. 29 (2002) 425–430.
[21] G.P. Bettinetti, P. Mura, F. Melani, M. Rillosi, F. Giordano, J. Inc. Phenom. 25 (1996) 327–338.
[22] S. Lin, Y. Kao, Int. J. Pharm. 56 (1989) 249–259.
[23] G. Dollo, P. Corre, M. Chollet, F. Chevanne, M. Bertault, J. Burgot, R. Verge, J. Pharm. Sci. 88 (1999) 889–895.