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# Note

## Determination by high-performance liquid chromatography of the binding properties of charged β-cyclodextrin derivatives with drugs

NICOLE THUAUD, BERNARD SEBILLE*, ANDRÉ DERATANI and GÉRARD LELIEVRE
*Laboratoire de Physico-Chimie des Biopolymères, Unité Mixte No. 27, CNRS, Université Paris Val de Marne, 2–8 Rue Henri Dunant, 94320 Thiais (France)*
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One of the most important properties of cyclodextrins is their ability to form inclusion complexes with numerous molecules that they take up in their central hydrophobic cavity. The selective chemical modification of cyclodextrins (CDs) provides opportunities to modify their complexing behaviour. For instance, the introduction of a hydrophobic surface to the parent CD may enhance considerably their association constants with several guests[1]. Tabushi *et al.*[2] used this to prepare a 'molecular design for artificial enzymes'. Moreover, the specific binding of anionic guests, such as drugs to aminated cyclodextrins in which both hydrophobic and electrostatic interactions participate, has been reported[3–9]. The binding and catalytic properties of these derivatives are stronger than those of the parent CD.

CDs are used in pharmaceutical research essentially for improving drug stability, dissolution rates and bioavaibility[10]. However, the application of β-CDs in the pharmaceutical field depends on their aqueous solubility. Thus various functional groups have been incorporated in the CD molecules. Methylated[11–15] and hydroxyalkylated[15] derivatives have been prepared and improved the solubilizing and binding properties for drugs compared with the parent CD.

This work was undertaken in order to study the complexing behaviour towards basic, acidic and neutral drugs of two β-CD derivatives, highly soluble in water and bearing either negative or positive electric charges.

## EXPERIMENTAL

### *Materials*

β-CD hydrate was a gift from Roquette (Lestrem, France). The drugs were purchased from Sigma (St. Louis, MO, U.S.A.)

### *Preparation of the sodium salt of the carboxymethyl ether of β-CyD (β-CyDCME)*

This derivative was prepared according to the method of Lammers *et al.*[17] in alkaline solution with monochloroacetic acid. From an initial 15 g of β-CD hydrate, 9

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g of derivative were recovered after twice precipitating with methanol and drying at 90°C under vacuum. The product was free from sodium chloride (C < 0.1% by elemental analysis); 3.16 ± 0.20 anionic substituents per molecule of derivative were measured by titration of carboxylate groups with perchloric acid in acetic acid.

*Preparation of the hydroxypropyltrimethylammonium ether of β-CyD (β-CyDN$^+$)*

This cationic derivative was prepared in our laboratory according to the patented method of Parmerter *et al.*[18] and modified by Deratani *et al.*[19]. The results of elemental analysis (C 45.7, H 7.58, N 3.69, C 8.99, Na < 0.1%) confirmed the results of argentimetry, which gave 4.6 monosubstituents per molecule, and absence of salt. The molecular weight is thus 1832.

*HPLC apparatus*

The apparatus was the same as that used previously[16]. The columns were packed with commercial LiChrosorb Diol (10-μm particle diameter and 100 A pore diameter) support (Merck, Darmstadt, F.R.G.).

Several 10-cm long columns were necessary to separate the drug from the β-CD derivative, depending on the drug and on its concentration in the mobile phase; therefore three columns were used for some hydrocortisone experiments, whereas one or two were sufficient in the other instances.

A Waters Assoc. Model 401 difference refractometer was used for detecting β-CD and its derivatives. A Beckman Model 103 variable-wavelength UV detector was used for drug monitoring.

*Binding measurements*

These were done using the Hummel and Dreyer[20] method adapted by us for β-CD–drug binding studies with high-performance liquid chromatographic (HPLC) columns[16].

The columns were equilibrated and eluted with successive concentrations of the drug under study in 0.1 *M* phosphate buffer (pH 7.4). These concentrations were varied in the range $1 \cdot 10^{-5}$–$7 \cdot 10^{-4}$ *M* for hydrocortisone and $6 \cdot 10^{-5}$–$3 \cdot 10^{-3}$ *M* for the other drug.

Small aliquots of β-CD or its derivatives were injected into the column, usually 50 μl of 2 g/l solutions. However, in the hydrocortisone binding measurements, 0.2 g/l CD solutions were used and gave narrow, well separated peaks, because of the higher binding capacity of the host molecules for this drug.

## RESULTS AND DISCUSSION

Both β-CD derivatives are eluted at about the void volume of the columns, like β-CD itself, as monitored by refractive index (RI) detection (Fig. 1). It is easy to compare the binding capacity of the various host molecules to one drug from observation of the negative peak in Fig. 2a or b: a known molar amount of injected β-CDCME binds fewer molecules of indomethacin (or warfarin) than does β-CD, whereas β-CDN$^+$ binds more (Fig. 2a). Opposite effects are observed with propranolol (Fig. 2b).

The negative peak areas in Fig. 2a and b allow one to calculate according to a

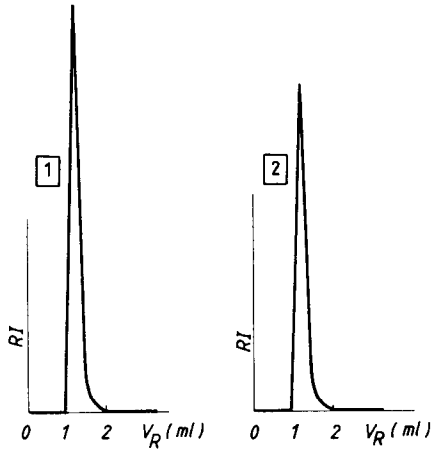


Fig. 1. Chromatograms of $\beta$-CD and $\beta$-CD derivatives on a 10 cm x 4.7 cm I.D. column packed with LiChrosorb Diol. Eluent, 0.1 M phosphate buffer (pH 7.4). Samples: 50 $\mu$l of 8 g/l solutions in phosphate buffer; differential refractometer, sensivity 32 ×. Peaks : 1 = $\beta$-CD; 2 = $\beta$-CDCME or $\beta$-CyDN$^+$. $V_R$ = Retention volume.

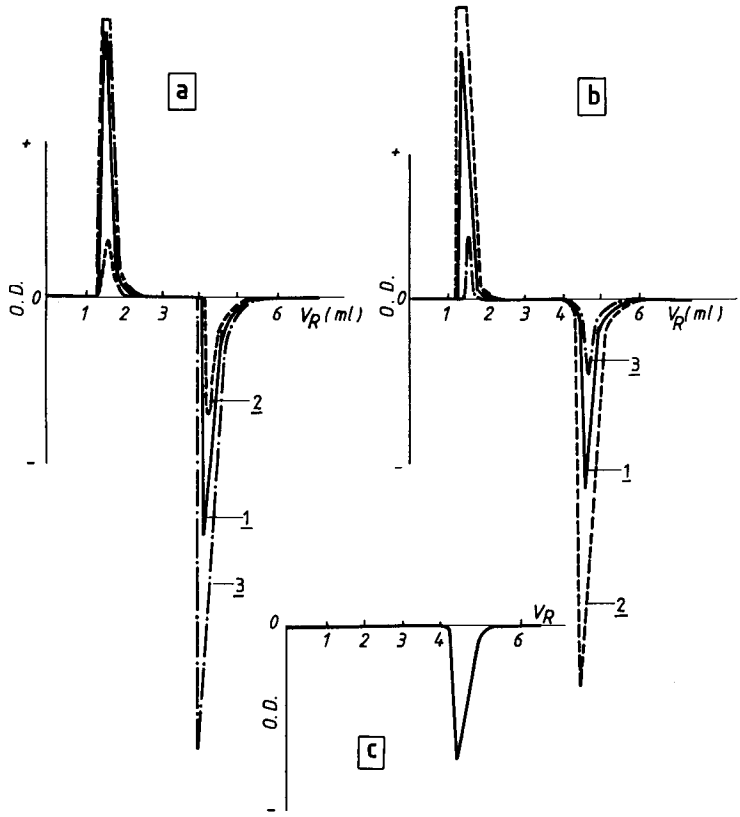


Fig. 2. Hummel and Dreyer[20] chromatograms obtained for inclusion complexation measurements of (a) warfarin and (b) propranolol on a 10-cm long column. Eluent, $10^{-4}$ $M$ solution of the drug in phosphate buffer; all samples injected as 50 $\mu$l of 3.3 m$M$ solutions. Peaks: 1 = $\beta$-CD; 2 = $\beta$-CyDCME; 3 = $\beta$-CyDN$^+$. (c) Calibration obtained by injection of 50 $\mu$l of phosphate buffer under the above experimental conditions; the obtained peak gives the signal of a deficit in $5 \cdot 10^{-9}$ mol of drug.

prior calibration obtained by injecting pure buffer (Fig. 2c), the amount of drug that has been complexed by the host molecule. The area of the positive peak, where the host molecule and its inclusion complex emerge, is not identical with that of the negative peak, because of the difference in UV molar absorption for the bound and unbound drug.

It must be emphasized that the HPLC technique used here requires only small amounts of β-CD derivatives for the determination of their complexing properties. This is advantageous over other methods based on solubility or spectroscopic experiments. Some β-CD binding constants measured previously by these methods have been reported for comparison and validation of the HPLC method[16]. However, there are no results in the literature concerning the binding of β-CD derivatives.

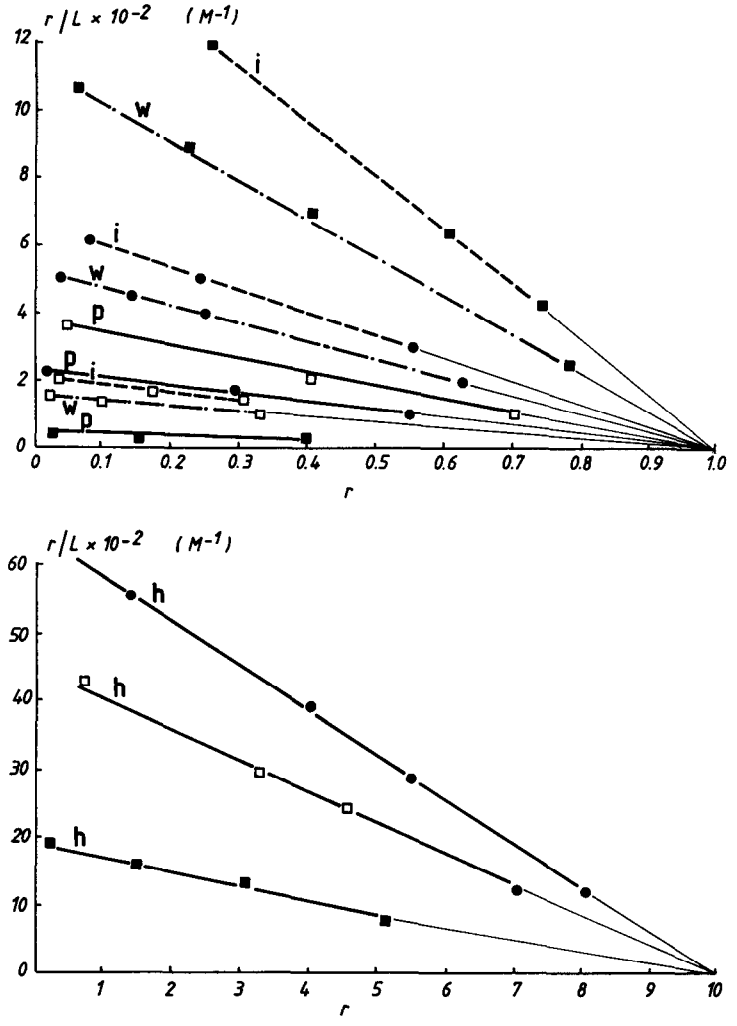


Fig. 3. Scatchard plots obtained for complexation of β-CD derivatives with drugs: h = hydrocortisone; i = indomethacin; w = warfarin; p = propranolol. Host molecules: ●, β-CD; ■, β-CyDN$^+$; □, β-CDCME. $r$ = Guest/host molar binding ratio.

TABLE I

ASSOCIATION CONSTANTS OF DRUG–β-CD DERIVATIVE COMPLEXES AT pH 7.4
The ± values are confidence intervals.

| *β-CD* | *Warfarin* ($pK_a$ = 5.1) | *Indomethacin* ($pK_a$ = 4.5) | *Propranolol* ($pK_a$ = 9.45) | *Hydrocortisone* |
|---|---|---|---|---|
| β-CD | 520 ± 30 | 620 ± 50 | 220 ± 20 | 6200 ± 50 |
| β-CDCME | 150 ± 10 | 250 ± 20 | 400 ± 40 | 4600 ± 50 |
| $\beta$-CDN$^+$ | 1150 ± 50 | 1500 ± 50 | 50 ± 10 | 2000 ± 50 |

Warfarin and indomethacin are acidic drugs, with $pK_a$ 5.1 and 4.5, respectively. They are negatively charged under the experimental pH conditions, whereas propranolol, a basic drug with $pK_a$ 9.5, is positively charged under these conditions. Hence $\beta$-CDN$^+$ has a greater binding capacity than β-CD for negatively charged drugs and a smaller capacity for positively charged drugs. Opposite effects are observed for β-CyDCME. There is evidence for attractive electrostatic interactions between oppositively charged hosts and guests and repulsive effects between identically charged couples.

The Scatchard plots[21], obtained by modifying the concentration of the eluting drug solution (L) are presented in Fig. 3. They converge towards the abscissa at a value of 1, indicating the formation of only 1:1 inclusion complexes, as is usually the case for inclusion of guests in β-CD. The association constants were determined from the slopes of the straight lines, and are reported in Table I. A 2–3-fold increase in their values is observed in the enhanced binding of β-CD derivatives with respect to β-CyD itself. The order of magnitude of the observed phenomena is the same as that reported by Matsui *et al.*[9] between charged dyes and sulphonato and pyridino β-CD derivatives.

The present results provide additional new examples of inclusion complex stabilization by the formation of cooperative electrostatic interactions. Other experiments have been carried out concerning the complexation of hydrocortisone, a noncharged hydrophobic drug.

A decrease in the stability of the inclusion compound formed with β-CD is observed with the quaternary ammonium ether derivative (Table I). A decreased hydrophobicity of the β-CD molecule, due to the presence of the substituent groups, is probably responsible for this phenomenon. This result has to be compared with the decrease in stability of the inclusion complex formed between the undissociated form of an azo dye and a trimethylammonium β-CD derivative, reported previously[4]. Such correlations between the stabilities of the complexes and the structure of the host CD derivatives permit approaches to the design of drug-complexing molecules.

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