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EFFECT OF HYDROTROPIC SUBSTANCES ON THE COMPLEXATION OF CLOTRIMAZOLE WITH ß-CYCLODEXTRIN

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ABSTRACT

The phase diagrams of clotrimazole/ß-cyclodextrin (ß-CD) in phosphate buffer, pH=7.1, containing 0.5 M of various hydrotropic agents were constructed. The water structure disruptors, urea and nicotinamide, increased the intrinsic solubility of the antimycotic drug clotrimazole while the water structure forming agents, sorbitol and fructose, decreased the solubility. Concerning the complex constant between clotrimazole and ß-CD, it was the other way around. The connection between the slopes of the phase diagrams, the intrinsic solubility of clotrimazole and the complex constant was discussed. Nicotinamide decreased the solubility of ß-CD in the buffer solution. The results reported in this study are in disagreement with the claim that addition of water structure forming agents to cyclodextrin solutions can be used to increase the total solubility of drugs.

INTRODUCTION

Cyclodextrins are cyclic oligosaccharides. The three major cyclodextrins are α-, ß- and γ-cyclodextrin, comprising 6, 7 and 8 glucopyranose units, respectively (1). Due to their ability to form inclusion complexes with drugs, cyclodextrins have a great potential as pharmaceutical excipients. The complexation may increase the bioavailability, the water solubility and the stability of the drug or it may mask the taste (1). The degree of complexation between the drug and the cyclodextrin in solution is of importance as far as bioavailability, water solubility, stability and masking of taste are concerned. If the drug is ionizable, the complex constant can be varied by changing the pH in the solution. It is a general statement that ions are weak complex forming agents (1). The complex constant may also be varied by using different types of cyclodextrins, i.e. chemically modified cyclodextrins. Addition of agents able to compete with the drug molecules for the cyclodextrin cavities may decrease the apparent complex constant between drug and cyclodextrin (2). Another way of varying the degree of complexation is by the addition of agents with a positive or negative hydrotropic effect (3,4,5).

In this study the influence of hydrotropic agents on the complexation between clotrimazole and ß-cyclodextrin (ß-CD) was investigated. Clotrimazole is a lipophilic antimycotic drug used both locally and systemically. Complexation between clotrimazole and cyclodextrins has been reported previously (6).

MATERIALS

Clotrimazole, ß-CD, D-fructose, nicotinamide and n-nonylamine were purchased from Sigma Chemical Co.(St.Louis, Mo.,USA). Urea was purchased from Mecobenzon (Copenhagen,DK) while sorbitol of a pharmaceutical grade was a gift from Fertin Laboratories A/S (Vejle,DK). All other chemicals were of analytical grade.

METHODS

Phase Diagrams

Solubility measurements were carried out according to the method described by Higuchi and Connors (7). To 10 ml 0.05 M ammonium phosphate buffer solution, pH=7.1, or to 10 ml of the buffer solution containing 0.5 M hydrotropic agent, various amounts of ß-CD were added. The solutions were heated gently to dissolve the ß-CD. When the solutions had reached room temperature, 23±2°C, 10 mg of clotrimazole were added to each tube. The suspensions were rotated for at least 7 days after which equilibrium was reached. The suspensions were filtered through 0.2 μm Sartorius cellulose acetate membrane filters. The concentration of clotrimazole in the filtrates was determined immediately after the filtration, using the HPLC method described later.

HPLC method

The concentration of clotrimazole in the filtered samples was estimated by an HPLC method using a Merck/-Hitachi model 655A-11 pump, a Merck/Hitachi UV-monitor model 655A-22, a Rheodyne model 1725 injection valve fitted with a 20 μl sample loop, a Merck chromatointegrator 2000, a Merck lichrospher 100 RP 18 reverse phase column (4mmx125mm) and a Merck guard column lichrosorb RP 18 (4mmx10mm). The detection wavelength was 230 nm. The mobile phase consisted of 75 % methanol, 25 % aqueous 0.01 M diammonium hydrogen phosphate / 0.02 M ammonium dihydrogen phosphate buffer and 0.005 M n-nonylamine to avoid tailing. The retention time was 4.4 min. Before the analysis the filtered solutions were diluted (1:1) with methanol. The solvent for the standards consisted of 0.05 M ammonium phosphate buffer, pH=7.1, and methanol (1:1).

Differential Scanning Calorimetry

To 100 ml 0.05 M ammonium phosphate buffer, pH=7.1, containing 0.5 M nicotinamide and 18 mg/ml ß-CD, various amounts of clotrimazole (20, 40, 60, 80 and 100 mg) were added. The samples were rotated for at least 10 days. The

precipitate was isolated by paper filtration, and it was washed with a few drops of water before it was dried in vacuum over phosphor pentoxide for 24 hours. The dried material was studied by differential scanning calorimetry (DSC) using a Perkin Elmer TAC 7/PC Instrument Controller and Perkin Elmer multitasking software. Nitrogen was used as carrier gas, the scan speed was 10°C/min. The sample size was in the range 1-5 mg. Samples consisting of pure clotrimazole and pure ß-CD were run too.

ß-CD solubility in nicotinamide solution

Various amounts of ß-CD (0, 20, 40, 60, 80, 100, 120, 140, 160 and 180 mg) were added to 10 ml samples of ammonium phosphate buffer containing 0.5 M nicotinamide. The samples were heated gently to dissolve the ß-CD, afterwards they were stored for 72 hours at 23±2°C. The samples were examined for precipitate.

RESULTS AND DISCUSSION

Clotrimazole/ß-CD solubility curves for the various solvents were constructed. The slope, the intercept with the y-axis and the apparent stability constant ($K_{1,1}$) are shown in table 1. The slopes are corrected for the water content of ß-CD. $K_{1,1}$ was calculated from the solubility data using the formula:

$$K_{1,1} = \text{slope} / S_o (1 - \text{slope})$$

in which slope is the slope of the initial linear part of the solubility curves, and S_o is the intrinsic solubility of the drug (7). In this study 0 - 1.5×10^{-3} M was used as the initial linear part of the curves, the correlation coefficient was at least 0.987. The intercept with the y-axis was used as an estimate of S_o (8), because the intrinsic solubility of clotrimazole in some of the solvents was under the limit for quantitation (0.5 µg/ml). The type of the solubility curves is shown in table 1 too. Solubility curves can be classified into two major categories. If a plateau

TABLE 1
Parameters from the Clotrimazole/ß-CD Solubility Curves in 0.05 M phosphate buffer, pH=7.1, containing various Water Structure Modifiers. The parameters are the Average of two Determinations.

Water Structure Modifier	Intercept S_o ($Mx10^6$)	Slope ($M/Mx10^3$)	$K_{1,1}$ (M^{-1})	Type of diagram
Buffer	0.61	1.94	3215	A_l
Nicotinamide 0.5 M	5.1	2.01	400	B_s
Urea 0.5 M	0.80	2.45	3079	A_l
Fructose 0.5 M	0.39	1.65	4303	A_l
Sorbitol 0.5 M	0.26	1.57	6063	A_l

region of the solubility curve is observed after an initial rise in solubility, the curve is classified as a B_s type. Type A solubility curves are obtained when the solubility of the substrate increases with increasing ligand concentration over the whole concentration range. A linear relationship is designated as an A_l type. A_p and A_n types of curves exhibit a positive or a negative curvature, respectively (6,7).

The intercepts shown in table 1 indicate that the intrinsic solubility of clotrimazole is dependent on the type of hydrotropic agent used. The results correlate fairly well with the lyotropic serie of nonelectrolytes: sucrose < sorbitol < mannitol / water / urea < methylacetamide < nicotinamide. The substances in the serie are arranged in the order of increasing disruptive effect on the water structure (5), and it is obvious that substances with a positive hydrotropic effect, urea and nicotinamide, increased the solubilization of clotrimazole while sorbitol and fructose with a negative hydrotropic effect, i.e. a water structure forming effect, decreased the intrinsic solubility of clotrimazole, table 1. Especially the solubility increasing effect of nicotinamide is obvious, and it

may at least to some extent be due to complexation between nicotinamide and clotrimazole. The calculated slopes, a measure of the solubilizing capacity of ß-CD, show that agents with a water structure forming effect decreased the slope while urea with a disruptive effect on the water structure increased the slope compared with the slope in phosphate buffer solution. Nicotinamide did not fit into the pattern because the slope in 0.5 M nicotinamide solution was smaller than expected. Exactly the same pattern was observed by Müller and Albers who investigated the effect of hydrotropic agents on the complexation of methyltestosterone and 2-hydroxypropyl-ß-cyclodextrin (2-HP-ß-CD) (5). Müller and Albers also reported that sorbitol decreased the intrinsic solubility of methyltestosterone while urea and nicotinamide increased the solubility.

It may be difficult to see why the slopes of the solubility curves are influenced by the addition of hydrotropic agents. The formula (7):

$$K_{1,1} = \text{slope} / S_o (1 - \text{slope})$$

may be reduced to :

$$K_{1,1} = \text{slope} / S_o,$$

as far as clotrimazole/ß-CD solubility curves are concerned, because (1 - slope) ≃ 1. The slopes reported in table 1 are equal to or smaller than 2.45×10^{-3} M/M.

$$\text{slope} = K_{1,1} \times S_o$$

That is, the slope is directly proportional to the complex constant and to the intrinsic solubility of clotrimazole. The reason why nicotinamide does not fit into the pattern concerning the correlation between hydroptropic effect and slope is the fact that nicotinamide decreased the size of the apparent complex constant ($K_{1,1}$), a decrease which outbalanced nicotinamides effect on the intrinsic solubility of clotrimazole, table 1.

The effect of water structure modifiers, i.e. sorbitol, urea an nicotinamide, on the complexation of methyltestosterone an 2-HP-ß-CD is shown in table 2. The data in table 2 are based on results reported by Müller and Albers (5).

TABLE 2
Effect of Water Structure Modifiers on the Complexation of Methyltestosterone and 2-HP-ß-CD. The data are based on results reported by Müller and Albers (5).

Water Structure Modifier	Intercept S_o ($Mx10^4$)	Slope (M/M)	$K_{1,1}$ (M^{-1})
Distilled water	4.36	0.648	4222
Sorbitol 50% (w/v)	0.96	0.548	12632
Urea 50% (w/v)	19.4	0.761	1638
Nicotinamide 50% (w/v)	530.0	0.535	22

The formula:

$$K_{1,1} = \text{slope} / S_o (1 - \text{slope})$$

can not be simplified for the methyltestosterone/2-HP-ß-CD solubility curves, because the slope is at least 0.535 M/M, table 2. The formula mentioned above can be transformed into:

$$\text{slope} = K_{1,1} \times S_o / (1 + K_{1,1} \times S_o)$$

When $K_{1,1} \times S_o \rightarrow \infty$ slope $\rightarrow$ 1 and

when $K_{1,1} \times S_o \rightarrow 0$ slope $\rightarrow$ 0

Concerning the effect of nicotinamide on the slope of the methyltestosterone/2-HP-ß-CD solubility curves, the conclusion is the same as for the clotrimazole ß-CD system, that is, nicotinamides effect on $K_{1,1}$ outbalanced the effect on the intrinsic solubility of methyltestosterone.

Indirect evidence of the effect of hydrotropic agents on the complex constants between ergocalciferol and ß-CD and between p-nitrophenol and ß-CD indicated that agents with a disruptive effect on the water structure decreased the size of the complex constant, while agents with a water structure forming effect increased the size. In the paper (3) and in the patent (4) it is claimed that the increased complex constant caused by the addition of water structure forming agents stabilized ergocalciferol in ß-CD solution. In

TABLE 3
Total Solubility of Clotrimazole and Methyltestosterone in Cyclodextrin Solutions, to which Hydrotropic Agents are added. The Methyltestosterone data are based on results reported by Müller and Albers (5).

Water Structure Modifier	Methyltestosterone (mg/ml)
2-HP-ß-CD 10% (w/v) in water	13.5
- plus 5% (w/v) sorbitol	13.3
- plus 10% (w/v) sorbitol	13.2
- plus 20% (w/v) sorbitol	12.8
- plus 50% (w/v) sorbitol	11.1
- plus 50% (w/v) urea	16.1
- plus 50% (w/v) nicotinamide	26.9
	Clotrimazole (µg/ml)
ß-CD 1.8% (w/v) in buffer	10.8
- plus fructose 9.0% (w/v)	9.1
- plus sorbitol 9.1% (w/v)	8.7
- plus urea 3.0% w/v)	13.7
- plus nicotinamide 6.1% (w/v)#	7.0

The solubility of ß-CD was 0.9% (w/v).

addition it is claimed that the increased complex constant increases the total solubility of a drug in cyclodextrin solution, and that the principle, addition of water structure forming agents to cyclodextrin solutions, may be used to prepare concentrated drug solutions (4). At least as far as clotrimazole and methyltestosterone are concerned, the principle can not be used, because water structure forming agents, i.e. sorbitol and fructose, decreased the total solubility of the drugs in cyclodextrin solution, table 3.

The clotrimazole ß-CD solubility curve in 0.5 M nicotinamide buffer solution turned out to be a B_S type phase diagram, figure 1.

The plateau region of the diagram was not caused by precipitation of a clotrimazole/ß-CD complex but was due to the

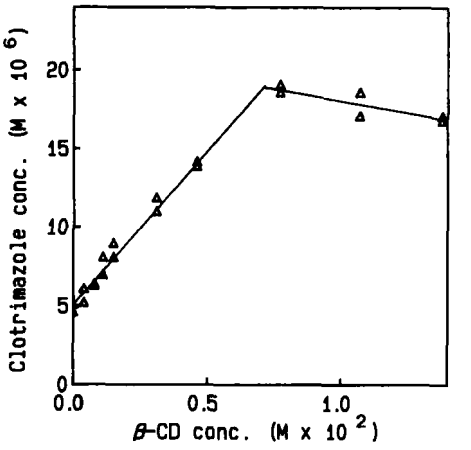

FIGURE 1
Clotrimazole/ß-CD solubility curve in 0.5 M nicotinamide buffer solution, pH=7.1.

limited solubility of ß-CD in 0.5 M nicotinamide buffer solution. The ß-CD solubility studies showed that the ß-CD solubility was between 8 and 10 mg/ml which is in agreement with the solubility curve, figure 1. Additional information was obtained about the solid phase formed in 0.5 M nicotinamide buffer solution during clotrimazole/ß-CD solubility studies by DSC analysis. Even when only 20 mg of clotrimazole was added to 100 ml 0.5 M nicotinamide buffer solution containing 18 mg ß-CD per ml, pure clotrimazole (m.p. 143-144°C) was detectable by DSC in the dried precipitate, isolated after at least 10 days of equilibration, data not shown. It can not be excluded that the decreased solubility of ß-CD in nicotinamide solution was caused by the formation of a nicotinamide/ß-CD complex. Complexation between nicotinamide and ß-CD could at least partly explain the low apparent complex constant, 400 M^{-1}, between ß-CD and clotrimazole in 0.5 M nicotinamide solution, table 1. Pharr et al. reported that urea increased the solubility of ß-CD and γ-cyclodextrin in water while it decreased the solubility of α-cyclodextrin (8). Further

studies are needed to fully understand the effect of water structure disruptors on the solubility of cyclodextrins.

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