JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. 2003; **16:** 121–126
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/poc.582

Complexation of flutamide by native and modified cyclodextrins

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Received 25 April 2002; revised 10 July 2002; accepted 9 August 2002

ABSTRACT: Complexation of flutamide (FM), the drug used in treatment of prostate cancer, with α-, β- and γ-cyclodextrins (CDs), heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD), heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD), 2-hydroxypropyl-β-CD (2HP-β-CD) and carboxymethyl-β-CD (CM-β-CD) was studied. For all CDs the stability constants were determined by the solubility method. UV spectrophotometry and polarography were used in particular cases. The values of the stability constants increased in the order: α-CD ≈ γ-CD < β-CD ≈ CM-β-CD < TM-β-CD ≈ 2HP-β-CD < DM-β-CD. For complexes of FM with β-CD and TM-β-CD, the thermodynamic parameters of complexation were determined from the temperature dependence of the corresponding stability constants. For α-, β-, γ- and TM-β-CD, calculations using HyperChem6 software by the MM^+ force field were carried out to gain some insight into the host–guest geometry. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: flutamide; cyclodextrins; inclusion complexes; stability constants

INTRODUCTION

Cyclodextrins (CDs) are truncated cone-shaped cyclic oligosaccharides built up from six (α-CD), seven (β-CD) and eight (γ-CD) D-glucopyranose units linked by α-1,4 glycosidic bonds.[1] The most important property of these molecules is the ability to form host–guest inclusion compounds with a variety of substrates.[1,2] Through the formation of inclusion complexes the physical, chemical and biological properties of guest molecules can be altered.

The most common pharmaceutical application of CDs is to enhance the solubility, stability and bioavailability of drug molecules.[3–5] The effect of CDs on drug absorption is largely dependent on the magnitude of the stability constant and the dissolution rate of the inclusion compound. The poor water solubility of β-CD (1.85 g per 100 cm^3 at 20 °C) is often the main drawback for its application in pharmacy. Recently, various kinds of CD derivatives have been prepared so as to extend the inclusion capacity as novel drug carriers.[5–7] Most CD derivatives are highly water-soluble products. Among the chemically modified CDs, methylated and hydroxyalkylated CDs have received considerable attention because their physicochemical properties and aqueous solubility are significantly changed and the inclusion behavior is largely magnified, depending on the degree of substitution.[8]

Flutamide (FM), 2-methyl-*N*-[4-nitro-3(trifluoromethyl)phenyl]propanamide (Fig. 1), is a non-steroidal anti-androgen drug commonly used in advanced prostate cancer.[9–11] FM was found to exhibit large differences in bioavailability following oral administration owing to its poor water solubility.

The aim of this work was to study the influence of native and modified CDs on the complexation of FM by the determination of the corresponding stability constants in the hope of improving some of the pharmaceutical properties of FM. To gain some insight into the intermolecular interactions accompanying the complexation, we estimated the thermodynamic parameters of complexation and performed molecular modeling studies using the HyperChem6 program. Studies were carried out with native α-, β- and γ-CD and modified CDs, heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD), heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD), 2-hydroxypropyl-β-CD (2HP-β-CD) and carboxymethyl-β-CD

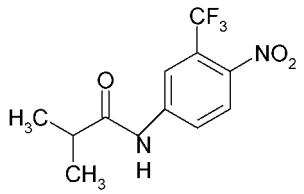

Figure 1. Structure of flutamide

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(CM-β-CD). The stability constants were determined by the UV–visible spectroscopy, polarography (for α,- β and γ-CD) and the solubility method.

EXPERIMENTAL

Materials. α-CD was purchased from Serva (Heidelberg, Germany) and β-CD, DM-β-CD (purity >98%), TM-β-CD, CM-β-CD (average degree of substitution DS $\approx$ 3) and 2HP-β-CD (DS = 2.7) from Cyclolab (Budapest, Hungary). They were used without additional purification. FM was supplied by Anpharm (Warsaw, Poland) and was used as received. Deionized water from a Milli-Q system (Millipore, Bedford, MA, USA) distilled additionally from a quartz still was used for solution preparation.

Spectrophotometric measurements. The absorption spectra were measured in 1 cm quartz cuvets using a Caryl spectrophotometer (Varian) equipped with a thermostatically controlled cell compartment. The change in absorption was measured as a function of CD concentration. The concentration of FM was held constant at 10^{-4} mol dm^{-3} for all the solutions. Six replicate solutions were prepared for each concentration of CD. Fresh solutions were prepared for each replicate run and the stock solutions were also frequently freshly prepared. In the reference cuvete was always the same concentration of CD as in the studied solution. The data were analysed by a statistical treatment.[12]

Solubility measurements. Solubility studies were carried out according to the method of Higuchi and Connors.[13] An excess amount of FM (3 mg) was added to 5 cm^3 of water containing various concentrations of the studied CDs. The suspensions were shaken in screw-capped vials at 25 $\pm$ 0.2 °C. After 6 days, when the equilibrium had been reached, the contents of each vial were centrifuged, filtered through a Schott funel (pore diameter 16–40 μm) and the concentration of FM in the filtered solution was measured by UV spectrophotometry at 302 nm. All the data were the averages of at least three determinations.

Polarographic measurements. The reduction of FM at a dropping Hg electrode from solutions of 0.1 mol dm^{-3} NaCl in the absence and in the presence of increasing concentrations of α-, β- and γ-CDs was studied by a polarographic method. All experiments were carried out in a three-electrode system at 25 $\pm$ 0.5 °C. The counter electrode was a Pt cylinder and a 1 mol dm^{-3} NaCl calomel electrode was used as the reference electrode. The polarographic curves were recorded from deaerated solutions using a measuring system constructed from an EP-20A potenstiostat, an EG-20 function generator (both produced by Elpan, Lubawa, Poland) and an XY-recorder.

RESULTS AND DISCUSSION

Spectrophotometric studies

The absorption spectrum of FM exhibits two maxima at 227 and 302 nm. With a progressive increase in the concentration of the studied CDs both absorption bands decreased and shifted slightly toward longer wavelengths. Figure 2 shows the UV spectrum of 10^{-4} mol dm^{-3} FM (saturated solution) in the absence and in the presence of increasing concentrations of TM-β-CD. The presence of two isosbestic points at 244 and 336 nm indicates the formation of a complex with 1:1 stoichiometry. The UV spectra in the presence of other CDs were similar. In the case of α-CD and γ-CD, the changes in absorbance were so small that they did not allow the accurate determination of the stability constants.

The stability constants were calculated using the Benesi–Hildebrand and Scatchard equations.[14] The plots were linear with typical regression coefficients exceeding 0.999, which indicates the presence of one complex with 1:1 stoichiometry. The association constants were also calculated using a non-linear least-squares regression analysis.

Solubility studies

The phase solubility diagrams of FM in aqueous solutions of studied CDs obtained at 25 °C are shown in Fig. 3. The solubility of FM increased linearly as a function of CD concentration and over the range of concentrations studied showed the features of an A_L type following Higuchi and Connors' classification.[13] The increase in solubility can be attributed to the formation of inclusion complexes between FM and the studied CDs characterized by greater solubilities than that of FM alone. As the slope of the solubility curves is less than unity, it can be assumed that the stoichiometry of inclusion complexes is 1:1. The stability constants of the inclusion complexes were calculated from the straight-line diagrams according to the equation

$$K = s/s_0\ (1 - s) \qquad (1)$$

where s_0 is the solubility of FM in the absence of CDs (the intercept) and s denotes the slope of the straight line.

The solubility of FM in the presence of β-CD in water was determined at 37 °C by Adel *et al.*[15] The value of K estimated by us from their data is included in Table 2.

Polarographic studies

Owing to the presence of the nitro group, the molecule of FM is electroactive. Electroreduction of FM from

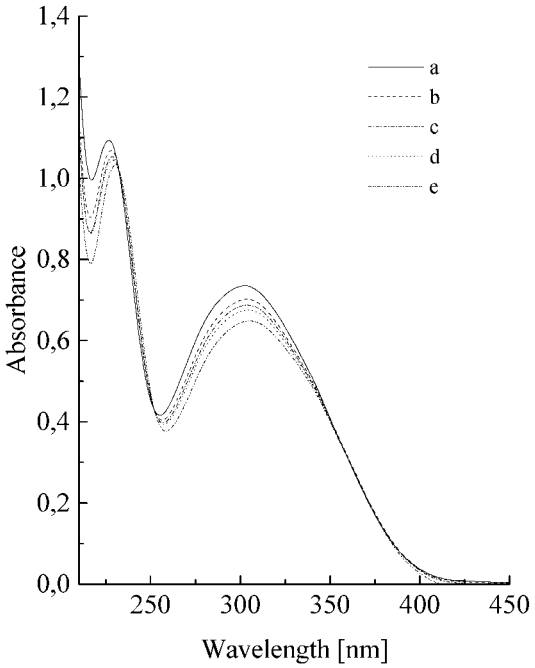

Figure 2. Absorption spectra of flutamide (10^{-4} mol dm^{-3}) in the absence and presence of various concentrations of TM-β-CD at 25°C. Concentrations of TM-β-CD: (a) 0; (b) 1×10^{-3}; (c) 1.5×10^{-3}; (d) 2×10^{-3}; (e) 5×10^{-3} mol dm^{-3}

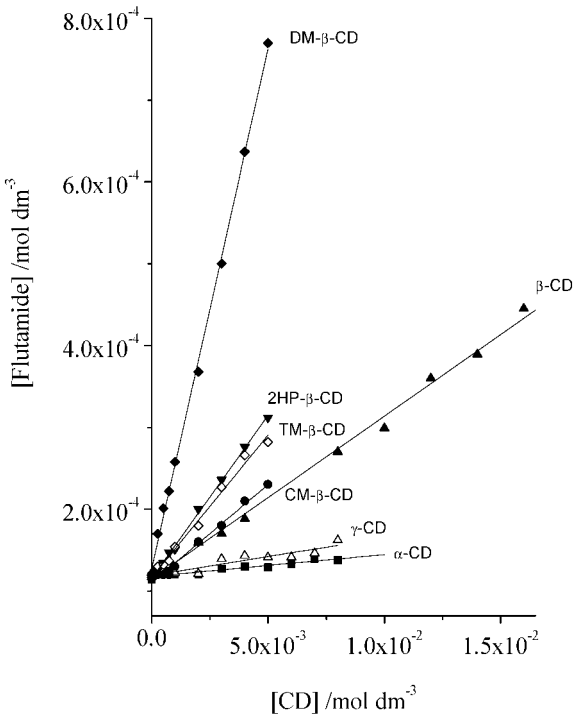

Figure 3. Phase-solubility diagrams of flutamide in the presence of increasing concentrations of the studied cyclodextrins at 25°C: (■) α-CD; (△) γ-CD; (▲) β-CD; (●) CM-β-CD; (◊) 2-HP-β-CD; (▼) TM-β-CD; (◆) DM-β-CD

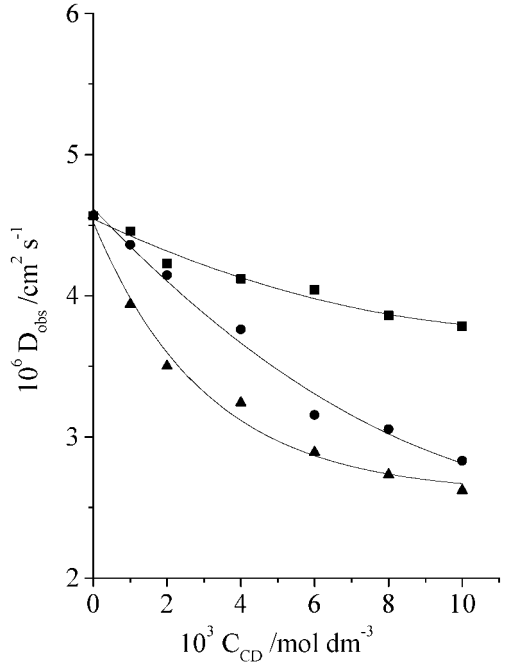

Figure 4. Changes in the observed diffusion coefficients, D_{obs}, of flutamide vs α-CD (■), β-CD (●) and γ-CD (▲) concentration

aqueous solutions of 0.1 mol dm^{-3} NaCl in the absence and presence of α-, β- and γ-CDs was studied polarographically. This salt was chosen because it is not complexed by CDs.[16,17] The diffusion coefficients, D_f, of FM and the observed diffusion coefficients, D_{obs}, in the presence of increasing concentrations of CDs were determined from the polarographic limiting instantaneous diffusion currents using the Ilkovič equation.[18] The changes in D_{obs} vs CD concentration are shown in Fig. 4. The method by which the stability constants were calculated has been described previously.[19] This method also allows the calculation of the diffusion coefficient, D_c, of the guest molecule with the host. The calculated values of D_c for FM with α-, β- and γ-CD were 1.48×10^{-6}, 6.98×10^{-7} and 1.08×10^{-7} cm^2 s^{-1}, respectively.

Stability constants and thermodynamic parameters

The stability constants of FM with native and modified CDs determined by UV spectrophotometry, polarographic and solubility methods are given in Table 1 together with the literature data. Inclusion of FM in β-CD and the stoichiometry of 1:1 has been confirmed by Sortino *et al.*[20] using the induced circular dichroism (ICD) method. The value of K_S that they determined is consistent with that obtained by us using the UV spectrophotometric method. Zuo *et al.*[21] provided experimental evidence that FM is included in HP-β-CD with the

Table 1. Apparent stability constants, K_S, of inclusion complexes of flutamide with CDs determined by various methods at 25 °C

	K_S (dm^3mol^{-1})				
Cyclodextrin	Polarography	Solubility	UV–Vis	ICD	NMR
α-CD	32 (±7)	24 (±3)	—		
β-CD	258 (±8)	200 (±16)	252 (±27)	300 (±50)[20]	
γ-CD	37 (±2)	35 (±8)	—		
CM-β-CD	—	211 (±10)	—		
TM-β-CD	—	354 (±5)	315 (±43)		
HP-β-CD		318[21]			357[21]
2-HP-β-CD	—	308 (±12)	375 (±1)		
DM-β-CD	—	1236 (±52)	1254 (±14)		

aromatic ring inserted in the hydrophobic inner cavity of HP-β-CD. The stoichiometry of the complex derived from NMR and solubility data was 1:1. The values of K_S (Table 1) are similar to our data for 2-HP-β-CD.

As can be seen from the data in Table 1, the stability constants obtained by various techniques are in fairly good agreement with each other and the magnitude of stability constant increases in the order α-CD ≈ γ-CD <β-CD ≈ CM-β-CD <TM-β-CD ≈ HP-β-CD ≈ 2HP-β-CD <DM-β-CD.

The results presented indicate that the size of the cyclodextrin cavity plays an important role in the complex formation. The size of the α-CD cavity can be too small and that of γ-CD too large to obtain a good fit of the guest molecule. Methylation extends the hydrophobic depth of the cavity of β-CD since most of the methyl groups attached to O-2 and O-6 point away from the center of the cavity.[22] The lower stability constant with TM-β-CD than with DM-β-CD suggests that the 3-*O*-methyl groups in TM-β-CD apparently reduce the necessary interaction between FM and the host molecule. A significant increase in the stability constants of many drugs in the presence of DM-β-CD in comparison with β-CD has been reported.[8,23]

The stability constants of FM with β-CD and TM-β-CD determined as a function of temperature by UV spectrophotometry and the thermodynamic parameters of the inclusion process estimated from the temperature dependence of the stability constants using the van't Hoff relation are presented in Table 2.

From the thermodynamic values in Table 2, it can be seen that for both CDs the stability constants decrease with increase in temperature and the complexation process is enthalpy controlled as for many other drug–cyclodextrin complexes.[24] Taking into account the uncertainty in the determination of the thermodynamic parameters ΔH, ΔS and ΔG, their values are similar for both CDs. For the FM–HP-β-CD complex,[21] for which the stability constants increased with temperature increase, the values of ΔH and ΔS were positive. Negative values of ΔH and ΔS indicate that the van der Waals forces are important in the complexation process. Despite the negative ΔH and ΔS values, the hydrophobic interaction also seems to contribute essentially to the association. This is supported by the experimental data indicating that an expansion of the hydrophobic region of the CD cavity enhances the substrate binding. The role of the hydrogen binding seems to be less important because although the permethylation makes the host molecule incapable of forming intramolecular hydrogen bonds, the stability constant with TM-β-CD is higher than that with β-CD.

Molecular modeling

Calculations by the MM^+ force field using HyperChem6 software were performed in order to obtain some global information about the geometry of the host–guest complexes and to find which residue of the FM molecule is mainly affected by CD. Simulations were performed for α-, β-, γ- and TM-β-CDs using the conjugate algorithm included in the Hyperchem6 software package with convergence criteria of 0.001 kcal mol^{-1} (1 kcal = 4.184 kJ). Simulations started from the crystallographic host geometries[25–27] which were minimized. The crystal-

Table 2. Apparent stability constants, K_S, of inclusion complexes of FM with CDs determined at different temperatures and thermodynamic parameters

	K_S ($dm^3\ mol^{-1}$)								
Cyclodextrin	5 °C	10 °C	15 °C	20 °C	25 °C	37 °C	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔG (kJ mol^{-1})
β-CD	554 (±28)	485 (±48)	349 (±43)	303 (±9)	252 (±27)	146[15]	−29.2 (±1.6)	−52.0 (±5.5)	−13.7 (±3.2)
TM-β-CD	812 (±81)	625 (±47)	520 (±15)	410 (±93)	315 (±42)		−31.1 (±1.1)	−56.2 (±4.0)	−14.3 (±2.2)

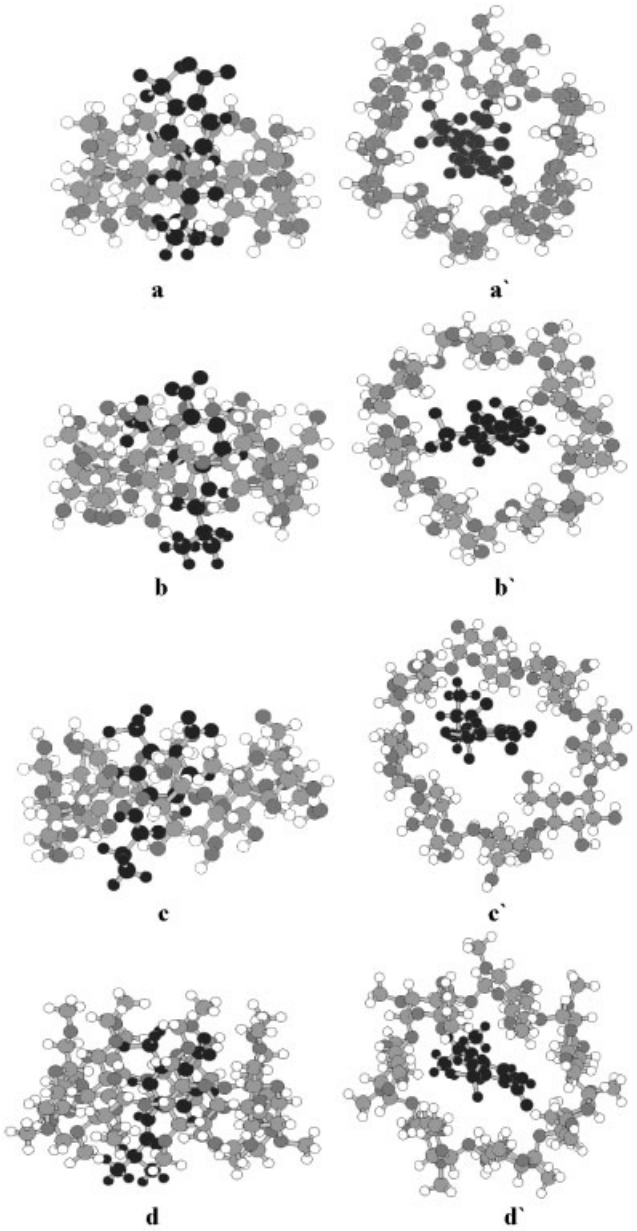

Figure 5. Minimized geometries of the inclusion complexes of flutamide with (a) α-CD, (b) β-CD, (c) γ-CD and (d) TM-β-CD, and the corresponding upside perpendicular views [(a′)–(d′)]. Flutamide molecule is shown by black circles

lographic geometry of FM[28] was also minimized. Subsequently the guest was pushed through the CD cavity for various starting geometries, i.e. the aromatic or aliphatic moiety pointing towards the macrocycle. The resulting energies of the minimum geometries were calculated and compared with the energy for the separated host–guest pair. The lowest energy conformations of the α-CD–FM complex was found at $-88\,\mathrm{kJ\,mol^{-1}}$, of the γ-CD–FM complex at $-94\,\mathrm{kJ\,mol^{-1}}$, of the β-CD–FM complex at $-107\,\mathrm{kJ\,mol^{-1}}$ and of the TM-β-CD–FM complex at $-125\,\mathrm{kJ\,mol^{-1}}$, indicating that the inclusion complex formation with TM-β-CD is the most energetically favorable. This enabled us to identify the sterically feasible complex geometries, which are presented in Fig. 5 together with the upside perpendicular views. The resulting structures show that the FM molecule is included in the cavities with the aromatic ring and the aliphatic part is only buried partly. In the case of α-CD [Fig. 5(a) and (a′)] part of the aromatic ring with two hydrophobic substituents (NO_2 and CF_3) is protruding from the cavity. This could explain the very weak complexation of FM by α-CD. In the case of β-CD [Fig. 5(b) and (b′)] the aromatic ring with the CF_3 substituent is well fitted to the cavity with the NO_2 group protruding. In γ-CD [Fig. 5 (c) and (c′)] the molecule of FM is less tightly bound in the cavity and in such a position that the CF_3 and NO_2 substituents are only partly buried. This may be the reason why the complexation of FM with γ-CD is weaker than that with β-CD. The cavity of TM-β-CD is deeper than that of β-CD because many methyl groups are located at both ends of the cavity, and the whole FM molecule, except the methyl groups of the aliphatic chain, is buried in the host [Fig. 5(d) and (d′)]. This may explain the higher stability constant of FM with this CD in comparison with β-CD.

The difference in the stability constants between TM-β-CD and DM-β-CD may be ascribed to the change in the shape of the cavity. In the permethylated β-CD, methyl groups introduced to the O-3 position enlarge the O-2, O-3 side of the cavity and also make the O-6 side narrower. The effect of such a conformational change can influence the geometry of host–guest interactions and cause a shallower penetration of the aromatic part of the FM molecule in to the cavity in TM-β-CD than DM-β-CD.

CONCLUSIONS

The FM molecule forms inclusion complexes with all the CDs studied. The stoichiometry of the complexes is 1:1. The stability constants determined by UV spectrophotometric, polarographic and solubility methods are in fairly good agreement. The size of the CD cavity plays an important role in the complex formation. The size of the α-CD cavity is too small and that of γ-CD too large to obtain a good fit of the guest molecule, and therefore the corresponding stability constants are low. The stability constants with methylated CDs are higher than with the native β-CD owing to the extension of the hydrophobic depth of the cavity. The difference in the stability constants between TM-β-CD and DM-β-CD may be ascribed to the change in the shape of the cavity.

Acknowledgements

The authors are indebted to Anpharm for providing

flutamide as a gift. We are also grateful to Mrs S. Pawlowska for technical assistance.

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