ELSEVIER

International Journal of Pharmaceutics 129 (1996) 63–71

international journal of pharmaceutics

Inclusion complexation of tolbutamide with β-cyclodextrin and hydroxypropyl-β-cyclodextrin

F. Veiga[a,*], J.J.C. Teixeira-Dias[b], F. Kedzierewicz[c], A. Sousa[a], P. Maincent[c]

[a] *Laboratório de Tecnologia Farmacêutica, Faculdade de Farmácia, Universidade de Coimbra, Coimbra Portugal*

[b] *Unidade de Investigacão em Química-Física Molecular, Departamento de Química, Faculdade de Ciências e Tecnologia da Universidade de Coimbra, Coimbra Portugal*

[c] *Laboratoire de Pharmacie Galénique et Biopharmacie, Faculté des Sciences Pharmaceutiques et Biologiques, Université de Nancy I, Nancy France*

Received 7 March 1995; revised 12 June 1995; accepted 26 June 1995

Abstract

Inclusion complexes of tolbutamide with β-cyclodextrin and hydroxypropyl-β-cyclodextrin were prepared using different methods: kneading, coprecipitation and freeze-drying. Inclusion complexation in aqueous solution and in solid phase state was studied by the solubility method, X-ray diffractometry, thermal analysis and Raman spectroscopy. The solubility of tolbutamide increased as a function of cyclodextrin concentration, showing B_S and A_L type diagrams for β-cyclodextrin and hydroxypropyl-β-cyclodextrin, respectively. The dissolution rate of tolbutamide/cyclodextrin complexes were investigated and compared with those of the physical mixtures and pure drug. The dissolution rate of tolbutamide from the inclusion complexes was much more rapid than tolbutamide alone.

Keywords: Tolbutamide; Cyclodextrin; Kneading; Freeze-drying; Coprecipitation; Dissolution rate

1. Introduction

Tolbutamide (1-butyl-3-(*p*-tolysulfonyl) urea (TBM), is used as an oral hypoglycemic agent. The drug is practically insoluble in water and its dissolution is supposed to be the rate-limiting step for its absorption from the gastrointestinal tract (Miralles et al., 1982).

Several reports in the literature have shown an increase in the TBM dissolution rate when it forms solid dispersions with water-soluble carriers such as urea, PEG, dextrose and mannitol (Miralles et al., 1982; McGinity et al., 1984).

Natural cyclodextrins are cyclic oligosaccharides, containing six (α-cyclodextrin), seven (β-cyclodextrin) or eight (γ-cyclodextrin) α-1,4-linked glucopyranose units, with hydrophilic outer surfaces and a hydrophobic cavity (Saenger, 1980; Szejtli, 1988). Cyclodextrins are able to form inclusion complexes with several poorly water-soluble compounds. These inclusion complexes have been shown to improve stability (Andersen and

* Corresponding author: Faculdade de Farmácia de Coimbra, Rua do Norte, 3000 Coimbra, Portugal. Tel.: + 351 39 4109998; Fax: + 351 39 27126 E-mail: fveiga@cygnus.ci.uc.pt.

SSDI 0378-5173(95)04243-4

Bundgaard, 1984; Loftsson et al., 1989), solubility, dissolution rate (Pitha et al., 1986; Blanco et al., 1991) and bioavailability (Chow and Karara, 1986; Vila-Jato et al., 1988). They also can reduce side effects associated with some drugs (Otero-Espinar et al., 1991; Lin et al., 1994).

As an inclusion complexant agent, β-cyclodextrin (β-CD) improves the solubility and dissolution rate of TBM (Gandhi and Karara, 1988; Vila-Jato et al., 1988; Kedzierewicz et al., 1990). However, the low aqueous solubility of β-CD (about 1.8% w/v, at 25°C) suggested the use of more water soluble complexant agents such as hydroxypropyl-β-cyclodextrin (HP-β-CD), whose solubility in water is 50% (w/v) at 25°C. The use of this cyclodextrin derivative is well known for parenteral administration and it is used also for oral applications, because it is very well tolerated (Brewster and Bodor, 1990; Brewster et al., 1990; Coussement et al., 1990; Mesens et al., 1991).

The aim of this study was to improve the solubility and dissolution rate of TBM in aqueous solution (pH 2) and thereby improve its bioavailability. This was attained through the formation of inclusion complexes with β-CD and HP-β-CD. TBM:β-CD and TBM:HP-β-CD complexes were prepared by coprecipitation, kneading and freeze-drying methods and the effects of the β-CD and HP-β-CD concentration on the solubility of TBM in different media (distilled water, pH 2 and 7 buffers) were determined. X-ray diffraction, DSC and Raman spectroscopy were used to characterize the complexes in the solid state.

2. Materials and methods

2.1. Materials

Tolbutamide (Sigma, St. Louis, USA), β-cyclodextrin (Roquette Frères, Lestrem, France) and 2-hydroxypropyl-β-cyclodextrin (Janssen, Beerse, Belgium), with a molar substitution of 0.39 were used as received. All other reagents and solvents were of analytical grade and double distilled water was used throughout the study.

2.2. Phase solubility studies

Solubility studies were performed as described by Higuchi and Connors (1965). An excess of TBM was added to 50-ml volumetric brown glass flasks. Cyclodextrins solutions of different concentrations (0.001–0.028 and 0.005–0.1 M for β-CD and HP-β-CD, respectively) were prepared at pH 2, pH 7 and in distilled water. A constant volume (20 ml) of various cyclodextrin solutions was added to each flask. The flasks were sealed and brought to solubility equilibrium at room temperature after shaking over a period of 2 weeks.

After equilibrium had been reached, the content of each flask was filtered through a Millipore membrane (HA 0.45 μm). The filtered solutions were appropriately diluted and the amount of dissolved TBM was determined spectrophotometrically at 229 nm (Shimadzu UV-160 spectrophotometer). The studies were carried out in triplicate.

2.3. Preparation of the physical mixture

Physical mixtures, TBM:β-CD (1:2) and TBM:HP-β-CD (1:1), were prepared by simple blending in a glass mortar.

2.4. Preparation of solid inclusion complexes

2.4.1. Kneading

Stoichiometric quantities of TBM and cyclodextrins were triturated with a small amount of a ethanol:water mixture (1:3). The slurry was kneaded for 60 min and then dried under vacuum at 40°C.

2.4.2. Freeze-drying

Stoichiometric quantities of TBM and cyclodextrins were dissolved in distilled water with a small amount of ammonia (25%) to aid dissolution of TBM. The solution was frozen by immersion in shell freezer and freeze-dried over 24 h in a Lyph-lock 6 apparatus (Labconco). No trace of ammonium was detected in the resulting mixture with Nessler reagent.

2.4.3. Coprecipitation

The solid complex was obtained by mixing appropriate amounts of TBM and β-CD in distilled water. The quantities were calculated from the descending curvature of the phase solubility diagram at the point (indicated by an arrow in Fig. 1), where no solid drug existed and the solubility of β-CD was not exceeded. The mixture was shaken at room temperature for 2 weeks. The complex, precipitated as a white powder, was filtered, washed with a small amount of distilled water and then dried under vacuum at 40°C for 24 h.

This method was not applicable to the TBM:HP-β-CD system (A type phase solubility diagram), because of the formation of a soluble inclusion complex.

2.5. Characterization of the physical mixture and inclusion complexes

2.5.1. X-Ray diffraction

X-Ray diffraction patterns were collected on an Enraf-Norius powder diffractometer equipped with a horizontal mounted INEL CPS120 curved position-sensitive detector. Cu-Kα_1 radiation was selected by a bent quartz-crystal monochromator and each pattern was collected during about 24 h.

2.5.2. Differential scanning calorimetry (DSC)

The measurements (Shimadzu, model 50) were obtained using aluminum sample pans at a scanning speed of 10°C/min under a nitrogen stream from 25 to 250°C.

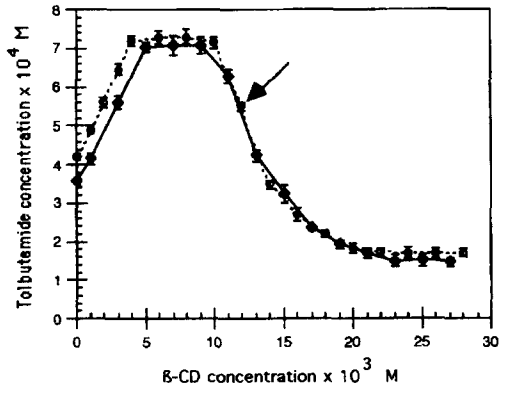


Fig. 1. Phase solubility diagram of TBM:ß-CD system at room temperature. Each point: mean ± S.D.

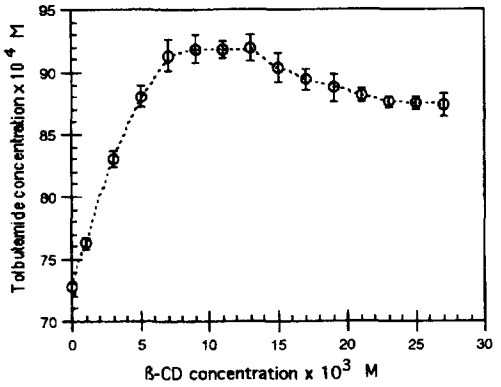


Fig. 2. Phase solubility diagram of TBM:ß-CD system in pH 7 buffer at room temperature. Each point: mean ± S.D.

2.5.3. Raman spectroscopy

The Raman spectra were recorded on a Spex 1403 double spectrometer. The 514.5-nm line of an argon ion laser (Spectra Physics, model 2020-03) was used as Raman excitation.

2.6. Dissolution studies

Dissolution studies were performed according to the USP XXII method with the apparatus 2, in 1000 ml of pH 2 buffer (Eu. Ph.) at 37 ± 5°C and at a rotational speed of 75 rev/min. Powdered samples (sieve fraction 90–160 μm) containing 25 mg of TBM (corresponding to about 20% of saturated concentration), or its equivalent in complex or physical mixture were added to the medium. The dissolution rates of TBM were measured in an apparatus (Hanson Research) connected to the spectrophotometer by a peristaltic pump, so that the absorbance was monitored automatically at 229 nm. All samples were analyzed at least six times.

3. Results and discussion

The phase solubility diagrams for the complex formation between TBM and cyclodextrins in

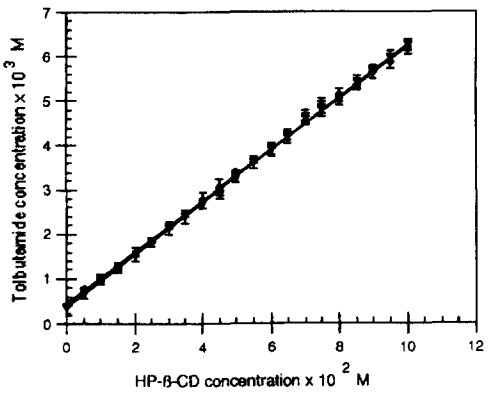


Fig. 3. Phase solubility diagram of TBM:HP-ß-CD system at room temperature. Each point: mean ± S.D.

different media (Figs. 1 and 2, Fig. 3 and Fig. 4), are B_S and A_L type diagrams for β-CD and HP-β-CD, respectively, following the Higuchi and Connors (1965) classification.

In the case of β-CD (Figs. 1 and 2), the solubility diagrams in various media show a typical curve, whose initial rising portion is followed by a plateau region and, finally, the concentration of TBM decreases as the solid microcrystalline complexes precipitate. The stoichiometry of the complexes in solid phase was analysed on the basis of data in the plateau region of solubility diagrams and was estimated to be 1:2 (TBM:β-CD). Chemical assay of precipitates confirmed the observed stoichiometry. Similar results were obtained by Chow and Karara (1986) and Kedzierewicz et al. (1990). For distilled water and pH 2 buffer solution, the solubility curves show similar patterns and are almost coincident. For the pH 7 buffer solution, the slope of the initial straight line portion and the S_o value (the solubility of TBM in absence of β-CD) are larger than the corresponding values for the other media.

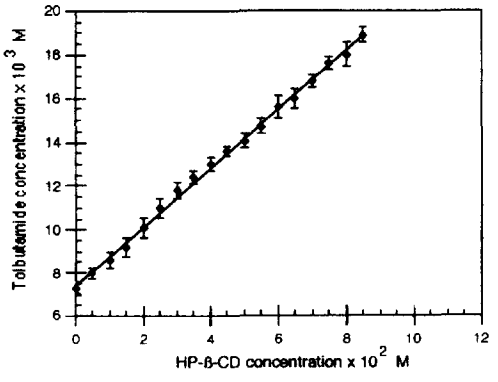


Fig. 4. Phase solubility diagram of TBM:HP-ß-CD system in pH 7 buffer at room temperature. Each point: mean ± S.D.

Concerning the HP-β-CD, the plots (Figs. 3 and 4) show that the aqueous solubility of the drug increases linearly as a function of the cyclodextrin concentration. For the distilled water and the pH 2 buffer solution, the straight lines display similar slopes and are almost coincident. For the pH 7 buffer solution, both the slope and the S_o values are larger than the corresponding values for the other media.

Table 1 summarizes the apparent stability constants of the complexes for each medium. The apparent stability constants (K_s) were calculated according to Higuchi and Connors (1965) from the initial straight line portion of the solubility diagrams, assuming that a 1:1 complex is initially formed (the slope is smaller than 1 for both cyclodextrins). Calculation of K_s was carried out according to Eq. 1 where S_o = intercept.

$$K_s = \text{slope}/S_o(1 - \text{slope}) \quad (1)$$

The influence of pH on apparent stability constants and on the efficacy of the solubility-enhancing effect is reported in Table 1. The increase in pH values leads to a decrease in apparent stability constants, indicating that apparent stability constants of TBM inclusion complexes are larger in the less ionized form (pK_a = 5.4), whereas the number of TBM per cyclodextrin (mol/mol) is greater at pH 7. This study shows that both the unionized and the ionized forms of TBM interact with β-CD and HP-β-CD. However, the interaction of the ionized form is weaker than for unionized species. Similar results were observed for the complexation of flufenamic acid (Otagiri et al., 1984), phenytoin (Menard et al., 1988) and indomethacin (Backensfeld et al., 1991) with β-CD or HP-β-CD.

Table 1
Summary of findings from the phase solubility studies

Cyclodextrin	Medium	K (M^{-1})	S_o (M^{-1})	Molar ratio	r (n)
ß-Cyclodextrin	Water	195.7	4.17×10^{-4}	0.0754	0.999 (3)
	pH 2 buffer	212.1	3.53×10^{-4}	0.696	0.999 (3)
	pH 7 buffer	50.2	73.7×10^{-4}	0.270	0.995 (3)
HP-ß-Cyclodextrin	Water	144.8	4.27×10^{-4}	0.0582	0.999 (3)
	pH 2 buffer	174.0	3.61×10^{-4}	0.0590	0.999 (3)
	pH 7 buffer	21.0	72.7×10^{-4}	0.135	0.999 (3)

3.1. X-ray diffraction

Powder X-ray diffractometry is a useful method for the detection of cyclodextrin complexation in powder or microcrystalline states. The diffraction pattern of the complex is supposed to be clearly distinct from that of the superposition of each component if a true complex exists.

Fig. 5 displays the powder X-ray diffraction patterns of TBM, β-CD and HP-β-CD alone and inclusion complexes prepared by different methods in comparison with that of a physical mixture

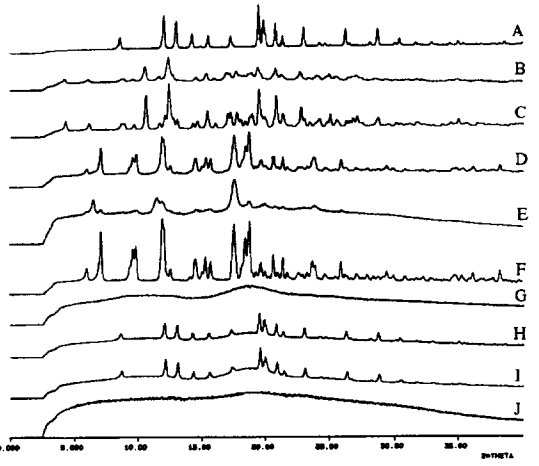


Fig. 5. X-ray diffraction patterns of: (A) TBM; (B) ß-CD; (C) physical mixture of TBM and ß-CD; (D) TBM:ß-CD inclusion complex by kneading method; (E) TBM:ß-CD inclusion complex by freeze-drying; (F) TBM:ß-CD inclusion complex by coprecipitation method; (G) HP-ß-CD; (H) physical mixture of TBM and HP-ß-CD; (I) TBM:HP-ß-CD inclusion complex by kneading method; (J) TBM:HP-ß-CD inclusion complex by freeze-drying.

at the same molar ratio. The diffraction patterns of physical mixtures and of the TBM:HP-β-CD kneaded mixture are simply superpositions of the patterns of the components, while those of the inclusion complexes show peaks different from those of the physical mixture, indicating the formation of new structure.

The X-ray diffractograms of TBM:β-CD complexes have a crystalline structure when obtained by kneading and coprecipitation, but present fewer and less intense peaks than when obtained by freeze-drying.

The diffraction pattern of TBM:HP-β-CD prepared by freeze-drying is completely diffuse, similar to that of HP-β-CD. However, the peaks of TBM present in the physical and kneaded mixtures, have disappeared indicating that TBM interacts with HP-β-CD and is transformed in an amorphous compound.

3.2. Differential scanning calorimetry (DSC)

The DSC thermograms of TBM show two endothermal peaks, one at 39.6°C and the major one at 129.2°C (Fig. 6). Due to a dehydration process β-CD and HP-β-CD present broad endothermic peaks around 80 and 60°C, respectively. The physical mixtures of TBM:β-CD and TBM:HP-β-CD, and the TBM:HP-β-CD kneaded mixture all show an endothermic peak near 129°C, due to fusion of the drug. In the freeze-dried complexes, the results show the absence of the characteristic endothermic melting peak at 129.2°C, as compared to the physical mixtures in which this peak is clearly visible. The

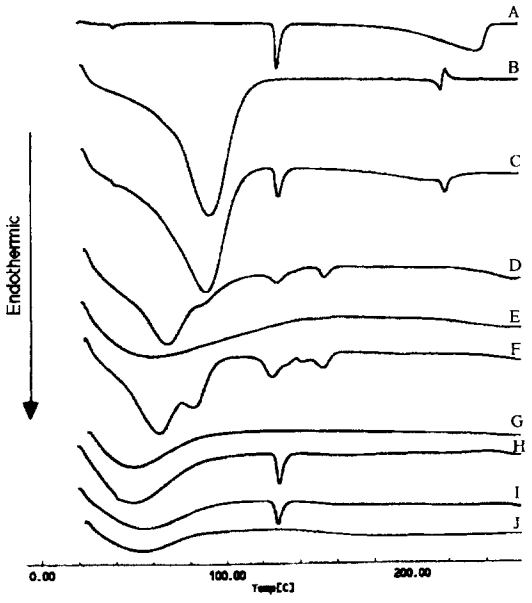


Fig. 6. Fig. 6. DSC Thermograms of: (A) TBM; (B) ß-CD; (C) physical mixture of TBM and ß-CD; (D) TBM:ß-CD inclusion complex by kneading method; (E) TBM:ß-CD inclusion complex by freeze-drying; (F) TBM:ß-CD inclusion complex by coprecipitation method; (G) HP-ß-CD; (H) physical mixture of TBM and HP-ß-CD; (I) TBM:HP-ß-CD inclusion complex by kneading method; (J) TBM:HP-ß-CD inclusion complex by freeze-drying.

disappearance of the endothermic peaks of TBM is attributed to the amorphous state or inclusion complexation or both (Kurozumi et al., 1975).

The thermograms of TBM:β-CD complexes prepared by coprecipitation and kneading display a broad endothermal band between 50 and 100°C due to the dehydration of the complexes. However, the endothermal peak near 126°C, for the complexes obtained by coprecipitation and by kneading, could still reflect the presence of a few drug crystals in the preparation but the height of this endotherm is reduced in comparison with pure TBM. The endothermal peaks near 155°C are due to the shift of melting point of TBM to a higher temperature. These results can provide an indication of the formation of inclusion complexes between TBM and both cyclodextrins (Uekama and Otagiri, 1987; Kedzierewicz et al., 1994).

3.3. Raman spectroscopy

Since β-CD and HP-β-CD have no Raman bands in the region of carbonyl stretching vibration (1650–1780 cm^{-1}), the following discussion will be focused on the region of TBM carbonyl stretching bands near 1670 cm^{-1}.

Table 2 presents the carbonyl stretching Raman frequencies for TBM, for physical mixtures TBM:cyclodextrins and for the various TBM:cyclodextrins compounds. The discussion of the presented frequencies can be done in terms of the following canonical structures (Fig. 7).

An increased contribution of structures (II) leads to lengthening of the C=O bond and to a reduction of the electronic density in this bond, i.e., to a lower stretching frequency. Hence, the spectral feature occurring at the highest frequencies, i.e., at ca. 1734 cm^{-1}, should be ascribed to the carbonyl group in a predominantly apolar environment, whereas the features occurring at lower frequencies, i.e., at ca. 1718 and 1670 cm^{-1}, should correspond to differently polarized carbonyl groups, the lowest frequency being ascribed to a more strongly polarized C=O bond. This reasoning, together with the assumption of a predominantly apolar environment for the included guest molecules, leads to the following conclusions of practical importance.

(1) The weak and broad Raman feature at ca. 1734 cm^{-1} should be considered as a spectroscopic 'signature' for the inclusion process, whereas the bands at ca. 1674 and 1718 cm^{-1} correspond to differently hydrogen bonded carbonyl groups of TBM molecules.
(2) Complex formation with inclusion of TBM is indicated by the presence of the 1734-cm^{-1} Raman feature which occurs in all cases, except for TBM:HP-β-CD obtained by kneading. The assignment of the relatively weak and broad 1734 cm^{-1} band is further supported by the absence of this feature in the spectra of TBM and of the physical mixtures.
(3) In the above mentioned cases of complex formation the occurrence of carbonyl stretching Raman bands at frequencies other than the highest at ca. 1734 cm^{-1} suggests the existence of TBM molecules whose carbonyl groups are differently polarized by hydrogen bonding type of interactions.

Table 2
Carbonyl stretching Raman frequencies

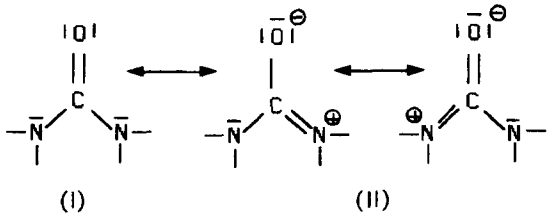
	υ C=O (cm^{-1})		
TBM (solid)	1670	1712 (w)	
Physical mixture of TBM and ß-CD	1674	1712 (w)	
Physical mixture of TBM and HP-ß-CD	1674	1712 (w)	
TBM:ß-CD (kneading)	1673 (vw)	1718	1735
TBM:ß-CD (freeze-drying)		≈1712	1731
TBM:ß-CD (coprecipitation)	1673 (vw)	1718	1734
TBM:HP-ß-CD (kneading)	1670	1711	
TBM:HP-ß-CD (freeze-drying)		1706,1718 (vw)	≈1734 (vw)

(4) The 1712 cm^{-1} Raman feature in the spectra of TBM solid disappears when the sample is prepared by the freeze-drying method. In this method ammonium hydroxide was used, resulting in the formation of an ammonium salt with TBM and in the probable breaking of hydrogen-bonding type interactions between TBM molecules. Hence, the 1712 cm^{-1} feature is herein tentatively ascribed to carbonyl groups of hydrogen-bonded TBM molecules in the solid.

3.4. Dissolution studies

Fig. 8 shows the dissolution rate profiles of TBM alone, from inclusion complexes and physical mixtures. It is evident that both the complexes and physical mixtures exhibit a faster dissolution rate than the free drug.

The amount of TBM dissolved after 20 min is presented in Table 3. It can be seen that the increase in dissolution rate of TBM is ca. 12-fold greater when under the form of inclusion complex, either obtained from β-CD by kneading, coprecipitation and freeze-drying, or from HP-β-CD by freeze-drying.



(I) (II)

Fig. 7. Canonical structures of carbonyl group.

The significant enhancement in dissolution rate of the complex obtained from βCD by kneading and coprecipitation may be due to an increase in solubility and marked reduction in crystallinity. For freeze-drying complexes, the increase of dissolution rate can be attributed, besides an increase in solubility, to the surfactant-like properties of cyclodextrins, which can reduce the interfacial tension between TBM and the dissolution medium, leading to a higher dissolution rate. Furthermore, it might be due to the high energetic amorphous state of freeze-drying products (Erden and Celebi, 1988).

The increase in the dissolution rate of TBM when physically mixed with β-CD (ca. 4.5-fold

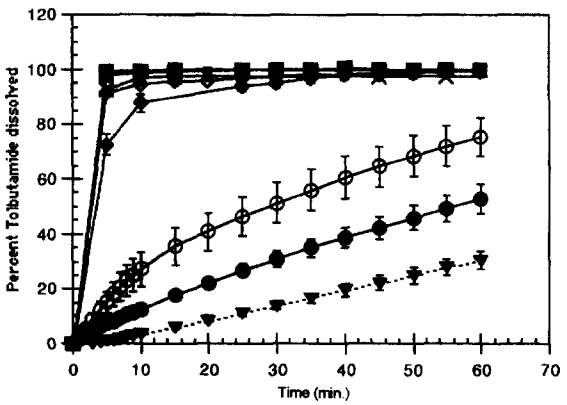


Fig. 8. Dissolution profiles of tolbutamide and its ß-CD and HP-ß-CD systems in pH 2 buffer at 37°C. Each point: mean ± S.D. ▼, TBM alone; ○, physical mixture of TBM and β-CD; ●, physical mixture of TBM and HP-β-CD; ◇, TBM:β-CD complex (kneading method); ◆, TBM:HP-β-CD complex (kneading method); ■, TBM:β-CD complex (freeze-drying method); □, TBM:HP-β-CD complex (freeze-drying method); ×, TBM:β-CD complex (coprecipitation method).

Table 3
Mean percentages of TBM dissolved after 20 min in pH 2 buffer at 37°C

Method of preparation	ß-CD (%)	HP-ß-CD (%)
Pure TBM	8.5	8.5
Physical mixture	40.7	22.3
Kneading	95.8	90.1
Freeze-drying	100.0	100.0
Coprecipitation	97.2	—

greater) and HP-β-CD (ca. 2.5-fold greater) is possibly due to a local solubilization action operating in the micro-environment or the hydrodynamic layer surrounding the drug particles in the early stages of the dissolution process. Indeed, this type of cyclodextrin dissolves in a short time thus improving the wettability, and hence dissolution of the drug particles (Goldberg et al., 1966; Ismail, 1991).

The TBM:HP-β-CD kneading process displays a dissolution rate greater than the physical mixtures but smaller than other complexes. This behaviour suggests that the dissolution rate may be due to reduction in crystallinity of the powder but not due to any inclusion complexation process.

These results are in full accordance with physical characterizations of different formulations, which indicate that inclusion complexes are obtained for β-CD by kneading, coprecipitation and freeze-drying and for HP-β-CD by freeze-drying.

4. Conclusion

In solution, the ability of β-CD and HP-β-CD to form complexes with TBM is pH-dependent. The increase in ionization of TBM (pK_a = 5.4) results in a decrease in its apparent stability constants. Although the unionized and the ionized forms of TBM interact with both cyclodextrins, the ionized form interacts to a much lesser extent, suggesting that some chemical parameters are involved in the complexation.

The solid inclusion complexes of TBM with β-CD were obtained by kneading, freeze-drying and coprecipitation, while those with HP-β-CD were obtained by freeze-drying. Kneading of TBM with HP-β-CD was unable to produce an inclusion complex, however, with this mixture the observed dissolution rate of TBM was greater than that of the physical mixture, probably due to a reduction in crystallinity of the powder. The extent of the dissolution rate-enhancing effect was found to be dependent on the method used for the preparation of the mixture (complex or not).

From the experimental results it is concluded that TBM:cyclodextrin complexation results in an increase of solubility and dissolution rate for the drug, suggesting a possible enhancement of its oral bioavailability.

The 'in vivo' availability of TBM:CD inclusion complexes in rabbits from oral solid dosage forms is under investigation.

Acknowledgements

The authors would like to thank Prof. Maria José Almeida for X-ray diffraction studies. This work was supported by a grant (DB 2662/93) of Junta Nacional de Investigacão Científica e Tecnológica, Portugal.

References

Andersen, F.A. and Bundgaard, H., Inclusion complexation of metronidazole with β-cyclodextrin and its depression of anhydrate-hydrate transition in aqueous suspensions. *Int. J. Pharm.*, 19 (1984) 189–197.

Backensfeld, T., Müller, B.W. and Kolter, K., Interaction of NSA with cyclodextrins and hydroxypopylcyclodextrin derivatives. *Int. J. Pharm.*, 74 (1991) 85–93.

Blanco, J., Vila-Jato, J.L., Otero, F. and Anguiano, S., Influence of method of preparation on inclusion complexes of naproxen with different cyclodextrins. *Drug Dev. Ind. Pharm.*, 17 (1991) 943–945.

Brewster, M.E. and Bodor, N., Parenteral safety and uses of 2-hydroxypropyl-β-cyclodextrin. In Duchêne, D. (Ed.), *Minutes of the 5th International Symposium on Cyclodextrins*, Editions de Santé, Paris, 1990, pp. 525–534.

Brewster, M.E., Estes, K.S. and Bodor, N., An intravenous toxicity study of hydroxypropyl-β-cyclodextrin, a useful drug solubilizer, in rats and monkeys. *Int. J. Pharm.*, 59 (1990) 231–243.

Chow, D. and Karara, A., Characterization, dissolution and bioavailability in rats of ibuprofen-β-cyclodextrin complex system. *Int. J. Pharm.*, 28 (1986) 95–101.

Coussement, W., Van Cauteren, H., Vandenberghe, J., Vanparys, P., Teuns, G., Lampo, A. and Marsboom, R., Toxicological profile of hydroxypropyl-β-cyclodextrin in laboratory animals. In Duchêne, D. (Ed.), *Minutes of the 5th International Symposium on Cyclodextrins*, Editions de Santé, Paris, 1990, pp. 522–524.

Erden, N. and Celebi, N., A study of inclusion complex of naproxen with β-cyclodextrin. *Int. J. Pharm.*, 48 (1988) 83–89.

Gandhi, R.B. and Karara, A.H., Characterization, dissolution and diffusion properties of tolbutamide-β-cyclodextrin complex system. *Drug Dev. Ind. Pharm.*, 14 (1988) 657–682.

Goldberg, A.H., Gribaldi, M., Kanig, J.L. and Myersohn, M., Increasing dissolution rates and gastrointestinal absorption of drugs IV: chloramphenicol-urea system. *J. Pharm. Sci.*, 55 (1966) 1205–1211.

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

Ismail, S., Interaction of anti-convulsant drugs with alpha- and beta-cyclodextrins I. Methsumide. *S.T.P. Pharma Sci.*, 1 (1991) 321–325.

Kedzierewicz, F., Hoffman, M. and Maincent, P., Comparison of tolbutamide β-cyclodextrin inclusion compounds and solid dispersions. *Int. J. Pharm.*, 58 (1990) 221–227.

Kedzierewicz, F., Zinutti, C., Hoffman, M. and Maincent, P., A three years stability study of tolbutamide solid dispersions and β-cyclodextrin complex. *Proceedings of 13th Pharmaceutical Technology Conference*, Strasbourg, April, 1994.

Kurozumi, M., Nambu, N. and Nagai, T., Inclusion compounds of non-steroidal anti-inflammatory and other slightly water soluble drugs with a and β-cyclodextrins in powdered form. *Chem. Pharm. Bull.*, 23 (1975) 3062–3068.

Lin, S., Wouessidjewe, D., Poelman, M. and Duchêne, D., In vivo evaluation of indomethacin/cyclodextrin complexes gastrointestinal tolerance and dermal anti-inflammatory activity. *Int. J. Pharm.*, 106 (1994) 63–67.

Loftsson, T., Björnsdóttir, S., Pálsdóttir, G. and Bodor, N., The effects of 2-hydroxypropyl-β-cyclodextrin on solubility and stability of chlorambucil and melphalan in aqueous solution. *Int. J. Pharm.*, 57 (1989) 63–72.

McGinity, J.W., Maincent, P. and Steinfink, H., Crystallinity and dissolution rate of tolbutamide solid dispersions prepared by the melt method. *J. Pharm. Sci.*, 73 (1984) 1441–1444.

Menard, F.A., Dedhiya, M.G. and Rhodes, C.T., Studies of the effect of pH, temperature and ring size on the complexation of phenytoin with cyclodextrins. *Pharm. Acta Helv.*, 63 (1988) 303–308.

Mesens, J.L., Putteman, P. and Verheyen, P., (1991) Pharmaceutical applications of 2-hydroxypropyl-β-cyclodextrin. Pre clinical studies and current clinical developments. In Duchêne, D. (Ed.), *New Trends in Cyclodextrins and Derivatives*, Editions de Santé, Paris, pp. 369–407.

Miralles, M.J., McGinity, J.W. and Martin, A., Combined water-soluble carriers for coprecipitates of tolbutamide. *J. Pharm. Sci.*, 71 (1982) 302–304.

Otagiri, M., Uekama, K., Imai, T., Maeda, T., Takadate, A., Goya, S. and Janssen, L., Comparative tudy on inclusion complexation of β-cyclodextrin and tri-*O*-methyl-β-cyclodextrin with several drugs in aqueous solution. *Acta Pharm. Suec.*, 21 (1984) 357–366.

Otero-Espinar, F.J., Anguiano-Igea, S., Blanco-Mendes, J. and Vila Jato, J.L., Reduction in the ulcerogenicity of naproxen by complexation with β-cyclodextrin. *Int. J. Pharm.*, 70 (1991) 35–41.

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

Saenger, W., Cyclodextrin inclusion compounds in research and laboratory. *Angew. Chem. Int. Ed. Engl.*, 19 (1980) 344–362.

Szejtli, J., *Cyclodextrin Technology*. Kluwer Academic Publishers, Dordrecht, 1988, pp. 1–78.

Uekama, K. and Otagiri, M., Cyclodextrins in drug carrier systems. *CRC Crit. Rev. Ther. Drug Carrier Syst.*, 3 (1987) 1–40.

Vila-Jato, J.L., Blanco, J. and Torres, J.J., Biopharmaceutical aspects of the tolbutamide-β-cyclodextrin inclusion compound. *Il Farmaco, Ed. Prat.*, 43 (1988) 37–45.