

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. trimethylammoniumpropyl- 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. © 1998 Elsevier Science B.V. All rights reserved.

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

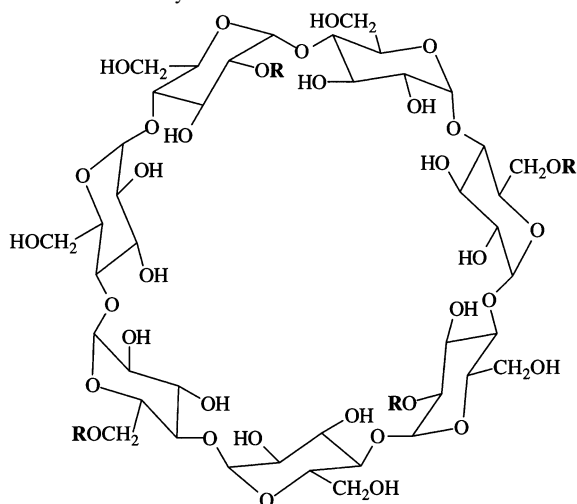
2.1. Materials

The cyclodextrins shown in Table 1 were used for this study. Carboxymethyl- β -cyclodextrin (CM-CD), trimethylammoniumpropyl- 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 ~ 2160) was kindly donated by CyDex (Kansas). The drug compounds were obtained from the following suppliers: acetyl salicylate, salicylic acid, cephalotin and diazepam from 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 μ l 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 μ m 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	$-\text{CH}_2\text{COO}^-$
SB-CD	CyDex	0.9	$-(\text{CH}_2)_4\text{SO}_3^-$
HP-CD	Wacker-Chemie	0.6	$-\text{CH}_2\text{CH}(\text{OH})\text{CH}_3$
A-CD	Wacker-Chemie	1.0	$-\text{COCH}_3$
M-CD	Wacker-Chemie	0.6	$-\text{CH}_3$
TMA-CD	Wacker-Chemie	0.5	$-(\text{CH}_2)_3\text{N}(\text{CH}_3)_3^+$

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-linear 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:

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

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:

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

and the stability constant can be written as:

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

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

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

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 too 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.

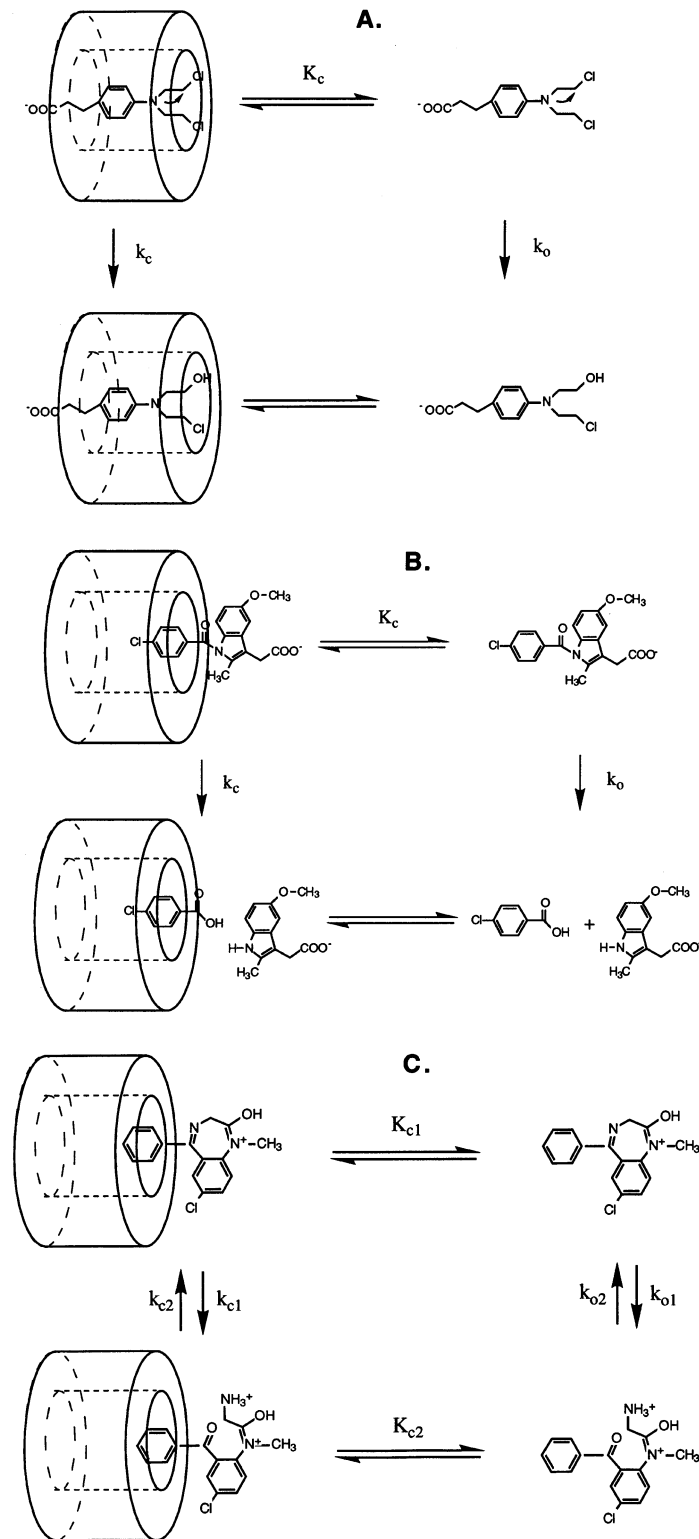


Fig. 1. Proposed structure of the drug–cyclodextrin complexes and the degradation pathways. (A) Chlorambucil, (B) indomethacin, and (C) diazepam. Diazepam is in equilibrium with its degradation product. The cyclodextrin is shown as a cylinder rather than a cone-shape, since it is frequently uncertain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

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