ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 52 (2001) 65–73

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

Complexation of phenytoin with some hydrophilic cyclodextrins: effect on aqueous solubility, dissolution rate, and anticonvulsant activity in mice

A. Latrofa[a], G. Trapani[a,*], M. Franco[a], M. Serra[b], M. Muggironi[b], F.P. Fanizzi[a,c], A. Cutrignelli[a], G. Liso[a]

[a]*Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università degli Studi di Bari, Bari, Italy*

[b]*Dipartimento di Biologia Sperimentale, Sezione di Neuroscienze, Università di Cagliari, Cittadella Universitaria Monserrato, Monserrato, Cagliari, Italy*

[c]*CARSO (Cancer Research Center), Valenzano, Bari, Italy*

Received 23 October 2000; accepted in revised form 9 February 2001

Abstract

The main objective of this study was to evaluate the influence of hydroxypropylated β- and γ-cyclodextrins and Me-β-cyclodextrin (HP-β-CD, HP-γ-CD, and Me-β-CD, respectively) on the dissolution rate and bioavailability of the antiepileptic agent, phenytoin (DPH). The corresponding solid complexes were prepared by a freeze-drying method and characterized by infrared spectroscopy, X-ray powder diffraction, and differential scanning calorimetry studies. Evidence of inclusion complex formation in the case of HP-β-CD was obtained by ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy. Drug solubility and dissolution rate in 0.05 M potassium phosphate buffer (pH 6) were notably improved by employing the β-CDs. Thus a 45% w/v HP-β-CD or Me-β-CD solution gave rise to an increase of dissolved drug of 420- and 578-fold, respectively. The Q_{10} (i.e. percentage of dissolved DPH at 10 min) was 5.2% for the pure drug and 93, 98, and 96% for DPH/HP-β-CD, DPH/HP-γ-CD, and DPH/Me-β-CD complexes, respectively. Moreover, it was found that in the maximal electroshock seizure test in mice the DPH/Me-β-CD complex exhibited anticonvulsant activity similar to DPH sodium salt (NaDPH).

Keywords: Phenytoin (DPH); Inclusion complexation; Hydroxypropylated β-cyclodextrin; Hydroxypropylated γ-cyclodextrin; Methyl-β-cyclodextrin; Water solubility; Dissolution rate; Maximal electroshock test

1. Introduction

Phenytoin (DPH) is an effective anticonvulsant drug which still remains one of the extensively used in epilepsy therapy [1]. Despite the existence of considerable experimental evidence for the usefulness of DPH in the treatment of several convulsive disorders, issues remain to be addressed concerning absorption, pharmacokinetic, and safety problems. Indeed, along with a non-linear pharmacokinetic and a narrow therapeutic index, DPH shows poor and erratic bioavailability following oral administration to patients [2,3]. This is probably due to its poor solubility in water and insufficient dissolution rate. For parenteral use, DPH has been formulated as a sodium salt in an aqueous alkaline medium at pH ~12 containing 40% propylene glycol and 10% ethanol [4]. Such a formulation allows concentrations as high as 50 mg/ml, but there is a serious risk of DPH precipitation at the injection site because, in physiological media (pH < 8), the sodium salt gives rise to the corresponding insoluble free acid [4,5]. The possibility that these issues can be addressed with methods employed for enhancing solubility and/or dissolution rate of poor soluble substances represents an opportunity for improving the therapeutic potential of DPH. For enhancing the dissolution rate of DPH, solid dispersions with water soluble carriers [6,7], complexation with cyclodextrins (CDs) [8–10], and many water-soluble prodrugs [4,11,12] have been developed. In particular, it has been recently reported by Savolainen et al. [13] that in dogs an approximate 2-fold increase in the bioavailability of DPH arises following oral administration of inclusion complexes with charged and neutral water-soluble cyclodextrins such as sulfobutylether-β-cyclodextrin (SBE_{7m}-β-CD) and hydroxypropylated β-cyclodextrin (HP-β-CD), respectively. However, the corre-

* Corresponding author. Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università degli Studi di Bari, Via Orabona 4, 70125 Bari, Italy. Tel.: +39-80-544-2764; fax: +39-80-544-2724.

E-mail address: trapani@farmchim.uniba.it (G. Trapani).

0939-6411/01/$ - see front matter
PII: S0939-6411(01)00144-8

sponding solid inclusion complexes have been prepared by freeze-drying a NaOH solution of DPH (pH 11.0) containing the appropriate CD. Thus, NaDPH has been used. More recently, Tanino et al. [14] reported on the effect of sugar-modified β-CDs on dissolution and absorption characteristics of DPH. These authors prepared such complexes by freeze-drying aqueous ammonium solutions, of unspecified strength, containing the cyclic oligosaccharide-CD and DPH in the equimolar ratio 1:1.

The main objective of the present study was to assess the effectiveness of some hydrophilic and neutral cyclodextrins (i.e. HP-β-CD, methyl-β-cyclodextrin (Me-β-CD), and 2-hydroxypropyl-γ-cyclodextrin (HP-γ-CD)) in enhancing the water solubility and dissolution rate of DPH as a free acid. Therefore, we prepared each complex in acidic medium and characterized them by the usual physicochemical methods, including Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC) and X-ray analysis. Phase solubility diagrams and dissolution profiles of the complexes were constructed and comparatively evaluated. Furthermore, an additional purpose of the present work was to explore the anticonvulsant activity of these CD-based formulations.

2. Materials and methods

2.1. Materials

2-Hydroxypropyl-β-cyclodextrin was obtained as a gift from Roquette (Cassano Spinola, Italy). Its substitution degree (5.88) was determined by us following the ^{1}H-nuclear magnetic resonance (NMR) method described in the literature [15]. 2-Hydroxypropyl-γ-cyclodextrin and randomly methylated-β-cyclodextrin having substitution degree of 0.6 and 1.8, respectively, were kindly donated from Waker-Chemie (Peschiera Borromeo, Italy). Their substitution degree was determined from the supplier by NMR analysis and not checked by us. DPH and 5-*p*-tolyl-5-phenylhydantoin (TPH) were purchased from Sigma-Aldrich (Milan, Italy). Tablets of Dintoina® (Recordati, Milan, Italy) containing NaDPH were purchased from a drugstore. Reagents used for the preparation of the buffers were of analytical grade. Fresh deionized water from all glass apparatus was used in the preparation of all the solutions. All the chemicals/reagents were used as obtained.

2.2. HPLC analysis

HPLC mobile phase was prepared from HPLC-grade methanol. High-performance liquid chromatography (HPLC) analyses were performed with a Water Associates (Vimodrone, Italy) Model 600 pump equipped with a Water 990 variable wavelength UV detector, a Waters 712 WISP autosampler, and a 20 μl loop injection valve. For analysis, a reversed phase Simmetry C_{18} (25 cm × 4.6 mm; 5 μm particles) column in conjunction with a precolumn insert was eluted with mixtures of methanol and deionized water (65:35). A flow rate of 0.8 ml/min was maintained. Quantification of the compounds was carried out by measuring the peak areas in relation to those of standards chromatographed under the same conditions. Standard curves were prepared at a wavelength of 225 nm using methanol as the solvent and were linear ($r^2 > 0.998$) over the range of concentrations of interest (23–470 μg/ml).

2.3. Preparation of drug/CD solid complexes

In preparing the solid DPH/CD inclusion complexes by freeze-drying method, DPH (3.4 mmol) was equilibrated with an equimolar amount of CD (3.4 mmol) in 20 ml of deionized water. The resulting mixture was stirred at room temperature for 5 days, then filtered through a 0.22-μm membrane filter, and the clear filtrate subjected to freeze-drying (Edwards (Trezzano sul Naviglio, Italy) model 680 freeze-drier). In similar way was prepared the solid 5-*p*-tolyl-5-phenylhydantoin (TPH)/HP-β-CD complex. The samples so obtained were stored in desiccator until their further manipulation. The incorporation degrees were calculated by HPLC analysis of each complex dissolved in 0.05 M potassium phosphate buffer, pH 6.

2.4. NMR measurements

^{1}H-NMR spectra of DPH, 5-*p*-tolyl-5-phenylhydantoin (TPH) and corresponding complexes with HP-β-CD were recorded at 25°C using a Bruker (Milan, Italy) AM 300 WB (300 MHz) spectrometer. ^{13}C-NMR and 2D NOESY experiments were performed on a Bruker AVANCE 500 DRX (500 MHz) instrument. The samples for NMR measurements were prepared by dissolving 34 mg of DPH, or DPH/HP-β-CD or MDPH in 0.6 ml of NaOD 0.2 N in D_2O. This mixture allows a suitable solubilization of the free drugs and complexes for these experiments. ^{13}C-NMR chemical shifts were referred to dioxane (67.8 ppm) as internal standard.

2.5. Fourier transform infrared spectroscopy

Fourier transform IR spectra were obtained on a Perkin–Elmer (Monza, Italy) 1600 FTIR spectrometer. Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 450–4000 cm^{-1} and the resolution was 1 cm^{-1}.

2.6. X-ray analysis

Powder X-ray diffraction patterns were recorded on a Philips (Monza, Italy) PW 1800 powder X-ray diffractometer using Ni-filtered, CuKα radiation, a voltage of 45 kV and a current of 25 mA.

2.7. Differential scanning calorimetry

DSC curves were obtained by a Perkin–Elmer DSC 7, equipped with a thermal analysis automatic program.

Aliquots of about 5 mg of each sample were placed in an aluminum pan of 50 μl capacity and 0.1 mm thickness, press-sealed with a not perforated aluminum cover of 0.1 mm thickness. An empty pan sealed in the same way was used as reference. Thermograms were measured by heating the sample from 30 and 330°C at a rate of 10°C/min, under a nitrogen flow of 20 cm^3/min. Indium was used as standard for calibrating the temperature. Reproducibility was checked running the sample in triplicate.

2.8. Solubility studies

Solubility measurements of DPH were carried out at 25°C using various CDs aqueous solutions. In these studies, the cyclodextrin solutions were made using 0.05 M potassium phosphate buffer, pH 6. In these conditions we can assume that DPH is as a free acid, taking into account its pK_a value (i.e. 8.3). A large excess of the antiepileptic agent was added to 2 ml of the appropriate CDs solution in screw-capped test tubes. The mixtures were vortexed for about 5 min and shaken in a thermostatically controlled water bath shaker for 4 days. Then, an aliquot of aqueous phase of each mixture was transferred to a 10-ml glass syringe preheated at the appropriate temperature and filtered through a 0.22-μm membrane filter (Millipore (Vimodrone, Italy), cellulose acetate) in thermostated test tubes. The filtrates were allowed to stand at 25°C until analyzed by HPLC. Samples were analyzed directly or diluted when needed with mobile phase. The injection volume was 20 μl. The apparent 1:1 stability constant (K_c) was estimated from the slope of the straight line of the phase-solubility diagram according to the following equation: $K_c = \text{slope}/S_0(1 - \text{slope})$ [16]. The solubility values (S_0) of DPH were directly determined in 0.05 M potassium phosphate buffer (pH 6) at 25°C.

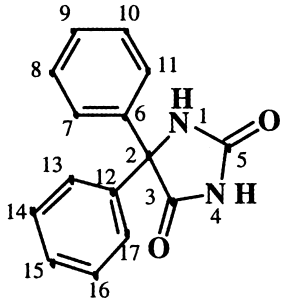
2.9. Dissolution studies

Dissolution experiments were carried out in triplicate with an Erweka (Seveso, Italy) DT dissolution test in deionized water at 37°C using the paddle method at a rotation speed of 60 rev./min. Samples of each preparation equivalent to 10 mg of DPH were added to the dissolution medium (400 ml of 0.05 M potassium phosphate buffer (pH 6.0) at 37 °C). At appropriate time intervals, 2 ml of the mixture were withdrawn, filtered through a 0.22 μm membrane filter (Millipore, cellulose acetate) in thermostated test tubes. Samples were withdrawn from a zone roughly midway between the surface of dissolution medium and the top of the rotating blade. The initial volume of dissolution was maintained by adding 2 ml of dissolution medium as such. About 1 ml of the clear filtrate was allowed to stand in bath at 37°C until analyzed by HPLC. The injection volume was 20 μl. The results were computed with a standard calibration curve of the drug prepared as above reported.

2.10. Pharmacological studies

Male CD-1 mice with body masses of 30 g (Charles River, Como, Italy) were used. Animals were housed in groups of 15–20 and kept in a temperature-controlled (23 ± 2°C) and humidity-controlled (65%) room on 12:12-h light/dark cycle (lights on 08:00–20:00 h). Food and water were freely avail-

Table 1
C chemical shifts corresponding to DPH in absence and presence of HP-β-CD



Carbon number	δ_{free}	$\delta_{complex}$	$\Delta\delta$[a]
2	75.46	Not detected	Not detected
3	193.28	191.20	−2.08
5	175.17	174.66	−0.51
6 and 12	143.24 and 141.88	142.84 and 142.05	−0.40 and 0.17
7, 11, 13, and 17	129.95 and 129.61	129.33 and 129.34	−0.62 and −0.27
8, 10, 14, and 16	128.42 and 128.17	128.13 and 127.75	−0.29 and −0.42
9 and 15	129.44 and 129.30	128.88 and 128.79	−0.56 and −0.51

[a] $\Delta\delta = \delta_{complex} - \delta_{free}$ DPH.

able, and the animals were acclimatized for > 7 days before use. Animal care and handling throughout the experimental procedure were in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC).

DPH/HP-β-CD and DPH/Me-β-CD complexes were suspended in distilled water with 0.5% (w/v) of Tween-80, while powdered tablets of NaDPH were used in water solution containing, for the sake of homogeneous comparison, the same amount of surfactant. Drugs were administered intraperitoneally at the equivalent dose of 12 mg/kg in a volume of 0.1 ml per 10 g of body mass. Drugs were administered to groups of six animals 30 min before induction of maximal electroshock (MES). Control mice received an equivalent volume of vehicle or vehicle and CD (HP-β-CD, Me-β-CD). Mice received an electroshock of 55 mA for 0.2 s with a frequency pulse/s of 50 Hz having a pulse-width duration of 0.4 ms, through intra-aural clip electrodes, sufficient to produce a hindlimb tonic extensor response in at least 67% of control animals. The complete suppression of the hindlimb extensor component of seizures was taken as evidence of anticonvulsant activity.

3. Results and discussion

3.1. NMR measurements

Evidence of inclusion complex formation for the DPH/HP-β-CD system was obtained by NMR spectroscopy. All the proton signals in the ^{1}H-NMR spectrum of the pure DPH are essentially located in a narrow range (7.1–7.3 ppm) which make proton assignments difficult. However, it was observed that in the ^{1}H-NMR spectrum of the DPH/HP-β-CD solid complex, the signals of the aromatic protons were split into two distinct groups. This might suggest that the drug molecule may interact with the CD cavity providing an inclusion complex. On the other hand, it was found that in the ^{1}H-NMR spectrum of TPH enantiomeric mixture, only a singlet attributable to protons of the methyl group occurs at 2.289 δ, whereas in the spectrum of the corresponding CD complex the methyl protons resonate at 2.335 and 2.348 δ, and this is clearly due to the chiral discrimination of TPH enantiomers induced by HP-β-CD complexation [17]. These data might that at least part of the DPH molecule

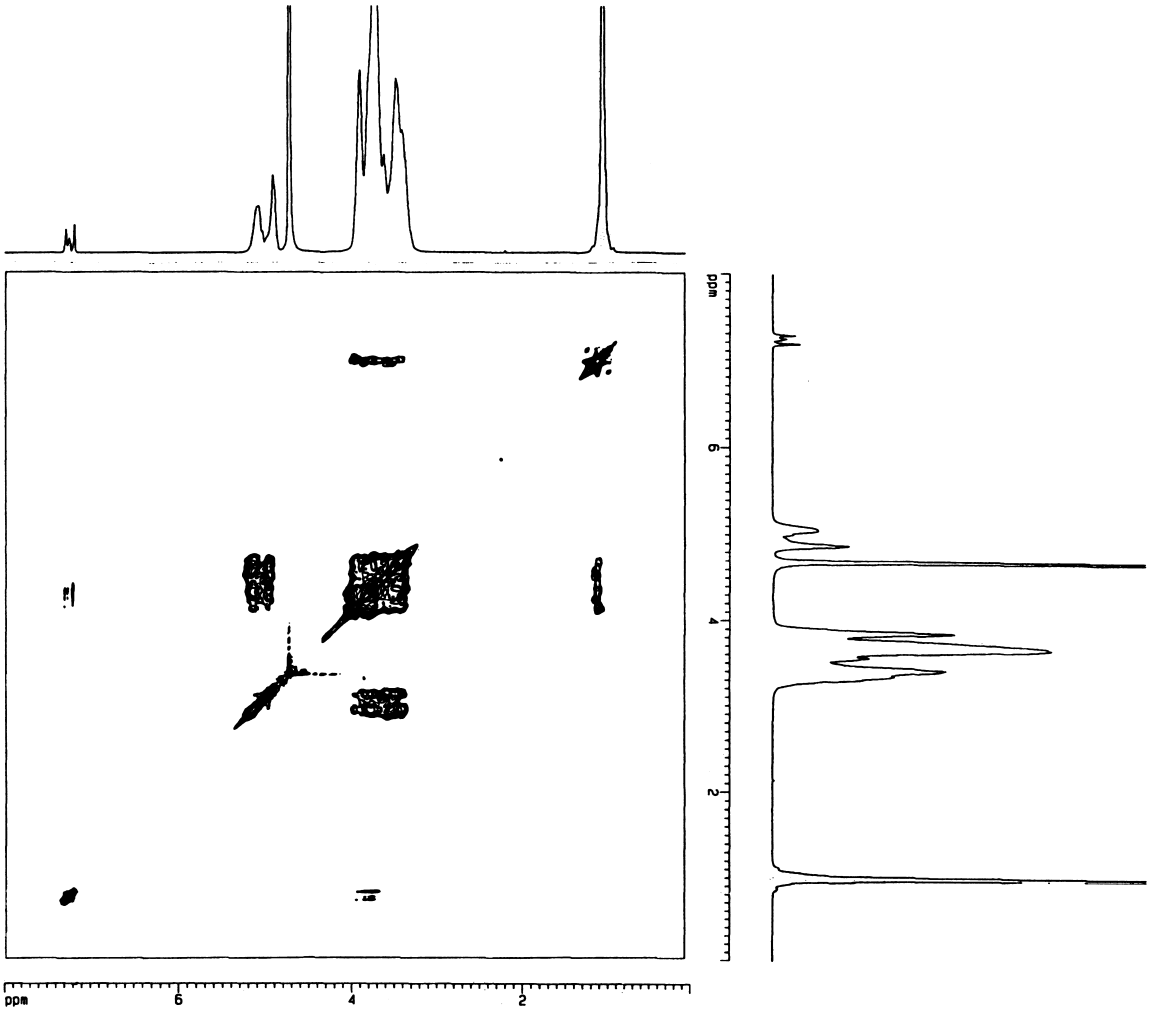


Fig. 1. 2D NOESY spectrum of DPH/HP-β-CD complex.

may be encapsulated from CD cavity. Further evidence of complex formation was obtained by a ^{13}C-NMR spectroscopic study. In this regard, it is well known that ^{13}C-NMR chemical shifts are very sensitive probes of molecular environments and they can be used to derive information on complexation [18,19]. The ^{13}C chemical shifts and carbon assignments made for DPH as well as the ^{13}C chemical shifts modifications upon complexation are shown in Table 1. For the guest molecule, the C-3 and aromatic carbons showed the most significant change in chemical shifts. These findings indicate that the C-3 and an aromatic ring of the DPH molecule should be located inside the cavity. To further support this suggestion, 2D NMR experiments on the DPH/HP-β-CD complex were performed. In the 2D NOESY spectrum of this complex (Fig. 1), cross-peaks between the aromatic protons of DPH and the H-3 proton of HP-β-CD were observed. These data are in good agreement with the inclusion mode suggested above.

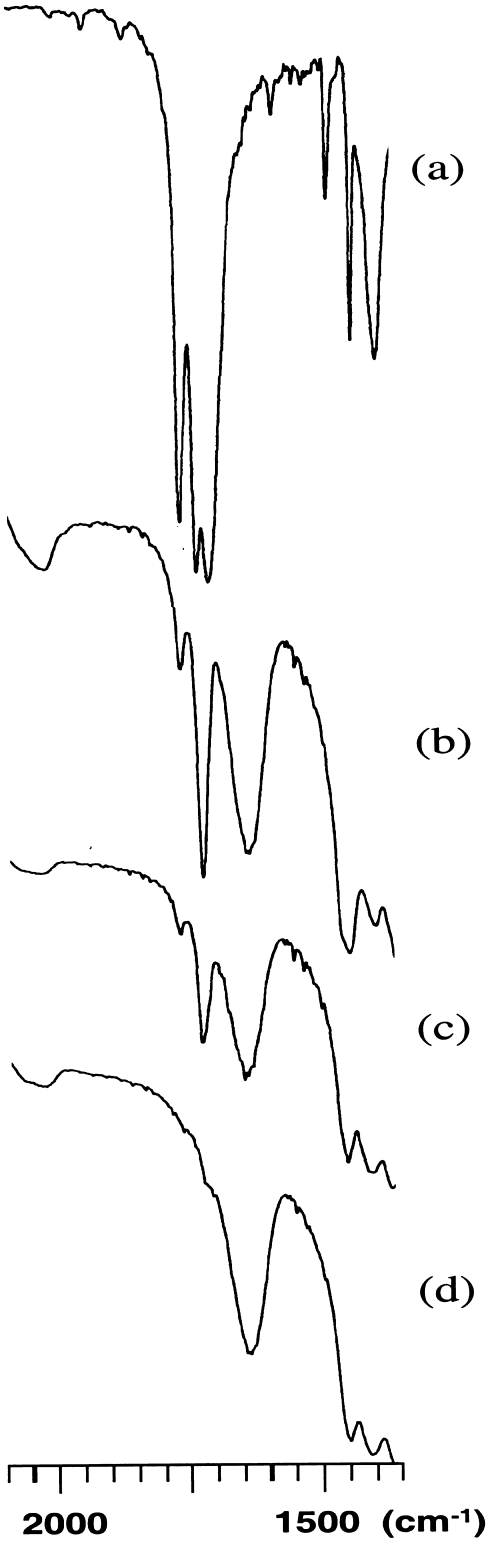


Fig. 2. FTIR spectra of DPH (a), DPH/Me-β-CD (b), DPH/HP-β-CD (c), DPH/HP-γ-CD (d), in the range 1400–2100 cm^{-1}.

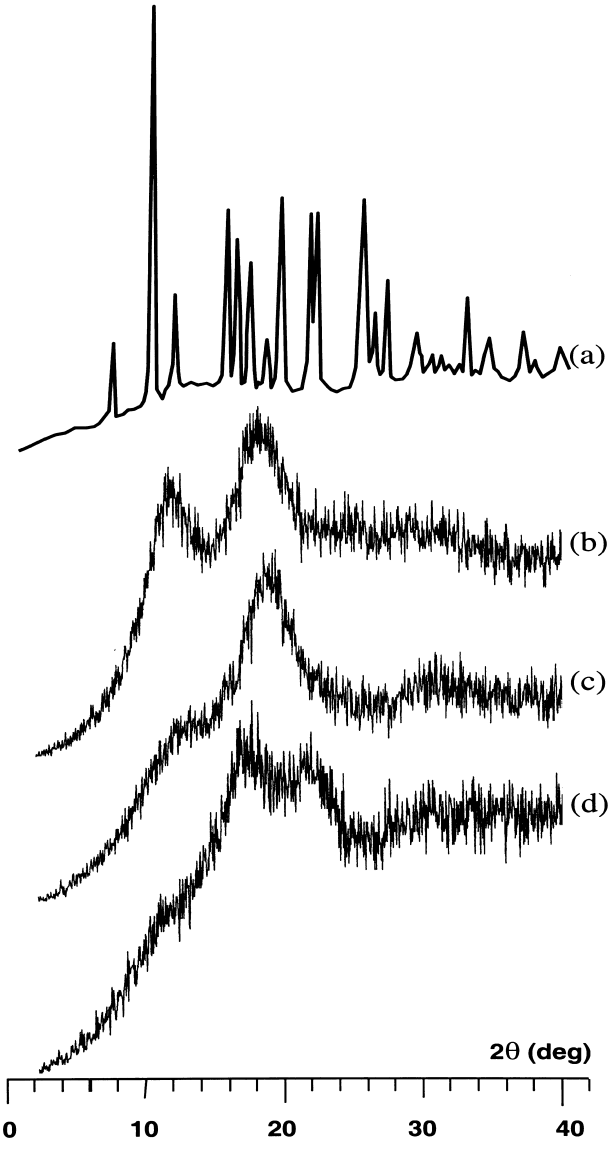


Fig. 3. X-ray diffraction patterns of DPH (a), DPH/Me-β-CD (b), DPH/HP-β-CD (c), DPH/HP-γ-CD (d).

3.2. Solid-state studies

The purpose of these studies was to estimate the possible interaction between DPH and CDs in the solid state as well as to evaluate the crystallinity degree of the DPH/CD systems.

3.2.1. Infrared spectroscopy

The FTIR absorptions in the range 1400–2100 cm^{-1} of DPH and corresponding solid CD complexes are shown in Fig. 2. The spectrum of DPH showed three strong absorption bands at 1716, 1738, and 1772 cm^{-1}, whereas in the spectra of DPH/HP-β-CD and DPH/Me-β-CD complexes, reduced absorption bands at 1727 and 1733 cm^{-1} are present together with a weak band at 1772 cm^{-1}. Such absorption bands are not detected in the DPH/HP-γ-CD complex. All the complexes showed a large absorption band in the range 1690-1720 cm^{-1}. The observed shifts of the DPH band occurring at 1738 cm^{-1} may suggest an interaction between DPH and CDs.

3.2.2. X-ray diffraction

Fig. 3 shows the X-ray diffraction patterns of DPH and corresponding complexes with CDs. In the X-ray diffractogram of DPH powder, sharp peaks at a diffraction angle of 2θ 8.51°, 11.27°,12.89°, 16.49°,17.19°, 18.10°, 20.28°, 22.31°, 22.78°, 25.76° are present and it suggests that the drug is present as a crystalline material. In contrast, the X-ray diffraction spectra of DPH-CD systems were characterized only by very large diffraction peaks. These results indicate that both DPH is no longer present as a crystalline material and its CD solid complexes exist in the amorphous state.

3.2.3. Differential scanning calorimetry

The thermograms of DPH and corresponding DPH-CD systems are shown in Fig. 4. The DSC curve of pure DPH showed a single endothermic peak at about 296°C, corresponding to the melting of the drug. Such a peak does not occur in the DSC curves of drug/CD complexes. These results confirm the suggestion from X-ray diffraction study, that DPH complexation with CDs leads to amorphous material.

3.3. Solubility studies

DPH is a weak acid with an apparent pK_a of 8.3 and is practically insoluble in water (i.e. 14 μg/ml at pH 7 and 20 μg/ml at pH 7.4 at 24°C) [20]. The intrinsic solubility (S_0) of DPH at pH 6 and at 25°C was 20 μg/ml, which is in good agreement with literature values [13,20] (Table 2). Solubility studies showed that the concentration of DPH at 25°C and pH 6.0 is notably affected by the presence of CDs. Thus, a 45% w/v HP-β-CD and Me-β-CD solutions provided for a 8.40 mg/ml and 11.57 mg/ml content of DPH corresponding to a 420- and 578-fold increase in the concentration of DPH, respectively. On the other hand, a 45% w/v HP-γ-CD solution provided for only a 1.20 mg/ml content of DPH. Therefore, the solubilizing effect of the hydrophilic cyclodextrins is in the following order: Me-β-CD > HP-β-CD > HP-γ-CD; moreover, the targeted solution concentration of 10 mg/ml, generally accepted for optimal oral and parenteral formulation, may be prepared by using only the DPH/Me-β-CD

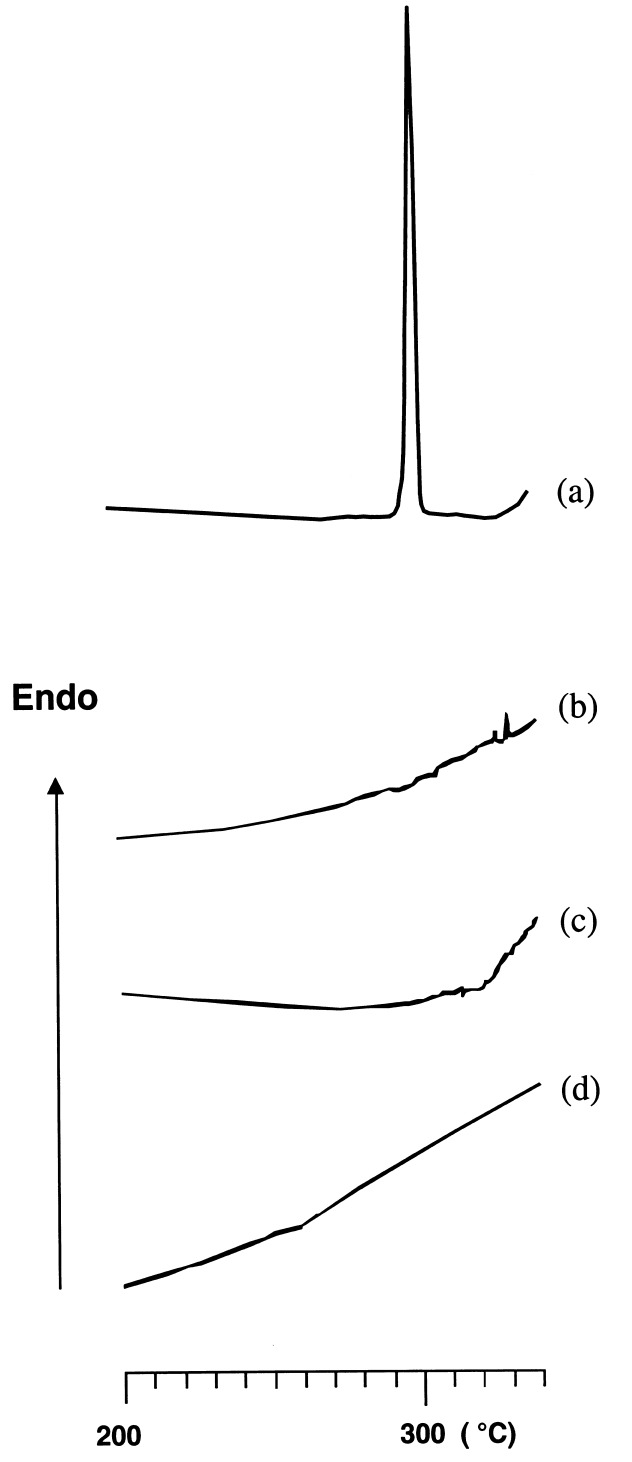


Fig. 4. DSC curves of DPH (a), DPH/HP-β-CD (b), DPH/Me-β-CD (c), DPH/HP-γ-CD (d).

complex. The phase-solubility diagrams obtained using a phosphate buffer at pH 6.0 containing various CD concentrations were linear and such profiles (not shown) according to Higuchi and Connors [16] are A_L-type. Because these profiles are characterized by a slope of less than 1 (i.e. 0.1378, 0.1117, and 0.0170 for Me-β-CD, HP-β-CD, and HP-γ-CD, respectively), it was assumed that the solubility increase is due to the formation of a 1:1 complex. The apparent stability constant values ($K_{1:1}$) (Table 3) were estimated from the slope of the straight line of the phase-solubility diagrams according to the following equation: $K_c = \text{slope}/S_0(1 - \text{slope})$ [16] where S_0 is the solubility value of DPH in phosphate buffer (pH 6.0). The stability constant values with the β-CDs were higher than that observed with γ-CD (1608 and 1936 M^{-1} for HP-β-CD, and Me-β-CD respectively versus 215 M^{-1} for HP-γ-CD), indicating that the complex stability depends on cavity size. Furthermore, the estimated $K_{1:1}$ value for the DPH/HP-β-CD complex was, as expected, higher than the values found by Savolainen et al. [13] (1215 M^{-1}at pH 7.4 and 352 M^{-1} at pH 11) and this should be due to the lower pH used in this study. Again, the estimated $K_{1:1}$ value for the DPH/Me-β-CD complex was higher than that found by Tanino et al. [14] (i.e. 1593.7 M^{-1}) for the 6-*O*-α-D-maltosyl-β-cyclodextrin recommended as a safe and potent additive for DPH. Table 3 also shows the DPH incorporation degrees in the solid complexes, with the highest values occurring for Me-β-CD and HP-β-CD systems (20.35 and 17.50 mg/g of complex, respectively). Moreover, our results indicate that the solubility enhancement of DPH/HP-β-CD complex is comparable to that found by Savolainen et al. [13] with NaDPH.

3.4. Dissolution studies

The results of these studies are shown in Fig. 5. As can be seen from the reported data, the rate of dissolution of pure DPH is very slow. The Q_{10}, Q_{30} and Q_{60} values [21] (i.e. percentage of dissolved DPH at 10, 30 and 60 min) were 5.2, 16.6, and 32.3%, respectively, for the pure drug. The dissolution of complexes is very fast in the initial stage and reaches more than 90% within about 10 min. The corresponding Q_{10}, Q_{30} and Q_{60} values, indeed, were 93, 93 and 91% for DPH/HP-β-CD, 98% for DPH/HP-γ-CD, and 100, 100, and 96% for DPH/Me-β-CD complexes, respectively. Thus, as for the dissolution rate the three complexes behave quite similarly, although DPH/HP-γ-CD and DPH/Me-β-CD exhibit slightly higher DPH fractions dissolved with respect to DPH/HP-β-CD. According to literature suggestions [22,23], a decrease in crystallinity and increase in solubility of the drug are considered important factors in determining the enhanced dissolution by complexation with CDs.

The dissolution profiles were also analyzed according to non-linear models using the MSFIT [24] computer program. Five common release models are implemented in this program (i.e. Baker–Lonsdale, Peppas, Hixon–Crowell, Higuchi, and first-order release kinetic models). It was found that all of these models failed to satisfactorily fit the dissolution profiles of DPH and its solid complexes. Furthermore, taking into account that DPH/Me-β-CD and DPH/HP-β-CD dissolution profiles show an initial rapid increase of drug concentration to a maximum followed by a slight decline to a plateau value, it appears that a non-intense supersaturation phenomenon could be involved. A dissolution profile surely involving supersaturated dissolution data has been observed earlier by Savolainen et al. [13] for the dissolution of this last complex at pH 7.4. Modeling of supersaturated dissolution data, based on the population growth model, has been recently proposed by Macheras et al. [25]. However, since in our cases the supersaturated maximum and plateau values are very close and not significantly different, it is not possible to estimate reliable Macheras parameters.

3.5. Pharmacological studies

To examine whether the notable increase in dissolution rate observed with CD-based formulations may lead to differences in pharmacological effects, we explored the anticonvulsant profiles of NaDPH, DPH/HP-β-CD and DPH/Me-β-CD complexes in the maximal electroshock seizure (MES) test, following intraperitoneal administration and given in equimolar doses (12 mg/kg) to mice. The effect of HP-β-CD or Me-β-CD was also evaluated. Due to the low drug incorporation degree, in this study the DPH/HP-γ-CD complex was not examined. Number of animals protected versus total number of animals tested as well as percentage of protection were recorded and the results are summarized in Table 4. Data indicate that CDs as such did

Table 2
Effect of various concentrations of CDs on DPH solubility at 25°C and pH 6.0[a]

HP-β-CD (% w/v)	*S* (mg/ml)	HP-γ-CD (%w/v)	*S* (mg/ml)	Me-β-CD (% w/v)	*S* (mg/ml)
0.0	0.020 (6.9)	0.0	0.020 (6.9)	0.0	0.020 (6.9)
4.5	0.69 (1.6)	4.5	0.09 (11)	4.5	0.75 (1.5)
9	1.40 (3.5)	9	0.15 (7.4)	9	1.61 (5.3)
18	2.90 (12)	18	0.33 (4.5)	18	3.50 (3.5)
27	4.70 (3)	27	0.49 (6)	27	5.62 (5.9)
36	6.50 (0.4)	36	0.87 (6.4)	36	8.18 (5.6)
45	8.40 (6)	45	1.20 (7.2)	45	11.57 (8)

[a] Mean of three determinations. Relative standard deviation (CV) values are reported in parentheses.

Table 3
Stability constants and degrees of incorporation by complexation of DPH with HP-β-CD, HP-γ-CD, and Me-β-CD in phosphate buffer (pH 6.0)

DPH/CD complexes	Apparent stability constant $K_{1:1}$ M^{-1}[a]	Degree of DPH incorporation (mg/g complex)
DPH/HP-β-CD	1608 (4.5)	17.50
DPH/HP-γ-CD	215 (6.9)	1.85
DPH/Me-β-CD	1936 (8)	20.35

[a] Mean of three determinations. Relative standard deviation (CV) values are reported in parentheses.

Table 4
Evaluation of the anticonvulsant effect in mice of DPH/HP-β-CD and DPH/Me-β-CD complexes

DPH formulations	No. of animals protected vs. total no. of animals tested (% protection)
Controls	0/6 (0)
HP-β-CD	0/6 (0)
Me-β-CD	0/6 (0)
NaDPH	6/6 (100)
DPH/HP-β-CD	4/6 (66)
DPH/Me-β-CD	6/6 (100)

not protect the animals from convulsions, while DPH/Me-β-CD complex exhibited anticonvulsant activity similar to NaDPH. This last compound, in contrast, resulted more active than DPH/HP-β-CD complex.

4. Conclusions

In this paper we demonstrated that complexation of DPH with hydrophilic and neutral cyclodextrins such as Me-, HP-β-CD and HP-γ-CD enhances both the solubility and the dissolution rate of the drug in aqueous media. Pharmacological evaluation in mice indicated that the DPH/Me-β-CD complex may be used in developing a new parenteral formulation with an in vivo performance similar to that of NaDPH. Since there is a direct relationship between drug plasma level and pharmacological effect, the implications of the in vivo data are that the bioavailability of DPH from the Me-β-CD-based formulation may be comparable to that of NaDPH. In addition, the present results suggest that Me-β-CD could represent a useful material in solubilizing DPH for oral administration. As for the safety profile of Me-β-CD, it is of particular interest, being different enough from that of other methylated-β-CDs such as Di-Me- and Tri-Me-β-CD. In fact, it is known that both high doses of Me-β-CD, up to 800 mg/kg weekly administered i.p., did not reveal any toxicity [26,27] and the LD_{50} value of Me-β-CD orally administered in mice is $>$ 15 000 mg/kg [27].

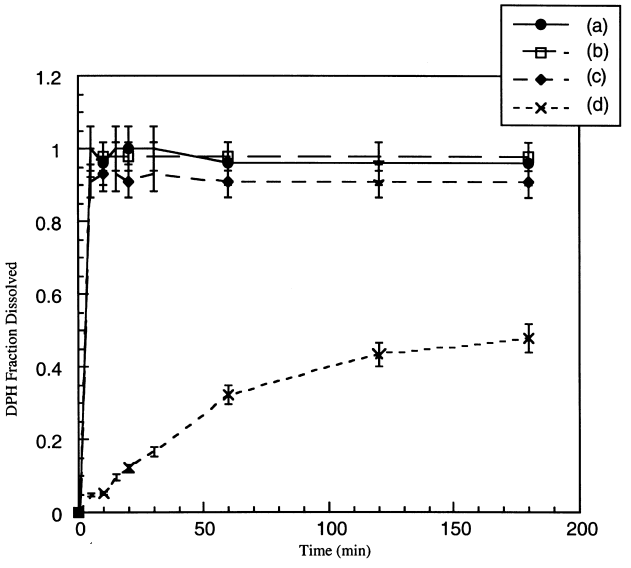


Fig. 5. Dissolution profiles of DPH/Me-β-CD (a), DPH/HP-γ-CD (b), DPH/HP-β-CD (c), DPH (d).

Acknowledgements

Thanks are due to Professor F. Stasi (Università degli Studi di Bari) for her help in recording the X-ray diffraction spectra. This work was supported by a grant from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST).

References

[1] T.W. Rall, L.S. Schleifer, Drugs effective in the therapy of the epilepsies, in: A. Goodman-Gilman, T.W. Rall, A.S. Nies, P. Taylor (Eds.), The Pharmacological Basis of Therapeutics, 8th ed, Pergamon, New York, 1990, pp. 439–443.
[2] K. Arnold, N. Gerber, G. Levy, Absorption and dissolution studies on diphenylhydantoin capsules, Can. J. Pharm. Sci. 5 (1970) 89–92.
[3] T. Suzuki, Y. Saitoh, K. Nishihara, Kinetics of diphenylhydantoin disposition in man, Chem. Pharm. Bull. 18 (1970) 405–411.
[4] S.A. Varia, S. Schuller, K.B. Sloan, V.J. Stella, Phenytoin prodrugs III: water-soluble prodrugs for oral and/or parenteral use, J. Pharm. Sci. 73 (1984) 1068–1073.
[5] K.E. Scriba, D.M. Lambert, Bioavailability of phenytoin and anticonvulsant activity after oral administration of phenytoin-bis-hydroxyisobutyrate to rats, Pharm. Res. 14 (1997) 251–253.
[6] S. Stavchansky, W.G. Gowan, Evaluation of the bioavailability of a solid dispersion of phenytoin in polyethylene glycol 6000 and a commercial phenytoin sodium capsule in the dog, J. Pharm. Sci. 73 (1984) 733–738.
[7] S. Yakou, S. Yamazaki, T. Sonobe, T. Nagai, M. Sigihara, Dissolution and bioavailability of phenytoin in phenytoin-polyvinylpyrrolidone-sodium deoxycholate coprecipitate, Chem. Pharm. Bull. 34 (1986) 3408–3414.
[8] P. Hsyu, R.P. Hedge, B.K. Birmingham, C.T. Rhodes, Studies of the interaction of β-cyclodextrin with ampicillin, methicillin and phenytoin, Drug Dev. Ind. Pharm. 10 (1984) 601–611.
[9] F.A. Menard, M.G. Dedhia, C.T. Rhodes, Studies of the effects of pH, temperature and ring size on the complexation of phenytoin with cyclodextrins, Pharm. Acta Helv. 63 (1988) 303–308.
[10] J. Blanchard, S.O. Ugwu, R. Bhardwaj, R.T. Dorr, Development and testing of an improved parenteral formulation of phenytoin using 2-

hydroxypropyl-β-cyclodextrin, Pharm. Dev. Technol. 5 (2000) 333–338.

[11] S. Varia, V.J. Stella, Phenytoin prodrugs VI: in vivo evaluation of a phosphate ester prodrug of phenytoin after parenteral administration to rats, J. Pharm. Sci. 73 (1984) 1087–1090.

[12] G.K.A. Scriba, D.M. Lambert, J.H. Poupaert, Anticonvulsant activity of DPH-lipid conjugates, a new class of DPH prodrugs, J. Pharm. Pharmacol. 47 (1995) 197–203.

[13] J. Savolainen, K. Jarvinen, L. Matilainen, T. Jarvinen, Improved dissolution and bioavailability of phenytoin by sulfobutylether-β-cyclodextrin ($(SBE)_{7m}$-β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) complexation, Int. J. Pharm. 165 (1998) 69–78.

[14] T. Tanino, T. Ogiso, M. Iwaki, Effect of sugar-modified β-cyclodextrins on dissolution and absorption characteristics of phenytoin, Biol. Pharm. Bull. 22 (1999) 298–304.

[15] J. Pitha, J. Milecki, H. Fales, L. Pannell, K. Uekama, Hydroxypropyl-β-cyclodextrin: preparation and characterization; effects on solubility of drugs, Int. J. Pharm. 29 (1986) 73–82.

[16] T. Higuchi, K.A. Connors, Phase-solubility techniques, Adv. Anal. Chem. Instrum. 4 (1965) 117–212.

[17] H. Holzgrabe, H. Mallwitz, S.K. Branch, T.M. Jefferies, M. Wiese, Chiral discrimination by NMR spectroscopy of ephedrine and N-methylephedrine induced by β-cyclodextrin, heptakis(2,3-di-O-acetyl)β-cyclodextrin, and heptakis(6-O-acetyl)β-cyclodextrin, Chirality 9 (1997) 211–219.

[18] H. Matsuda, K. Ito, Y. Sato, D. Yoshizawa, M. Tanaka, A. Taki, H. Sumiyoshi, T. Utsuki, F. Hirayama, K. Uekama, Inclusion complexation of p-hydroxybenzoic acid esters with 2-hydroxypropyl-β-cyclodextrins. On changes in solubility and antimicrobial activity, Chem. Pharm. Bull. 41 (1993) 1448–1452.

[19] G. Bettinetti, F. Melani, P. Mura, R. Monnanni, F. Giordano, Carbon-13 nuclear magnetic resonance study of naproxen interaction with cyclodextrin in solution, J. Pharm. Sci. 80 (1991) 1162–1169.

[20] P.T. Schwartz, C.T. Rhodes, J.W. Cooper, Solubility and ionization characteristics of phenytoin, J. Pharm. Sci. 66 (1977) 994–997.

[21] S. Anguiano-Igea, F.J. Otero-Espinar, J.L. Vila-Jato, J. Blanco-Mendez, Improvement of clofibrate dissolution by complexation with cyclodextrin, Int. J. Pharm. 135 (1996) 161–166.

[22] K. Uekama, F. Hirayama, Improvement of drug properties by cyclodextrins, in: C. Wermuth (Ed.), The Practice of Medicinal Chemistry, Academic Press, New York, 1996, pp. 793–825.

[23] J. Stzejtli, Medicinal applications of cyclodextrins, Med. Res. Rev. 14 (1994) 353–386.

[24] D.R. Lu, K. Abu-Iza, F. Mao, Nonlinear data fitting for controlled release devices: an integrated computer program, Int. J. Pharm. 129 (1996) 243–251.

[25] G. Valsami, A. Dokoumetzidis, P. Macheras, Modeling of supersaturated dissolution data, Int. J. Pharm. 181 (1999) 153–157.

[26] Y.P. Grosse, F. Bressolle, P. Rouanet, J.M. Joulia, F. Pinguet, Methyl-β-cyclodextrin and doxorubicin pharmacokinetics and tissue concentrations following bolus injection of these drugs alone or together in the rabbit, Int. J. Pharm. 135 (1999) 161–166.

[27] Waker Chemie, Technical data concerning Me-β-CD, Waker Chemie, 1997.