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Stabilisation of ionic drugs through complexation with non-ionic and ionic cyclodextrins

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Abstract

The effects of negatively charged (i.e. carboxymethyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin), positively charged (i.e. trimethylamoniumpropyl-β-cyclodextrin) and neutral cyclodextrins (i.e. hydroxypropyl-β-cyclodextrin, acetyl-β-cyclodextrin and randomly methylated β-cyclodextrin) on the chemical stability of various drugs were investigated. The degradation rate of each drug in aqueous cyclodextrin solutions was determined and the stability constant (K_c) of the drug–cyclodextrin complex and the degradation rate of the drug within the complex (k_c) was obtained by non-linear fitting of the data. Compared to drug complexes with neutral cyclodextrins, the values of K_c were from 20 to 1600% larger when the drug and cyclodextrin molecules carried opposite charges, but from 50 to 80% smaller when the molecules carried the same type of charge. The values of k_c were not affected by the charge of the cyclodextrin molecule. NMR studies of chlorambucil complexes indicated that the structure of the cyclodextrin complex was at least in some cases affected by the charge on the cyclodextrin molecules.

Keywords: Cyclodextrins; Ionic drugs; Complexation

1. Introduction

Cyclodextrins are cyclic oligosaccharides which are currently being investigated as pharmaceutical excipients, mainly as solubilizing and stabilising agents for lipophilic drugs in aqueous pharmaceutical formulations (Loftsson, 1995; Loftsson and Brewster, 1996). The cyclodextrin molecules have a hydrophilic outer surface and somewhat hydrophobic central cavity. Many drugs are solubilized in cyclodextrin solutions through formation of drug–cyclodextrin inclusion complexes. Because of the nature of the cyclodextrin complex, a large

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increase in the drug stability is frequently observed. However, in some cases the drug molecule interacts with the cyclodextrin hydroxyl groups in such way that the drug degradation is catalysed (Loftsson, 1995).

The most common natural cyclodextrins are, α-, β- and γ-cyclodextrins, which consist of 6, 7, and 8 glucose units, respectively. β-Cyclodextrin, and its derivatives, are the ones most commonly used for pharmaceutical applications since their central cavity has good affinity for many hydrophobic structures of drug compounds (Fromming and Szejtli, 1994; Loftsson and Brewster, 1996).

The parent β-cyclodextrin is not always ideal for drug formulations due to its moderate solubility and reported toxicity after parental administration (Brewster et al., 1989). Therefore, various water/soluble β-cyclodextrin derivatives have been synthesised and used as pharmaceutical excipients. The structure of many β-cyclodextrin complexes has been studied in detail by nuclear magnetic resonance (NMR) (Ueda and Nagai, 1979; Loftsson et al., 1993). The available cyclodextrin derivatives are not always suitable for such study since they consist of a mixture of a number of closely related derivatives and isomeric forms. It is often assumed that the nature of the cyclodextrin derivative complex is the same as that of the parent β-cyclodextrin complex, i.e. interaction between the drug and cyclodextrin is the binding in the hydrophobic cavity. However, it has been shown that cyclodextrin conformations are modified to accommodate for methyl groups in methylated cyclodextrins, thus slightly changing the shape of the cyclodextrin cavity. It is therefore possible that the variation in the stability constant (K_c) and the degradation rate for the complexed drug (k_c), can be explained by such changes in the shape of the cyclodextrin cavity.

In the present work, we investigated the ionic interaction contribution to the complexation of the recently available, ionic cyclodextrins with ionic drug compounds. The values of both k_c and K_c and degradation rate for the drug in buffer (k_o), could be obtained from a series of degradation studies. In this study, we used non-linear regression of the data rather than the previously reported linear regression (Loftsson, 1995) as this allowed better estimation of the error.

2. Materials and methods

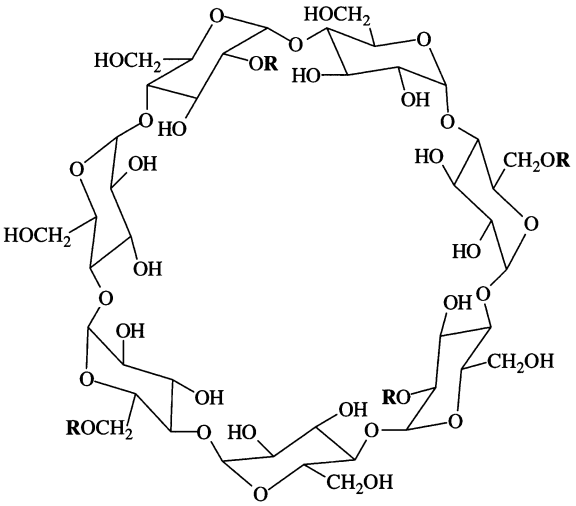
2.1. Materials

The cyclodextrins shown in Table 1 were used for this study. Carboxymethyl-β-cyclodextrin (CM-CD), trimethylamoniumpropyl-β-cyclodextrin (TMA-CD), hydroxypropyl-β-cyclodextrin (HP-CD), acetyl-β-cyclodextrin (A-CD) and randomly methylated β-cyclodextrin (M-CD) were kindly donated by Wacker-Chemie (Germany), and sulfobutylether-β-cyclodextrin (SB-CD, MW $\sim$ 2160) was kindly donated by CyDex (Kansas). The drug compounds were obtained from the following suppliers: acetyl salicyclate, salicylic acid, cephalotin and diazepam form Icelandic Pharmaceuticals (Iceland), indomethacin was purchased from Sigma Chemical Co. (USA), and chlorambucil was supplied by the courtesy of Wellcome Foundation Ltd. (UK). All other chemicals were commercially available chemicals of reagent or analytical grade.

2.2. Chromatography conditions for kinetic studies

A stock solutions of cephalotin was made in water, acetylsalicylic acid in ethanol, and chlorambucil, diazepam, indomethacin and phenobarbital in methanol. Between 10 and 7.5 μl of the drug stock solution were added to 1.5 ml of the cyclodextrin solutions, which were kept in a temperature controlled sample rack in an AS-4000 (Merck-Hitachi) autosampler, and the changes in the drug concentration with time were monitored by HPLC. The HPLC system consisted of Constametric 3000 (Milton Roy) solvent delivery system with a SP8450 (Spectra-Physics) variable wavelength detector, using a 150-mm, 4.6-mm I.D., 5 μm bead, C18 reverse-phase column. The initial concentration of the drug in the reaction media was 3.2×10^{-5} M for cephalotin, 5.7×10^{-5} M for acetylsalicylic acid, 2.3×10^{-5} M for diazepam, 3.8×10^{-5} M for indomethacin, 5.7×10^{-5} M for phenobarbital and 3.4×10^{-5} M for chlorambucil. The mobile phases, detection wavelengths and retention times for the different drugs were as follows: for cephalotin: acetonitrile/acetic acid/tetrahydrofuran/water (35:2:5:63 v/v), 260

Table 1
Structure of the cyclodextrin derivatives



Cyclodextrin derivative	Supplier	Substitution degree (DS)	**R**
CM-CD	Wacker-Chemie	0.5	$-CH_2COO^-$
SB-CD	CyDex	0.9	$-(CH_2)_4SO_3^-$
HP-CD	Wacker-Chemie	0.6	$-CH_2CH(OH)CH_3$
A-CD	Wacker-Chemie	1.0	$-COCH_3$
M-CD	Wacker-Chemie	0.6	$-CH_3$
TMA-CD	Wacker-Chemie	0.5	$-(CH_2)_3N(CH_3)_3^+$

nm, 2.1 min; for chlorambucil: acetonitrile/acetic acid/water (55:1:44 v/v), 257 nm, 3.6 min; for diazepam: methanol/acetic acid/water (65:1:34 v/v), 228 nm, 3.8 min; for indomethacin: acetonitrile/tetrahydrofuran/acetic acid/water (55:5:0.4:39.6 v/v), 256 nm, 3.4 min; and for phenobarbital: methanol/tetrahydrofuran/0.01 M phosphate (pH 7.7)/tetradecyltrimethyl ammonium bromide (51:5:44:0.02 v/v) 240 nm, 2.4 min.

2.3. Data fitting

The observed first-order rate constants in the aqueous cyclodextrin solutions (k_{obs}) or k_o for drug compounds other than diazepam was obtained from linear regression of the logarithm of the HPLC peak intensity plotted against time.

The data was fitted using non-liner fitting of the Kaleidagraph program (Synergy Software, USA) which uses Levenberg-Marquardt algorithm for fitting of a user-defined equation. All data was fitted to a 1:1 complex model, according to the equation:

$$k_{obs} = \frac{k_o + k_c \times K_c \times [CD]}{1 + K_C \times [CD]} \quad (1)$$

The values of k_c and K_c were obtained from the best fit, but the k_o was determined in aqueous buffer solutions containing no cyclodextrin.

2.4. NMR measurements

A stock solution of chlorambucil in CH_2Cl_2 was prepared. Sample (100 μl) of the stock solution was added to a glass vial, the solvent evaporated under a stream of nitrogen and the residue dissolved in cyclodextrin containing D_2O solution. The NMR spectra were recorded at 298 K in D_2O

buffered solutions on a Bruker AC 250 spectrometer using standard software for water suppression. For calibration, the water signal was fixed at 4.80 ppm. To diminish the hydrolysis of the drug, the NMR samples were prepared and equilibrated at 25°C for 10 min just before the spectra were recorded. The chlorambucil concentration varied from 0 to 4 mM. For the spectra, scans from 50 to 500 were necessary depending on the relative concentration of the drug cyclodextrin concentration ratio.

The observed change (Δ_{obs}) in the chemical shifts of the drug in cyclodextrin solutions was:

$$\Delta_{obs} = [D \cdot CD]\Delta_{CD}/[D]_t \quad (2)$$

and the stability constant can be written as:

$$K_c = \frac{[D \cdot CD]}{[D][CD]} = \frac{([D \cdot CD]/[D]_t)}{(1-[D \cdot CD]/[D]_t)([CD]_t/[D]_t - [D \cdot CD]/[D]_t)([D]_t)} \quad (3)$$

By combining Eqs. (2) and (3), Eq. (4) was obtained where the negative solution had been discarded.

$$\Delta_{obs} = \left(1 + \frac{([CD]_t K_c - K_c[D]_t + 1)}{2K_c[CD]_t}\right)\Delta_c - \left(\frac{\sqrt{([CD]_t K_c - K_c[D]_t + 1)^2 + 4K_c[CD]_t}}{2K_c[CD]_t}\right)\Delta_c \quad (4)$$

In these equations, [D] is the concentration of free drug, [D·CD] concentration of complexed drug, $[D]_t$ is the total drug concentration, [CD] and $[CD]_t$ are the cyclodextrin and total cyclodextrin concentrations and Δ_c is the change in chemical shift of the drug when complexed with cyclodextrin.

3. Results and discussion

3.1. Relative affinity of the charged drugs for the cyclodextrin cavity

The cyclodextrins had a marked effect on the degradation rate of chlorambucil, indomethacin and diazepam and thus the values of both k_c and K_c could be calculated for these drugs. The cyclodextrins had only minor effect on the degradation of acetylsalicylic acid, phenobarbital and cephalotin. Previously, we have shown that acetylsalicylate forms a complex with β-cyclodextrin at pH 1.0. (Loftsson et al., 1993). In our present study, we measured the degradation at higher pH (or pH 7.0) in order to have the drug in fully ionised form. Ionic acetylsalicylate apparently has very little affinity for β-cyclodextrin cavity as no effect on the degradation rate could be observed for any of the cyclodextrins tested. NMR study of salicylic acid also confirmed the lack of complexation at pH 7.0.

The degradation for negatively charged phenobarbital was measured at 50°C in an aqueous 0.1 M NaOH (pH 12.88), 75 mM cyclodextrin solutions. The rate of degradation was 25% slower in the aqueous SB-CD solution than in the pure buffer solution. The rate of degradation was 21% slower in aqueous HP-CD solutions, and 8% slower in the M-CD solutions, the effect was insignificant in the TMA-CD, and CM-CD solutions. K_c could not be estimated since the cyclodextrins had insignificant effect on the degradation rate below 10 mM cyclodextrin concentration. The decreased rate of degradation did not reflect any significant ionic interaction. Also at such high cyclodextrin concentrations, the effects observed could be due to secondary phenomena, such as changes in drug activity, rather than formation of a drug–cyclodextrin complex.

The experimental error (10%) in the determination of k_{obs} for cephalotin was to large to allow determination of the K_c values. Previously, it has been shown that cyclodextrin does form a complex with cephalotin at pH 6.5, but the difference between k_o and k_c was only about 10% (Loftsson and Johannesson, 1994).

3.2. Non-linear fitting of the degradation data

Fig. 1 shows the expected degradation pathways as previously reported (Connors et al., 1986) and the proposed structure of the complex for chlorambucil, indomethacin (Backensfeld et al., 1990) and diazepam. All the drugs are degraded via hydrolytic reaction in the aqueous solutions.

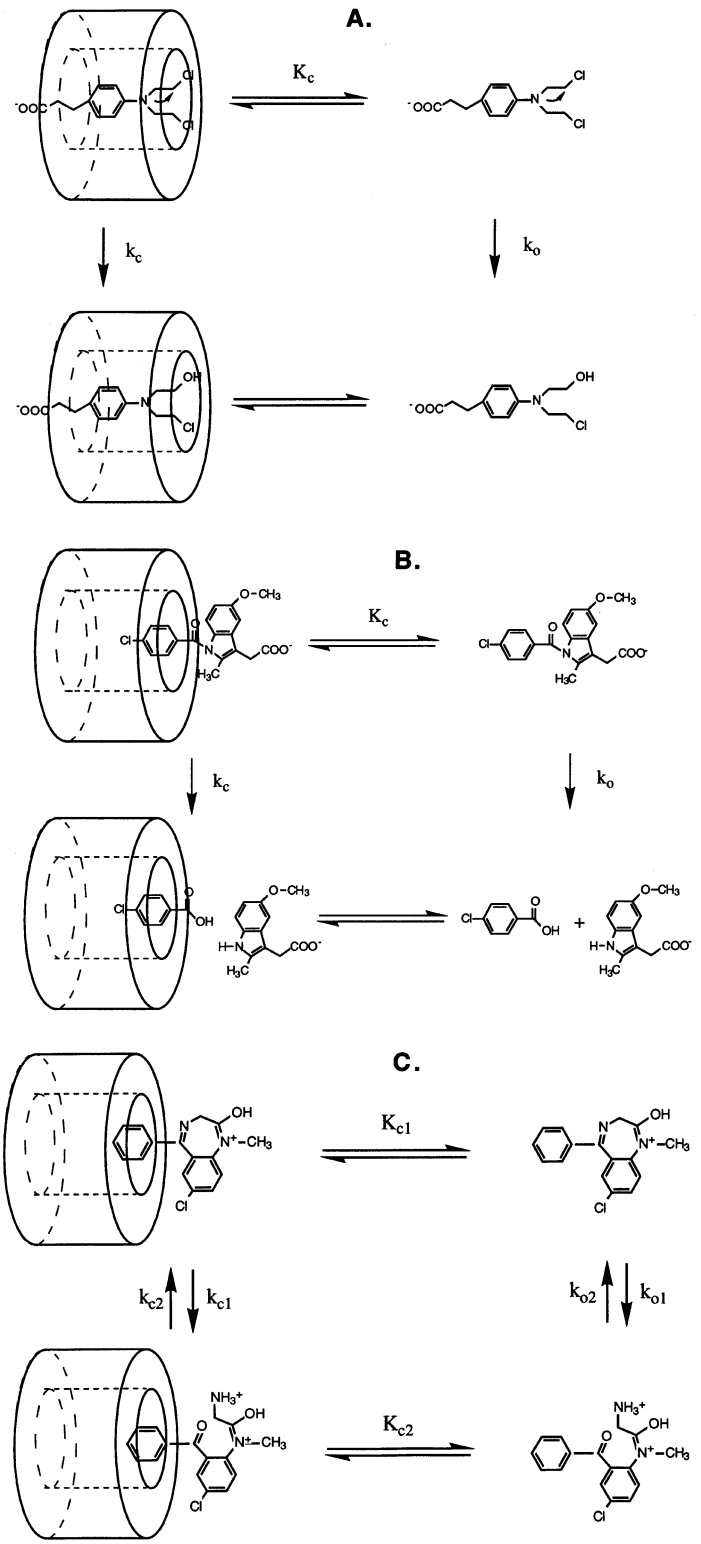


Fig. 1. Proposed structure of the drug–cyclodextrin complexes and the degradation pathways. (A) Chlorambucil, (B) indomethacin, and (C) diazepam. Diazepam is in equilibrium with its degradation product. The cyclodextrin is shown as a cylinder rather than a cone-shape, since it is frequently uncertain from which end the drug is entering the complex.

The overall observed degradation constant (k_{obs}) depends on the degradation constant for the drug in solution (k_o), and the degradation rate constant for drug within the complex (k_c) and the stability constant of the drug–cyclodextrin complex (K_c), as described by Eqs. (5) and (6):

$$-\frac{d[D]_t}{dt} = k_{obs}[D]_t = k_o[D] + k_c[D\cdot CD] \quad (5)$$

$$K_c = \frac{[D\cdot CD]}{[D][CD]} = \frac{[D\cdot CD]/[D]_t}{(1-[D\cdot CD]/[D]_t)[CD]} \quad (6)$$

In these equations, [D] is the concentration of free drug, [D·CD] concentration of drug–cyclodextrin complex, $[D]_t$ is the total drug concentration, and [CD] and $[CD]_t$ are the cyclodextrin and total cyclodextrin concentrations $[CD] \approx [CD]_t$. If $[D]_t \ll [CD]_t$, then

$$([D\cdot CD]/[D]_t) = \frac{K_c[CD]}{1+K_c[CD]} \quad (7)$$

Finally, Eq. (8) can be obtained by combining Eqs. (5) and (7)

$$k_{obs} = k_o\left(1-\frac{K_c[CD]}{1+K_c[CD]}\right) + k_c\left(\frac{K_c[CD]}{1+K_c[CD]}\right) = \frac{k_o + k_cK_c[CD]}{1+K_c[CD]} \quad (8)$$

Both pH and the temperature of the reaction media were chosen such that the degradation rates of the drugs were between 65 and 2 min^{-1}, and the drug molecules were either fully positively charged or fully negatively charged.

Fig. 2 shows how HP-CD affected the drug degradation. The graph shows k_{obs}/k_o versus [CD] with non-linear fitting, as this allows better visual comparison than the conventional Lineweaver-Burk method, where a linear regression of $k_o/(k_o - k_{obs})$ versus 1/[CD] is obtained (Loftsson, 1995). This method also puts equal weight on each data point, whereas the linear regression method will give increased weight to the lower concentration measurements, which can lead to larger errors, especially when K_c is small.

3.3. Effects of cyclodextrins on the equilibrium between diazepam and its hydrolysis product

The hydrolysis of diazepam is a reversible reaction. Whereas, chlorambucil and indomethacin are completely degraded in aqueous solution, an equilibrium will be established between diazepam and its degradation product. The degradation reaction is then described by the rate constants (k_{01} and k_{c1},) for the hydrolysis reaction and the rate constants (k_{02} and k_{c2}) for the dehydration reaction. K_{c1} and K_{c2} are the corresponding stability constants (Fig. 1.). Systems such as this have previously been described in some detail by Capellos and Bielski (1972). In this case, the concentration of the drug relative to the initial drug concentration is dependent on time according to the equation:

$$\frac{[\text{Diazepam}]}{[\text{Diazepam}]_o} = \frac{k_{obs1} + k_{obs2}\, e^{-(k_{obs1}+k_{obs2})t}}{k_{obs1}+k_{obs2}} \quad (9)$$

where k_{obs1} and k_{obs2} are the observed rate constants for each cyclodextrin concentration and $[Diazepam]_o$ is the initial diazepam concentration. Fig. 3 shows that whereas indomethacin is de-

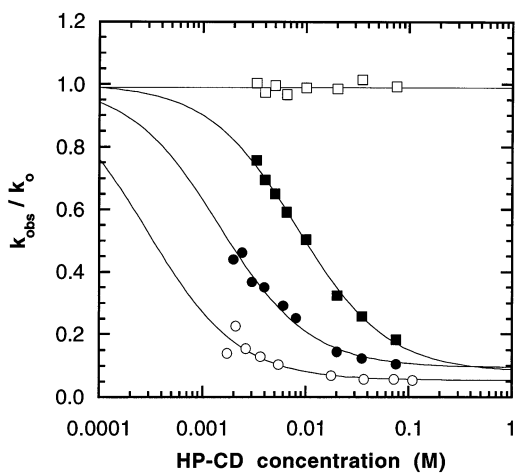


Fig. 2. Degradation rate relative to the HP-CD concentration. (○) Chlorambucil, (●) indomethacin, (□) rate of degradation of diazepam (k_1), and (■) rate of re-formation of diazepam (k_2).

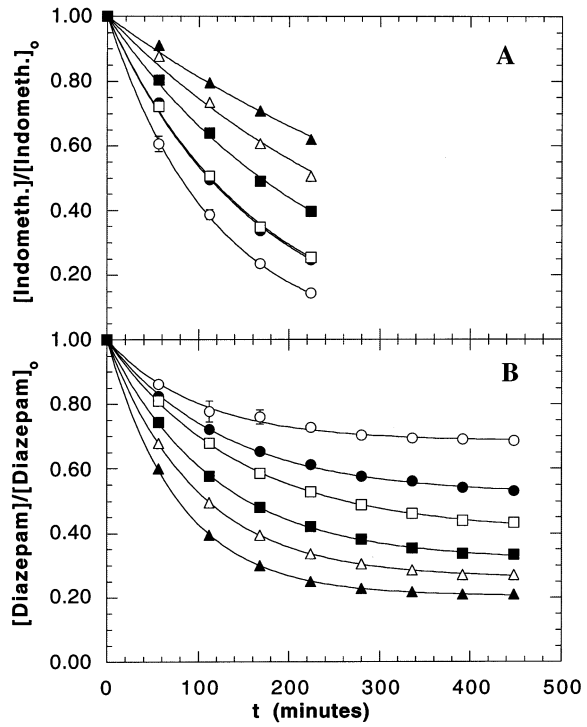


Fig. 3. Degradation profile for indomethacin (A) and diazepam (B) in CM-CD solutions. Diazepam: (○) pure buffer solution, (●) 5 mM CM-CD, (□) 10 mM CM-CD, (■) 20 mM CM-CD, (△) 35 mM CMCD, and (▲) 75 mM CM-CD. Indomethacin: (○) pure buffer solution, (●) 3.3 mM CM-CD, (□) 8 mM CM-CD, (■) 20 mM CM-CD, (△) 35 mM CM-CD, and (▲) 75 mM CM-CD solution. The buffer values were average of four measurements.

graded exponentially, the diazepam degradation progressed towards an equilibrium. In this case, cyclodextrin influenced the equilibrium between diazepam and the hydrolysis product to favour the latter compound, and therefore the initial rate of degradation increased with increasing cyclodextrin concentration. Fig. 2 shows that the rigid diazepam structure complexed poorly, whereas the more flexible degradation product formed cyclodextrin complex more easily.

3.4. Cyclodextrin charge and the stability constant for the complex

Table 1 shows the structures of the cyclodextrin derivatives. TMA-CD is positively charged and SB-CD is a strong acid and carries permanent negative charge even at a low pH. Titration showed that CM-CD had lower pK_a than the structurally similar carboxymethylcellulose, the respective values being 3.6 and 4.3 (Wade and Weller, 1994). This means that CM-CD is fully ionised above pH 5.

Table 2 shows the K_c and k_c value for the drug compounds with the various cyclodextrins. Negatively charged chlorambucil had less affinity for the two negatively charged cyclodextrins than for the neutral cyclodextrin, but the largest complexation was obtained with the positively charged TMA-CD. The stability constants of the indomethacin–cyclodextrin complexes were also smaller in the case of the negatively charged cyclodextrins than in the case of the neutral cyclodextrins.

The opposite trend was observed with the positively charged diazepam, but, because of small value of K_{c1}, it was difficult to interpret the data. The degradation product formed more easily a complex with cyclodextrins (larger K_{c2} values) and in this case the K_{c2} observed with SB-CD, which was the only cyclodextrin negatively charged at pH 2, was 5–17 times larger than with the neutral cyclodextrins and 9–3 times smaller with TMA-CD. Comparable observations have been made for SB-CD by other investigators (Okimoto et al., 1996)

3.5. Complexation of indomethacin with TMA-CD

Complexation of indomethacin with TMA-CD was somewhat different from the other drug–cyclodextrin complexes studied. In the beginning, when the cyclodextrin concentration was increased the degradation rate decreased, but then it increased again upon further increase in the cyclodextrin concentration (Fig. 4.). These changes could not be due to ionic strength changes since the ionic strength was equally high in other solutions containing ionic cyclodextrins in which the data was consistent with 1:1 complex formation. It has been shown that in the same solution there can co-exist more than one form of cyclodextrin inclusion complexes (Aki et al., 1996; Crouzy et

Table 2
Stability (K_c) and degradation constants (k_c) for the drugs in complex with cyclodextrin derivatives

Cyclodextrin	Charge	K_c ($\times 10^{-3}$, [M^{-1}])	k_c [min^{-1}]	k_c/k	R^2
Chlorambucil (40°C, pH 7.35)	−		65.3 ± 1.4*		
CM-CD	−	0.72 ± 0.03	3.2 ± 0.6	0.049	0.99
SB-CD	−	1.40 ± 0.05	1.0 ± 0.4	0.015	1.00
HP-CD	0	3.40 ± 0.72	3.6 ± 1.0	0.055	0.79
A-CD	0	2.62 ± 0.04	2.1 ± 0.1	0.032	1.00
M-CD	0	3.55 ± 0.53	3.3 ± 0.6	0.051	0.88
TMA-CD	+	4.25 ± 0.36	1.2 ± 0.4	0.018	0.95
Indomethacin (40°C, pH 9.8)	−		8.53 ± 0.36*		
CM-CD	−	0.07 ± 0.01	1.09 ± 0.51	0.12	0.97
SB-CD	−	0.26 ± 0.02	−0.09 ± 0.23	−0.01	0.98
HP-CD	0	0.69 ± 0.06	0.84 ± 0.13	0.09	0.97
M-CD	0	0.77 ± 0.09	1.42 ± 0.20	0.16	0.96
TMA-CD	+	Did not form 1:1 complex			
Diazepam (30.5°C, pH 2.0) K_{C1} and k_{c1}	+		2.5 ± 0.1*		
CM-CD	0	0.01 ± 0.00	25.0 ± 6.9	10.0	0.98
SB-CD	−	0.07 ± 0.06	1.7 ± 0.2	0.7	0.73
HP-CD	0	No effect			
M-CD	0	0.14 ± 0.05	3.0 ± 0.1	1.2	0.86
TMA-CD	+	No effect			
Diazepam (30.5°C, pH 2.0) K_{C2} and k_{c2}	+		6.0 ± 0.4*		
CM-CD	0	0.36 ± 0.07	2.2 ± 0.2	0.37	0.91
SB-CD	−	2.05 ± 0.26	0.0 ± 0.1	0.00	0.92
HP-CD	0	0.12 ± 0.01	0.5 ± 0.1	0.08	1.00
M-CD	0	0.18 + 0.01	0.5 ± 0.1	0.08	0.99
TMA-CD	+	0.04 ± 0.01	1.8 ± 0.2	0.30	0.99

* k_o for the drug at the indicated conditions.

al., 1996). In such cases, the observed values of K_c and k_c are the weighted averages of the different forms as long as all the complexes are of 1:1 stoichiometric ratio. If an 1:2 complex (where the drug molecule is complexed with two cyclodextrin molecules) is also formed it will dominate at higher cyclodextrin concentrations. If the charge–charge interactions are weak in the normal inclusion complex, such interaction could be increased by interaction with another cyclodextrin molecule (i.e. formation of 1:2 complex). This could explain the k_{obs} pattern seen for the indomethacin TMA-CD solutions. At higher concentration, a 1:2 complex could be formed with higher k_c values than the 1:1 complex predominant at lower concentrations.

3.6. The k_c values for drugs in a complex

No direct relationship between the cyclodextrin charge and the k_c could be observed. However, it was notable that k_c for SB-CD was always lower than for the other cyclodextrins and in the case of indomethacin and diazepam (i.e. for k_{c2}) not significantly different from zero. SB-CD is more highly substituted than the other CD molecules, with the exception of A-CD, with the charged groups extended far away from the cyclodextrin cavity. The environment around the cyclodextrin cavity could, therefore, offer better protection of the drug molecule.

3.7. NMR of chlorambucil complexes

The calculated NMR shifts for fully complexed molecules are shown in Tables 3 and 4. When chlorambucil formed a complex with the β-cyclodextrin the protons which are inside the cavity are shifted downfield, mainly due to the anisotropical effect from the aromatic moiety inside the cavity. Our measurements showed that the shift was largest for H′-5, and slightly larger shift for H′-6, than H′-4. This places the benzene ring near the narrow cavity (H′-6 end). The small shifts of chlorambucil H-1, H-2 and H-3 protons showed that these were outside the cavity. The aromatic protons are shifted downfield as they will in hydrophobic environment. The H-6 and H-7 protons, forming one singlet, will be inside the cavity and, thus, they were shifted upfield. The hydrophobic effect shifts the protons downfield but the data obtained in a $CDCl_3/F_3CCOOH$ media indicates that stronger hydrogen bonding of the nitrogen ion pair will have the opposite effect. The data therefore indicates that chlorambucil forms stronger hydrogen bonding inside the cavity, with a co-complexed water molecule or cyclodextrin –OH group, than outside the cavity. The lowest energy structure of the complex would therefore be as shown in Fig. 1, with the benzene ring closer to the narrow end of the cavity.

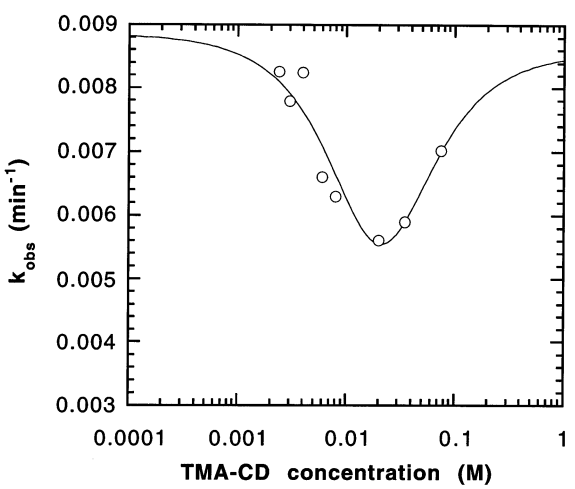


Fig. 4. Degradation profile for indomethacin in TMA–CD complex. The data could be fitted for 1:1, 1:2 complex system.

The cyclodextrin derivatives are mixtures isomers and, thus, all the NMR peaks obtained were broad. The NMR shifts of the derivatives could therefore not be studied, but the chlorambucil peaks were sharp. The cavity of the cyclodextrin derivatives should have similar structure as that of the parent β-cyclodextrin and similar shifts would therefore be expected for the chlorambucil molecule. The shifts for CM-CD, SB-CD, M-CD and HP-CD are similar to those obtained for β-cyclodextrin. That is a -0.163 to -0.204 ppm shift of H-5 and approximately 30% larger shift of H-4 (i.e. -0.222 to -0.281) and a positive shift of H-6,7 which confirmed that the position of chlorambucil within all these complexes was the same.

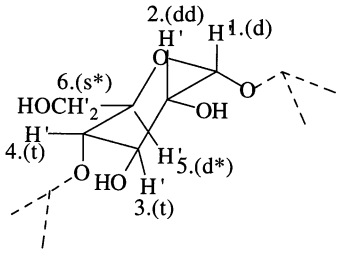
TMA-CD has a positive charge and therefore the most favourable position of the negatively charged acid group of chlorambucil is not far away from the positively charged cyclodextrin. The most favourable position of the drug should therefore be expected to be different from the other cyclodextrin complexes. NMR confirmed this prediction as indicated by the H-4 and H-5 shifts. This was also observed for A-CD, with lower shifts, a effect which could not be predicted. Hydrogen bonding could play some role in this complex formation.

Degradation of the other drugs in A-CD complexes was not studied as A-CD was prone to hydrolysis at higher pH and elevated temperatures, forming acetic acid which affected the pH.

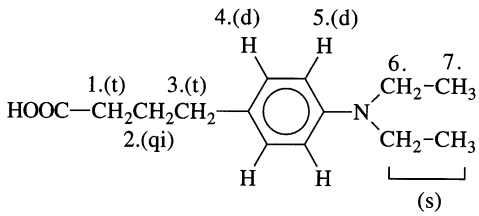
4. Conclusion

The ionic cyclodextrins form stronger complexes with counter-ionic drugs but weaker complexes with drugs carrying the same type of charge, compared to comparable complexes with non-ionic cyclodextrins. The charge–charge forces were usually not additive to the other forces involved in the complex formation. If the forces had been additive, one would expect at least a 10-fold increase in the value of the stability constant, i.e. K_c, which was not the case. The NMR studies indicate that this lower than expected increase in the stability constant could be due to changes in

Table 3
Shifts of the β-cyclodextrin protons when complexed with chlorambucil relative to buffer and the relative shifts of the chlorambucil protons



Cyclodextrin



Chlorambucil

β-Cyclodextrin	Chemical shift	Relative shift	
Proton	Buffer	Free	In complex
H′-1	5.096	0.000	−0.028
H′-2	3.993	0.000	−0.018
H′-3	3.907	0.000	−0.068
H′-4	3.890	0.000	−0.002
H′-5	3.674	0.000	−0.164
H′-6	3.609	0.000	−0.087

Chlorambucil	Chemical shift	Relative shift			
Proton	Buffer	Buffer	Methanol	CD_2Cl_2	CD_2Cl_2/F_3CCOOH
H-1	2.208	0.000	0.105	0.163	0.279
H-2	1.828	0.000	0.085	0.094	0.191
H-3	2.556	0.000	0.026	0.034	0.223
H-4	7.215	0.000	−0.113	−0.130	0.254
H-5	6.885	0.000	−0.161	−0.220	0.584
H-6	3.757	0.000	−0.041	−0.099	0.275
H-7	3.757	0.000	−0.041	−0.099	−0.186

* The H′-6 proton of β-cyclodextrin was observed as broad singlet. The relative shift of the H′-5 was calculated from the two peaks which were readily observable close to the H′-6 peak. The H-6 and 7 protons of chlorambucil formed a singlet in buffer and cyclodextrin solutions but a multiplet or two triplets in other solutions.

the location of the drug molecule within the cyclodextrin cavity. That is, when the cyclodextrin and drug molecules carry opposite charges, the drug molecule has to arrange itself within the cavity to allow for the ionic interactions, but at the same time the forces between the drug molecule and the cyclodextrin molecule within the cavity will be reduced. For example, in some cases the hydrophobic moiety of a drug molecule, which under normal conditions would be located well inside the cavity, will partly be located outside the cavity to allow for ionic interactions between the drug and the cyclodextrin molecules. In the case of indomethacin and TMA-CD, the ionic interaction and the drug–cyclodextrin interactions within the cavity appeared to be more or less competing with each other not allowing the two forces to operate simultaneously in a 1:1 complex.

No relationship could be observed between the charge on the cyclodextrin molecule and the rate of drug degradation within the cyclodextrin cavity

Table 4
Shifts (in ppm) of chlorambucil in cyclodextrin complexes relative to buffer

Proton	Cyclodextrin						
	B-CD	CM-CD	SB-CD	HP-CD	M-CD	A-CD	TMA-CD
H-1	0.006 ± 0.003	0.032 ± 0.004	0.022 ± 0.006	0.024 ± 0.001	0.001 ± 0.003	0.080 ± 0.004	0.037 ± 0.000
H-2	−0.035 ± 0.004	−0.025 ± 0.005	ND*	−0.024 ± 0.001	−0.051 ± 0.005	ND	0.037 ± 0.000
H-3	−0.013 ± 0.003	−0.016 ± 0.001	−0.027 ± 0.004	−0.008 ± 0.002	−0.033 ± 0.003	0.054 ± 0.003	0.017 ± 0.000
H-4	−0.222 ± 0.028	−0.281 ± 0.014	−0.247 ± 0.009	−0.230 ± 0.017	−0.266 ± 0.013	−0.121 ± 0.004	−0.207 ± 0.002
H-5	−0.177 ± 0.021	−0.201 ± 0.010	−0.163 ± 0.008	−0.172 ± 0.013	−0.204 ± 0.010	−0.119 ± 0.004	−0.205 ± 0.002
H-6,7	0.039 ± 0.003	0.067 ± 0.007	0.077 ± 0.004	0.057 ± 0.003	0.037 ± 0.002	0.069 ± 0.004	0.048 ± 0.002

* Could not be determined due to overlapping cyclodextrin peaks.

(i.e. the value of k_c). However, the value of k_c was generally smaller in the SB-CD solutions which indicates that the drug molecules were better protected against hydrolysis within the cavity of SB-CD than within the cavity of the other β-cyclodextrin derivatives tested.

Acknowledgements

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