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# Inclusion complexation of metronidazole benzoate with β-cyclodextrin and its depression of anhydrate–hydrate transition in aqueous suspensions

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## Summary

Metronidazole benzoate was found to form an inclusion complex with β-cyclodextrin (β-CyD) in aqueous solution and in the solid phase. A phase solubility diagram was obtained and an apparent 1 : 1 formation complex constant of $1.3 \times 10^3$ $M^{-1}$ was determined. A microcrystalline inclusion complex was isolated and shown to have the stoichiometric composition of 1 : 1.5 (drug: β-CyD). By inclusion complexation of the metronidazole ester with β-CyD the phase transition of the clinically used anhydrous form of the compound to the monohydrate occurring in aqueous suspensions was inhibited as was the marked crystal growth resulting from the phase transition. Besides increasing the physical stability of metronidazole benzoate suspensions the complexation with β-CyD protected the drug against photochemical degradation and decreased the rate of hydrolysis.

## Introduction

Cyclodextrins (CyD) or cycloamyloses are cyclic oligosaccharides containing 6 (α-CyD), 7 (β-CyD) or 8 (γ-CyD) α-(1,4)-linked glucose units. The important structural features of these compounds are their toroid or doughnut shape, their hydrophobic cavity and their hydrophilic faces (Bender and Komiyama, 1978; Saenger, 1980). In recent years, the cyclodextrins have received considerable attention in the pharmaceutical field because of their ability to form inclusion complexes

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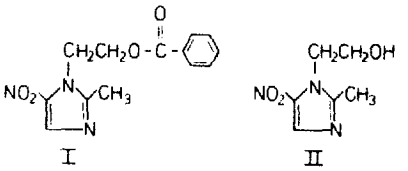
with various drug molecules either in the solid phase or in solution. Such inclusion complexation or 'molecular encapsulation' may be utilized in the pharmaceutical formulation to improve, e.g., the aqueous solubility, chemical stability or bioavailability (for reviews, see Saenger, 1980; Uekama, 1981; Szejtli, 1982).

In the present work the interaction of β-cyclodextrin (β-CyD) with metronidazole benzoate was investigated with the purpose of exploring the possibility of improving the physical stability of the drug in aqueous suspensions. Metronidazole benzoate (I) is a prodrug of metronidazole (II), and because of its poor solubility in water the benzoate ester is tasteless and is used clinically in the form of oral aqueous suspensions (Alestig et al., 1980; Houghton et al., 1982). However, such suspensions show physical stability problems when stored in the cold in that a marked crystal growth may occur (Dumex Ltd., Copenhagen, personal communication). This increase of the particle size has recently been shown to be due to a phase transition of the anhydrous form to the monohydrate form of the metronidazole ester (Hoelgaard and Møller, 1983). The monohydrate was further shown to be the thermodynamically stable form in water below 38°C. As will be shown in this paper, inclusion complexation of metronidazole benzoate with β-CyD may afford a convenient means to depress the anhydrate–hydrate transition and thus eliminate or reduce the crystal growth of aqueous suspensions.




## Materials and Methods

*Materials*

β-Cyclodextrin, metronidazole and metronidazole benzoate anhydrate were obtained from Dumex Ltd., Copenhagen. All other chemicals used were of analytical grade.

*Apparatus*

High-performance liquid chromatography (HPLC) was done with a Spectra-Physics Model 3500B instrument equipped with a variable wavelength detector and 10 μl loop injection valve. The detector was connected to a Servogor RE 541 potentiometric recorder. A column (250 mm × 4 mm i.d.) packed with LiChrosorb RP-8 (7 μm particles) (E. Merck, Darmstadt, F.R.G.) was used. Measurements of pH were done at the temperature of study using a Radiometer Type PHM 26 instrument. Ultraviolet spectra were recorded using a Shimazu UV-190 spectrophotometer and 1 cm cuvettes. Differential scanning calorimetry (DSC) was done with a Perkin-Elmer DSC-1 instrument equipped with an effluent gas detector, EGD, the sample size being approximately 10 mg and the scanning rate 8° · $min^{-1}$.

*Solubility studies*

Phase–solubility diagrams were obtained according to the method described by Higuchi and Connors (1965). Excess amounts of metronidazole benzoate (11.0 mg) were accurately weighed and added to 10 ml of aqueous solutions containing various concentrations of β-CyD in screw-capped vials. These were then rotated on a mechanical spindle at room temperature (24°C) for one week. It was ascertained that this was sufficient to ensure solubility equilibrium. Following equilibration, the contents of the vials were filtered. Aliquot portions of the filtrate were properly diluted and analyzed by HPLC for total metronidazole benzoate.

*Analysis of metronidazole benzoate*

An HPLC method was used to quantitate metronidazole benzoate (Bundgaard et al., 1983). The column was eluted at ambient temperature with methanol–0.005 M acetate buffer (pH 4.5) (65 : 35 v/v), the flow rate being 1.6 ml · $min^{-1}$. The column effluent was monitored at 319 nm. The ester showed a retention time of about 3 min whereas the product of ester hydrolysis, metronidazole, eluted with the solvent front. The content of metronidazole benzoate in the samples injected was determined by comparing the peak height to those of standards chromatographed under similar conditions. No interference from β-CyD was produced.

*Kinetic studies*

Kinetic measurements were carried out in aqueous buffer solutions at 24°C. The reaction solutions containing varying concentrations of β-CyD were kept in a constant temperature water bath in screw-capped vials. The reactions were initiated by adding 100 μl of an ethanolic solution of metronidazole benzoate to 10 ml buffer solution to give an initial concentration of about $10^{-4}$ M. At appropriate intervals samples were taken and analyzed by the stability-indicating HPLC procedure described above. Pseudo-first-order rate constants for the hydrolysis were determined from linear plots of the logarithm of remaining ester against time.

*Preparation of a solid complex*

A solid complex of metronidazole benzoate and β-CyD was obtained using conditions derived from the descending part of the phase–solubility diagram shown in Fig. 1: a mixture of 1 litre of a $1.35 \times 10^{-2}$ M β-CyD solution and 1.1 g (4 mmol) of metronidazole benzoate was stirred for 6 days at room temperature and then filtered to yield the complex as a microcrystalline precipitate. The complex was washed with water and dried overnight in vacuo over phosphorous pentaoxide.

*Stability studies*

The physical stability of the complex in water was investigated at 8°C and compared to that of free metronidazole benzoate. Sub-sieve range metronidazole benzoate anhydrate powder (0.5 g) was dispersed in 30 ml of water and placed at 8°C. The dispersion of the powder was accelerated by ultrasonic treatment. The solid complex (0.5 g) was treated in the same manner. At appropriate intervals the suspensions were examined visually for change in color and microscopically for

change in particle size. A qualitative test for hydrate formation was done on samples of the crystals. The samples were carefully dried in the air at room temperature for 4 h and then tested for hydrate water by detecting gas evolved when heated in a differential scanning calorimeter.

## Results and Discussion

*Inclusion complexation in solution*

The phase–solubility diagram obtained for metronidazole benzoate with β-CyD is shown in Fig. 1. The solubility curve can be classified as type $B_S$ (Higuchi and Connors, 1965) with a microcrystalline complex precipitating from the solutions at the higher β-CyD concentrations. The ascending part of the diagram is a straight line and may be ascribed to the formation of a 1 : 1 complex. The apparent formation constant for such a complex can be determined from the initial straight line portion according to the following equation (Higuchi and Connors, 1965):

$$K = \frac{\text{slope}}{S_0(1 - \text{slope})} \tag{1}$$

where $S_0$ is the equilibrium solubility of the substrate in the absence of cyclodextrin ligand and thus equal to the intercept of the plot shown in Fig. 1. A value of $1.3 \times 10^3\ M^{-1}$ was found for K.

Although the geometrics within the inclusion complexes in aqueous solutions cannot be accurately defined, it is reasonable to assume a 1 : 1 complexation because this would allow maximum contact of the hydrophobic portion of the organic substrate with the apolar cavity of β-CyD (cf. Griffiths and Bender, 1973). At the later part of the diagram a microcrystalline complex is precipitating from the

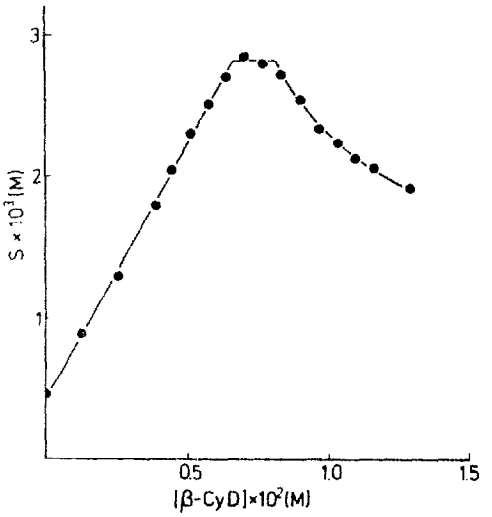



Fig. 1. Solubility (S) of metronidazole benzoate as a function of β-cyclodextrin concentration in water at 24 °C.

solutions. This solid complex is of a higher order than 1 : 1 because in the latter case the descending part of the solubility curve will only fall to a value being $S_0$ lower than the plateau region.

*Effect of complexation on rate of hydrolysis*

Inclusion complexation of metronidazole benzoate with β-CyD was also assessed kinetically by studying the influence of the cyclodextrin on the rate of hydrolysis of the ester at 24°C in a 0.05 M carbonate buffer of pH 10.40. For all solutions containing varying concentrations of β-CyD the disappearance of metronidazole benzoate displayed strict first-order kinetic behaviour. Fig. 2 shows the effect of β-CyD concentration on the observed pseudo-first-order rate constants ($k_{obs}$) for the ester hydrolysis. It is readily evident that β-CyD has a stabilizing effect. As seen in Fig. 2, $k_{obs}$ asymptotically approaches a minimum value as the β-CyD concentration is increased. This saturation behaviour is characteristic of reactions which proceed through a complex prior to the rate-determining step and may be accomodated by the mechanism illustrated in the following scheme:

$$\begin{array}{ccc} MB + CyD & \overset{K}{\rightleftharpoons} & MB\text{–}CyD \\ \downarrow k_0 & & \downarrow k_c \\ \text{Products} & & \text{Products} \end{array}$$

where MB represents metronidazole benzoate, MB–CyD the inclusion complex between the drug of β-CyD, $k_0$ and $k_c$ are the pseudo-first-order rate constants for the degradation of uncomplexed and complexed metronidazole benzoate, respectively, and K is the apparent formation constant for the complex. From this 1 : 1 complexation scheme the following rate expression may be derived (Griffiths and Bender, 1973):

$$k_{obs} - k_0 = -\frac{(k_{obs} - k_0)}{K[CyD]} + (k_c - k_0) \qquad (2)$$

Fig. 2.

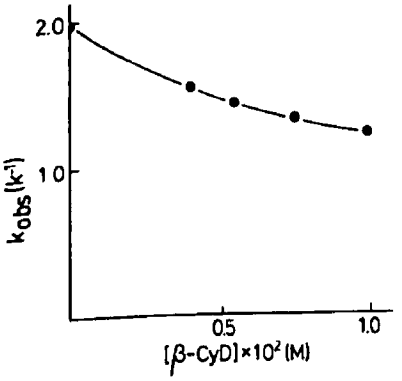



Fig. 3.

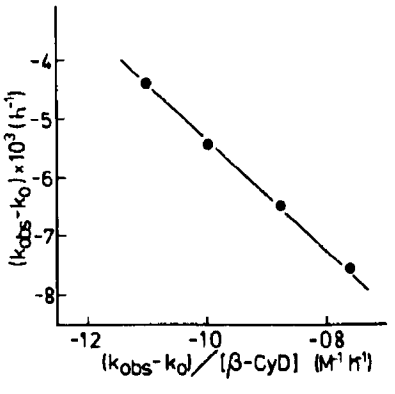



Fig. 2. The effect of β-cyclodextrin concentration on the pseudo-first-order rate constant for the hydrolysis of metronidazole benzoate at 24°C in 0.05 M carbonate buffer solution of pH 10.40.

Fig. 3. Plot of the rate data in Fig. 2 according to Eqn. 2.

In Fig. 3 the rate data of Fig. 2 are plotted according to Eqn. 2. From the slope and intercept of the plot the following values of $k_0$, $k_c$ and K were obtained:

$k_0 = 1.98 \times 10^{-3}\ min^{-1}$

$k_c = 5.1 \times 10^{-4}\ min^{-1}$

$K = 1.1 \times 10^{3}\ M^{-1}$

The value of the kinetically determined complex formation constant is seen to agree well with the value ($1.3 \times 10^{3}\ M^{-1}$) found from the solubility study.

Concerning the structure of the complex in solution, the stabilizing effect against hydrolysis observed by complexation indicates that the ester is included in the cyclodextrin cavity in such a way that the ester carbonyl grouping is partly protected against attack by hydroxide ions or cyclodextrin alkoxide ions. Benzoic acid is known to form inclusion complexes with cyclodextrins (Lewis and Hansen, 1973) and it is therefore likely that the benzoate moiety of the metronidazole ester is involved in the complex formation. This is further supported by the UV-spectra of metronidazole and the benzoate ester in β-CyD solutions. Whereas the spectrum of metronidazole is the same in aqueous solutions with or without β-CyD, the spectrum of the ester shows a clear bathochromic shift in the presence of β-CyD.

*Inclusion complexation in the solid state*

The microcrystalline complex isolated as described in the experimental section was shown by HPLC analysis to contain 13.9% w/w of metronidazole benzoate. The rest was considered to be β-CyD as no water could be driven off by drying at 140°C. The stoichiometric composition of the complex is therefore 1 : 1.5, i.e. one mole of the drug to 1.5 moles of β-CyD.

That the product is a true inclusion complex and not a simple physical mixture was substantiated on the basis of differential scanning calorimetry. As shown in Fig. 4 the thermograms of metronidazole benzoate and its physical mixture with β-CyD (the composition being 1 : 1.5 on a molar basis) show an endothermic peak at about 98°C corresponding to the melting of the drug. In contrast, this endothermic peak was not observed in the case of the assumed inclusion complex.

*Depression of metronidazole benzoate hydrate formation in aqueous suspension*

As reported by Hoelgaard and Moller (1983) a drastic increase in particle size is observed when aqueous suspensions of the anhydrous metronidazole benzoate are stored for a few weeks at low temperature (about 8°C), the crystal growth being a result of a phase transition of the anhydrous form to the monohydrate. By inclusion complexation with β-CyD it has now been found possible to depress this phase transition and consequently increase the physical stability of aqueous suspensions of metronidazole benzoate. Aqueous suspensions of sub-sieve range metronidazole benzoate (i.e. the clinically used anhydrous form) and of its β-CyD inclusion complex were stored at 8°C. As seen from the microphotographs in Fig. 5 the

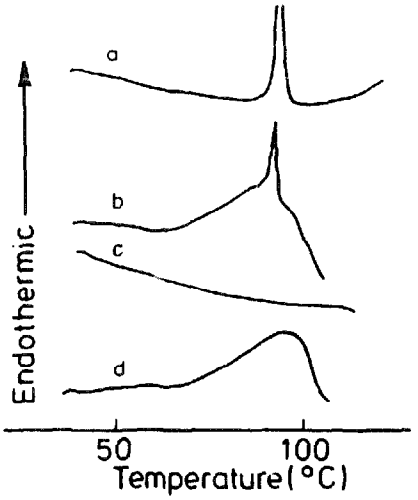



Fig. 4. Differential scanning calorimetry of metronidazole benzoate (a); a physical mixture of metronidazole benzoate and β-cyclodextrin (b); the complex of metronidazole benzoate with β-cyclodextrin (c); and β-cyclodextrin (d).

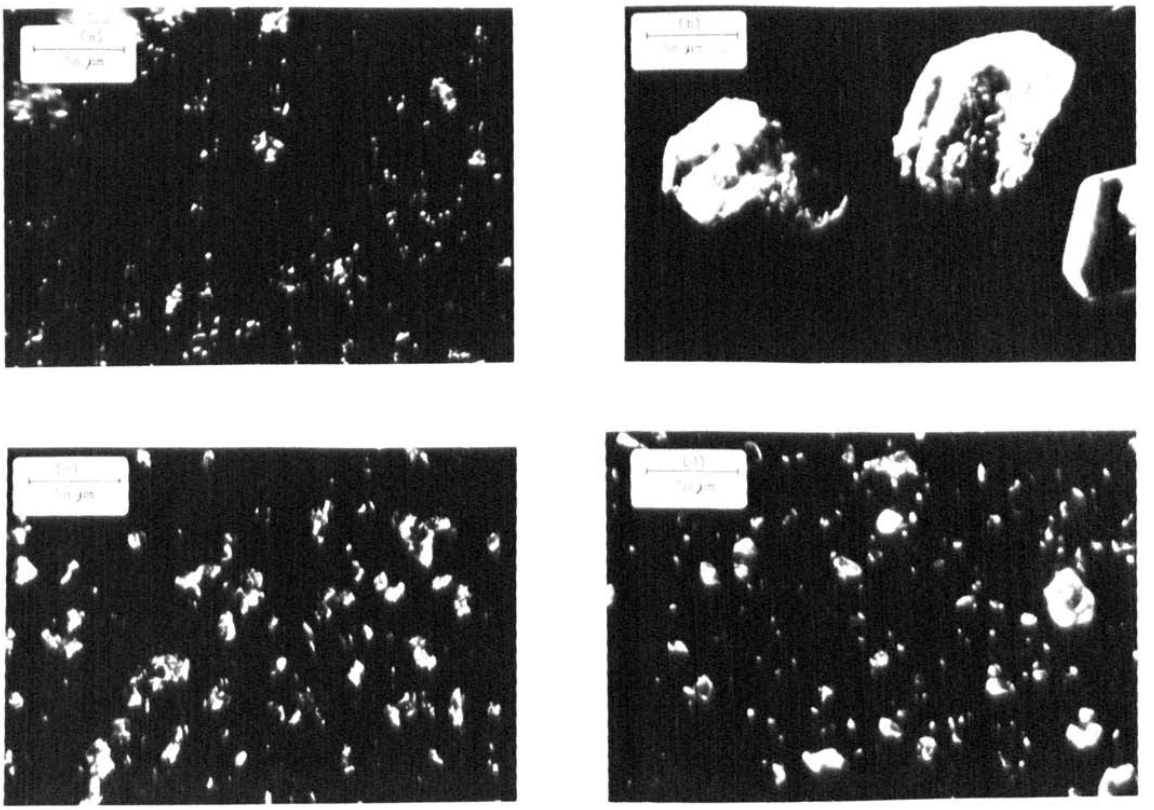


Fig. 5. Microphotographs of a freshly prepared suspension of metronidazole benzoate (anhydrate) in water (a); the same suspension after storage for 3 weeks at 8°C (b); freshly prepared suspension of the metronidazole benzoate complex with β-CyD (c); and the suspension (c) after storage for 6 months at 8°C (d).

suspension of the complex exhibits no significant particle growth after storage for 6 months at 8 °C. In contrast, a marked crystal growth is observed with the suspension prepared from pure metronidazole benzoate. After a storage time of 3 months crystals were isolated from both suspensions, washed with water and carefully dried in air for 4 h. Differential scanning calorimetry of the crystals from the suspension of metronidazole benzoate revealed two endothermic peaks (Fig. 6). Simultaneous effluent gas recording showed a peak in connection with the first endotherm at about 40 °C, indicating the escape of hydrate water (cf. Hoelgaard and Møller, 1983). In contrast, no peaks were seen in the DSC curve of the cyclodextrin complex (Fig. 6), demonstrating its superior stability over free metronidazole benzoate.

Besides increasing the physical stability of aqueous metronidazole benzoate suspensions inclusion complexation with β-CyD was found to protect the drug against photochemical degradation. When exposed to daylight, aqueous suspensions of the compound quickly turned yellow (i.e. within 2–3 days), presumably due to a photochemical surface oxidation on the crystals. On the contrary, suspensions of the inclusion complex remained colorless for more than 3 months when stored under similar conditions.

Inclusion complexation of drugs with cyclodextrins may be useful to solve various pharmaceutical formulation problems such as improvement of solubility, dissolution and chemical stability. The present findings of inhibition of hydrate formation and crystal growth in aqueous suspensions demonstrate an apparently new property to be attained by such complexation and thus extend the field of pharmaceutical application of inclusion complexation with cyclodextrins.

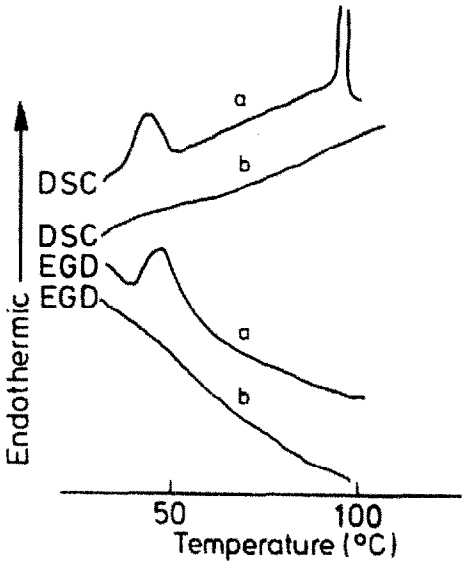



Fig. 6. Differential scanning calorimetry (DSC) of suspensions stored for 3 months at 8 °C: (a) metronidazole benzoate; (b) metronidazole benzoate complex with β-CyD. The simultaneous effluent gas recording (EGD) is also shown.

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