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Polycarboxylated Derivatives of β-Cyclodextrin

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Abstract

The synthesis of polycarboxylated derivatives of β-cyclodextrin obtained by reacting maleic anhydride and suitable thiolic compounds is described. The derivatives were characterised by potentiometric analyses and ^{13}C-NMR spectra. The products obtained show a great solubility in water, but also in ethanol and methanol and act as complexing agents for many bivalent metallic cations. These derivatives are probably biodegradable and non toxic.

Introduction

Cyclodextrins [1] are cyclic oligosaccharides which have the characteristic size of a truncated cone. Commonly, they are constituted by 6, 7 or 8 glucose rings linked to each other by a 1- $\rightarrow$ 4-α glucosidic bond and they are named α, β and γ cyclodextrins, respectively. The spatial disposition of the atoms in the molecule generates an inner hydrophobic cavity that allows the formation of inclusion compounds with organic molecules of suitable size and polarity and are stable even in solution.

Nowadays β-cyclclodextrin is produced in larger quantities and it is the cheapest available. It is useful in several applications and in particular in the pharmaceutical industry [2]. The principal limit to a wider application of β-cyclodextrin arises from its low solubility in water at room temperature: only 1.85 wt% [3]. This problem could be overcome by synthesizing more soluble derivatives by reacting the primary and/or secondary hydroxyl groups of the rim of the cyclodextrin with suitable molecules [4–5]. Often these modifications lead to β-cyclodextrin derivatives that form more stable inclusion compounds with an organic guest than with the parent cyclodextrin [6].

Less studied is the complexing ability of the cyclodextrins with positively charged molecules. However, considering the structure of the parent cyclodextrins, they do not seem suitable for the formation of stable complexes with metallic cations. Nevertheless it was found that CDs dramatically enhance the solubility of lanthanide cations in a basic environment. In this latter case the hydrophobic cavity of the cyclodextrin does not participate in the formation of the complex [7]. Polycarboxylated derivatives of β-cyclodextrin could be, on the other hand, good complexing agents for metallic cations.

Recently, our group has patented a synthetic procedure to derivatize polysaccharides to obtain complexants of calcium and magnesium cations with a low environmental impact [8].

Here we report the synthesis of new polycarboxylated derivatives of β-cyclodextrin which are highly soluble in water at room temperature and act as complexants of many metallic cations.

These latter compounds are probably biodegradable and non toxic.

Materials and methods

β-Cyclodextrin was a gift of Roquette-Italia (Cassano Spinola, Italy) and was dried at 120 °C to constant weight before use. Maleic anhydride, mercaptoethanol, cysteamine, mercaptosuccinic acid, mercaptoacetic acid and L-cystein were purchased from Aldrich (Germany) and used without further purification.

All the other chemical compounds and solvents (Merck-Germany) were ACS grade and used as delivered.

The FT-IR spectra were recorded on a Perkin-Elmer 1710 spectrophotometer. ^{13}C-NMR 400 MHz spectra were recorded on a Jeol 400 spectrometer in D_20.

Potentiometric titrations were carried out by dissolving a known amount of the cyclodextrin derivative and inorganic salt and titrating the solution with 0.1M NaOH by means of a pH meter (Hanna instruments HI 8521).

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Synthesis of β-CD—O(COCH=CH—COOH)$_n$ (maleic acid derivative of β-CD)

In a typical experiment 60 g (0.612 mol) of maleic anhydride were dissolved in 150 mL of DMSO at 90 °C. To this solution 10 g (0.0088 mol) of anhydrous β-cyclodextrin were added and the solution allowed to react under magnetic stirring for 2 hours. Once the reaction was completed, the solution was cooled to room temperature and then was slowly poured into 500 mL of ethyl acetate under vigorous stirring. The precipitate obtained was recovered by decantation and redissolved in methanol. The methanolic solution was added again to 500 mL of ethyl acetate and the precipitate recovered by filtration and put into a dessicator under vacuum. Small amounts of the sample obtained were dissolved in water and titrated with a NaOH solution to determine the degree of substitution.

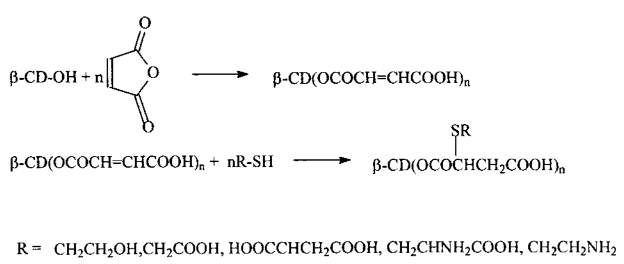
Synthesis of β-CD $\left(\begin{array}{l}\text{—OCOCH—CH}_2\text{—COOH} \\ \qquad\quad | \\ \qquad\quad \text{S—CH}_2\text{—COOH}\end{array}\right)_n$

In a typical experiment 10 g of unsaturated polycarboxylated β-cyclodextrin (obtained from reaction of maleic anhydride and β-cyclodextrin) was dissolved in 100 mL of DMSO. To this solution 5.9 mL of triethylamine was added and then 3 mL of mercapto acetic acid. The solution was allowed to react overnight at 25 °C. Once the reaction was completed, the solution was added to 500 mL of ethylacetate. The solid was recovered by filtration and dissolved in 100 mL of methanol and the solution was again slowly poured into 500 mL of ethyl acetate. The precipitate was recovered by filtration and stored in a dessicator under vacuum.

Other thiolic derivatives were similarly obtained.

Results and discussion

The synthetic procedure followed to produce the polycarboxylated derivatives of β-cyclodextrin is sketched in Scheme 1:



Scheme 1.

The first step is a typical nucleophilic substitution on the carbonyl group of the maleic anhydride by hydroxyls of the β-cyclodextrin. The reaction is carried out in DMSO at 90 °C for two hours and forms a compound which has an average degree of substitution (DS) per glucose unit of about 0.82. Obviously, varying the reaction conditions, and in particular the amount of maleic anhydride, leads to β-cyclodextrin derivatives with different DS values. The degree of substitution is easily determined simply by performing a potentiometric titration with NaOH solution of the recovered product.

These unsaturated β-cyclodextrin derivatives obtained are water soluble and show quite a good interaction with some metallic cations. For instance, they act as sequestrants for calcium cations: up to 100 mg of $CaCO_3$ per gram of unsatured polycarboxylated cyclodextrin.

The presence of a double bond in the polyacid derivatives obtained allows the easy grafting of the thiolic derivative under mild reaction conditions according to a typical nucleophilic addition to the double bond.

In fact, the presence of two electron withdrawing groups (ester group and carboxylic acid group) conjugated to the double bond, makes the latter particularly activated towards the reaction with the mercapto group, especially in the presence of a basic catalyst like triethylamine.

Carrying out the reaction of the thiol derivative with the suitable unsaturated polyacid derivative of β-CD, it is possible to obtain molecules with a significant number of carboxylic acid groups.

For example, starting from the above mentioned unsaturated polyacid which has a DS value as high as 0.82 per glucose unit, the reaction with the mercaptosuccinic acid leads to saturated polycarboxylated derivatives which have 2.46 COOH groups per glucose unit, i.e., more than 17 COOH residues per cyclodextrin molecule. Each residue on a single glucose unit closely resembles the molecule of citric acid, a well known complexing agent for several cations. Moreover, the relative stiffness of the cyclodextrin ring probably favours the complexation. The reaction has a wide range of applicability because several mercapto derivatives can be bonded to the unsaturated polyacid.

As sketched in Scheme 1, it is also possible to introduce hydroxyl or amine groups respectively in addition to COOH groups by using mercaptoethanol or cysteamine. Despite this, the reaction proceeds also with cysteine, thus allowing the introduction of amino acid residues on to the cyclodextrin ring.

The nucleophilic addition reaction is complete in 48 hours at room temperature. In fact, the ^{13}C-NMR spectrum (Figure 1) of the thiolic CD derivative obtained does not show the typical signal at about 130 ppm due to the carbon-carbon double bond (Figure 2). The disappearance of this signal after the addition of the thiolic derivative proves the completeness of the reaction. In any case, the insertion of the mercaptoacetic acid is easier in comparison with thiomalic acid addition, probably due to a steric hindrance factor.

The introduction of many carboxylic acid groups on the β-cyclodextrin ring highly modifies its solubility. In particular the product obtained by the addition of the thiomalic acid onto the double bond of the unsaturated polyacid of β-cyclodextrin is very soluble in water: up to 900 mg/mL at room temperature, that is about 50 times more soluble than the parent β-cyclodextrin!

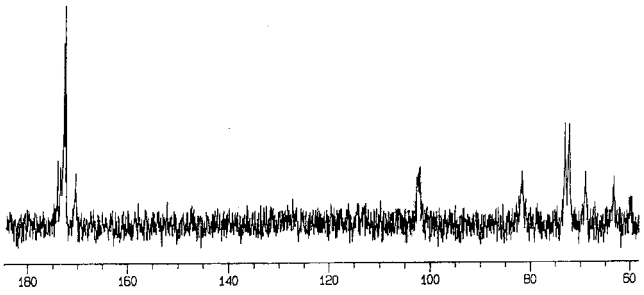


Figure 1. ^{13}C-NMR spectrum of the thiomalic acid derivative of β-cyclodextrin.

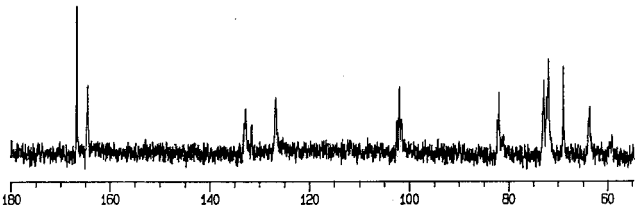


Figure 2. ^{13}C-NMR spectrum of the maleic acid derivative of β- cyclodextrin.

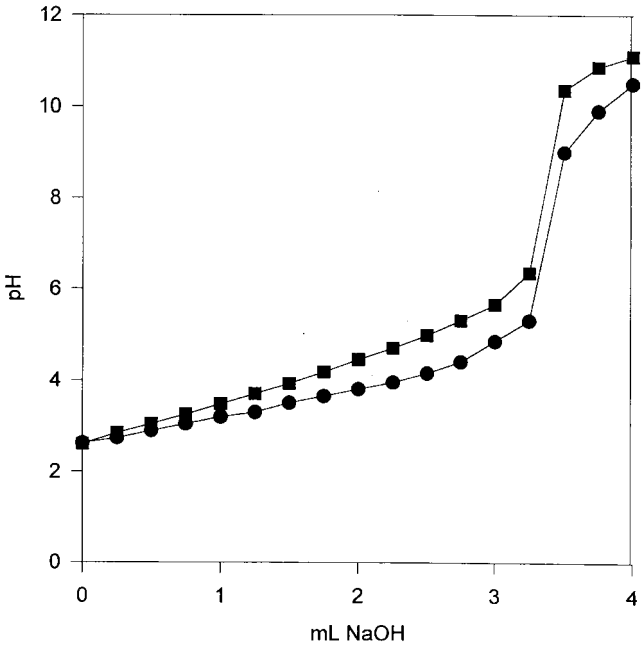


Figure 3. Titration curve for the thiomalic derivative of β-cyclodextrin. (■)in the absence of metal salt (●) in the presence of 5 mmol of Ca^{2+} salt.

Table 1 shows, moreover, that the thiomalic acid derivative of β-cyclodextrin is not only soluble in a polar aprotic solvent such as DMSO but also in methanol and ethanol, solvents in which β-cyclodextrin is insoluble.

In order to test the complexation power of the new saturated polycarboxylated β-CD derivatives, we have performed potentiometric titration of the COOH groups in the presence and in the absence of different molar amounts of selected metal salts. Initially thiomalic derivatives were chosen.

In fact, it is well known that the greater the interaction of the COOH group with metallic cations, the lower the initial pH value for a given β-CD derivative solution, because the cation strongly interacts with the COOH group and leads to a higher dissociation degree and, as a consequence, more acid compound.

Moreover, when the end point of the titration required a higher amount of the titrating base, this means that also OH groups interact with the testing cation. (i.e., the interaction of the metal cation with the oxygen of the OH group makes its proton more acidic). Thus, the shift to the right of the titration curve could be roughly considered as an evaluation of the degree of complexation of the selected cation by the polyacid derivative of β-CD.

Table 1. Solubility of β-cyclodextrin and of its thiomalic polycarboxylated derivative in some common solvents at room temperature

Solvent	β-cyclodextrin	Thiomalic derivative of β-cyclodextrin
Water	+	+
Methanol	−	+
Ethanol	−	+
Diethyl Ether	−	−
Chloroform	−	−
Ethyl Acetate	−	−
Acetone	−	−
DMSO	+	+
THF	−	−

Some bivalent metallic cations: Ca^{2+}, Cu^{2+}, Co^{2+}, Hg^{2+}, and Pb^{2+} were tested. Figures 3–7 show the results obtained. We point out that the results obtained from the titration curves must be considered with care since, often, the corresponding low soluble hydroxides are obtained. As a consequence the results obtained under heterogeneous conditions have only a qualitative value.

In particular Figure 3 shows the titration curve obtained by titrating the polycarboxylated β-CD derivative in the absence and in the presence of 5 mmol of Ca^{2+} cations. The shift to the right of the titration curve is evident and accounts for a participation of the hydroxyls of the β-cyclodextrin in the complexation of the Ca^{2+} cations. Moreover, the initial pH value of the CD solution is not significantly lowered, that is, the Ca^{2+} cations do not interact strongly with the COOH groups and, as a consequence, their acidity is not particularly enhanced. Generally speaking Ca^{2+} cations seem to influence little the titration curve of the thiomalic derivative of β-CD.

More effective is the Cu^{2+} cation. Figure 4 reports the titration curve of the thiomalic β-CD derivative in the absence and in the presence of different molar amounts of copper salt. We note that, in this case, the initial pH value is noticeably lower in the presence of copper salt and the curve is sharply shifted to the right. Despite this, the curve shows two inflections that could be attributed to the titration of two acids with different pKa values. The first one is surely due to the COOH groups whose acidity is enhanced by the interaction with copper cations. The second one is due to the complexation ability of the OH groups.

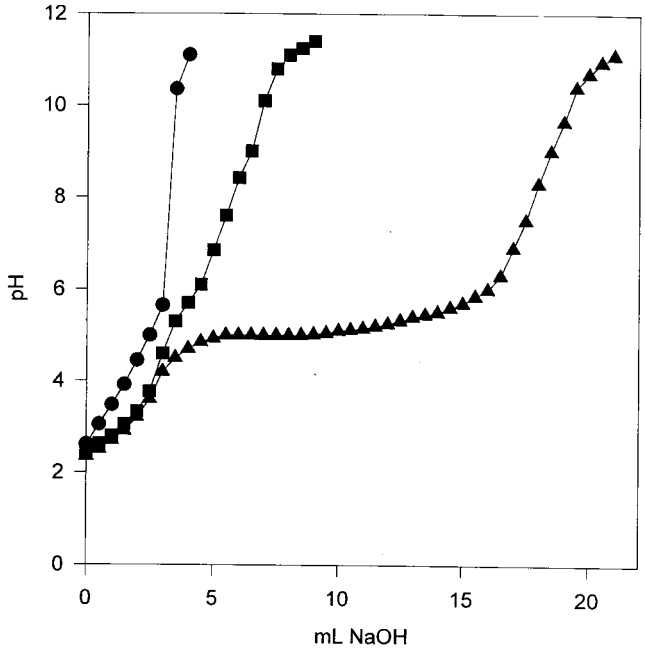


Figure 4. Titration curve for the thiomalic derivative of β-cyclodextrin (●) without metal salt (■) in the presence of 0.1 mmol of Cu^{2+} salt (▲) in the presence of 1 mmol of Cu^{2+} salt.

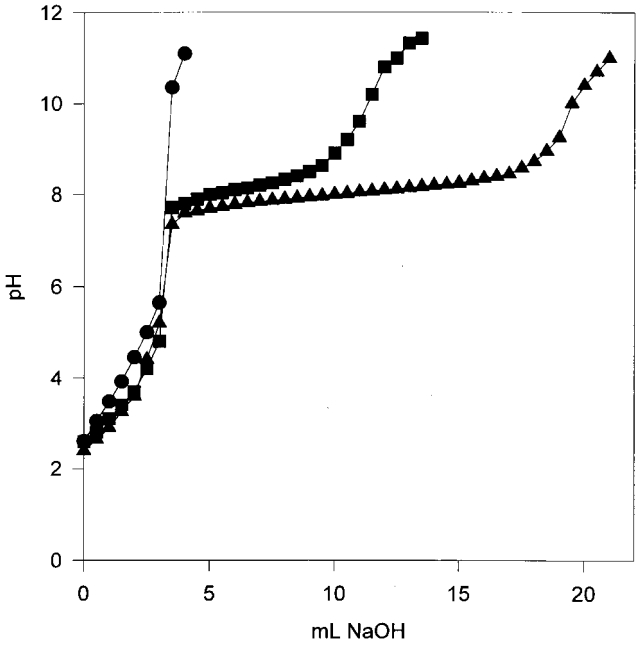


Figure 5. Titration curve for the thiomalic derivative of β-cyclodextrin (●) without metal salt (■) in the presence of 0.5 mmol of Co^{2+} salt (▲) in the presence of 1 mmol of Co^{2+} salt.

Table 2. % hydrolysis of the thiomalic acid derivative of β-cyclodextrin as a function of the reaction time and pH at room temperature

Time(hours) pH	0.5	1	2	3	5	8	17	24
2	–	–	–	3%	18%	40%	85%	100%
3	–	–	–	–	–	–	–	–
6	–	–	–	5%	17%	30%	60%	80%
7	–	–	5%	12%	25%	40%	70%	90%
12	15%	40%	90%	100%				

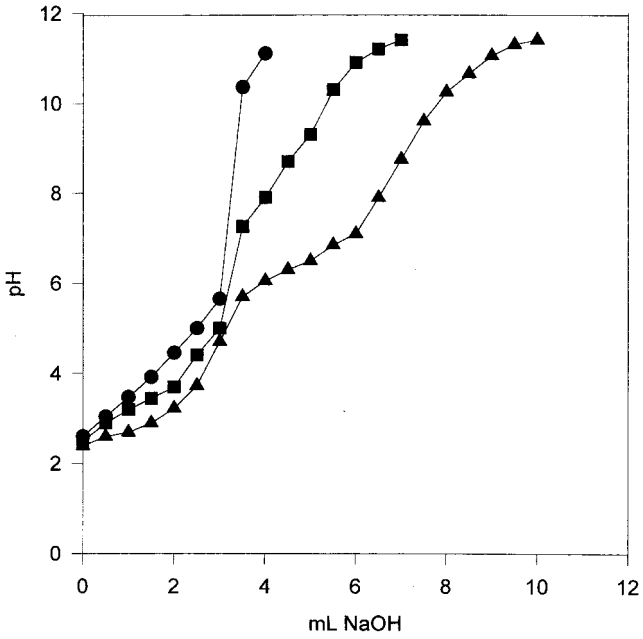


Figure 6. Titration curve for the thiomalic derivative of β-cyclodextrin (●) in the absence of metal salt (■) in the presence of 0.2 mmol of Pb^{2+} salt (▲) in the presence of 1 mmol of Pb^{2+} salt.

The fact that those effects are enhanced by increasing the metal salt concentration is probably due to the higher amount of the Cu^{2+} bound per polycarboxylated β-CD molecule. Since at least 2.5 COOH groups are present per glucose unit, probably every substituted β-CD molecule is able to complex several Cu^{2+} cations.

Very similar behaviour was observed for other bivalent cations such as Co^{2+} (Figure 5), but in this latter case no precipitation of the insoluble $Co(OH)_2$ was observed up to pH 9, thus indicating the great complexing ability of the cyclodextrin derivative towards Co^{2+} cations. A similar effect was also detected for Pb^{2+} (Figure 6) but precipitation occurred even at low pH.

A different behaviour was noted for Hg^{2+} (Figure 7). In fact, the titration curve was little affected by the presence of the Hg^{2+} salt, but in contrast, no precipitation was observed up to pH 10, thus revealing a great interaction of the Hg^{2+} cation with the reported new cyclodextrin derivative.

Finally it is possible to imagine a selectivity to a certain extent in the metal cation complexing ability of the polycarboxylated CD derivative and it seems plausible that by varying the residue on the CD rim, and by introducing other heteroatoms, a different complexation behaviour could be achieved. These studies are in progress.

The ester bond between the alkyl chain and the β-CD is clearly hydrolyzable both in acid and basic environments. However, stability tests performed under several working conditions allow us to establish that the hydrolysis reaction rate is higher, as predictable, in a basic medium (pH 12) than in an acid one (pH 2). In fact, Table 2 shows that carrying out the hydrolysis in a strong basic medium, the reaction

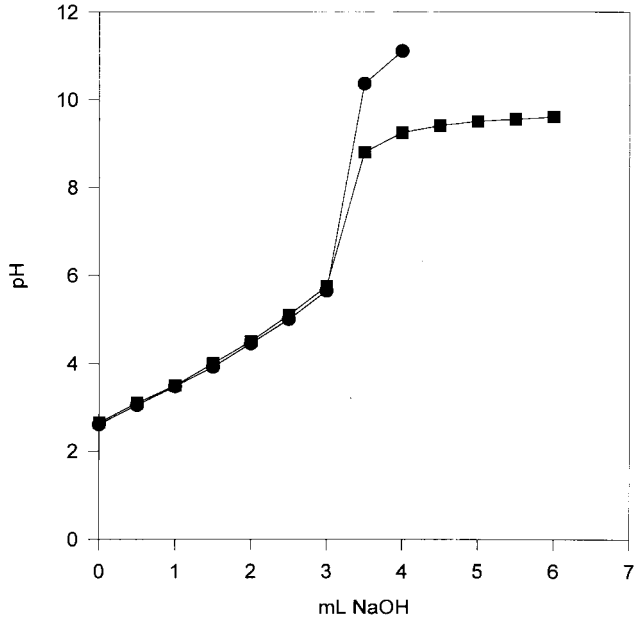


Figure 7. Titration curve for the thiomalic derivative of β-cyclodextrin (●) without metal salt (■) in the presence of 1 mmol of Hg^{2+} salt.

is over in only 5–6 hours, while in an acid environment at least 24 hours are required to complete the reaction. At moderate acid or basic pH more than 24 hours are required to completely hydrolyze the ester bond. Nevertheless, simple dissolution of the saturated polycarboxylated derivative of β-CD in water at room temperature does not lead to any relevant hydrolysis after 24 hours.

In any case TLC analyses of the hydrolysed mixtures showed the presence of the parent β-CD. This behaviour assumes particular importance when we consider that many commercial complexing agents are toxic and often are not biodegradable. Polycarboxylated derivatives of β-CD could be an effective alternative to these compounds.

Acknowledgement

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