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ORIGINAL ARTICLE

The protonation thermodynamics of cyclodextrin-containing polymers for drug inclusion

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Abstract A poly(amido-amine), PAA, bearing β-CD units in the side chain was synthesized by a polyaddition reaction of 1,4-bis-acryloyl-piperazine with 6-monodeoxy-6-monoamino-β-cyclodextrin (β-CD-NH_2). Unlike the simple β-CD-NH_2 with a greater basicity constant (log K = 8.60), the polymer revealed an unusual polyelectrolyte behaviour with a lower basicity constant (log K° = 6.29) of the tertiary nitrogen atom, that is strongly dependent on the degree of protonation α of the whole macromolecule. It follows the modified Henderson–Hasselbalch equation with n = 1.75, in a wide α-range. The greater (−46.1 kJ/mol) and the lower (−27.6 kJ/mol) enthalpy (ΔH°) changes of the compounds were in line with the protonation of a primary or a tertiary nitrogen atom. The calorimetric data suggested that the PAA protonation destroyed a packing structure formed by two rigid β-CD side chains interacting head-to-head. The UV spectrophotometric data showed that the PAA exhibits affinity towards the L-ascorbic acid at low pH (pH 2.46) with an isosbestic point at 241 nm and a slight blue shift of the maximum absorption of the ascorbic acid (244 nm) on PAA additions.

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Introduction

The potential uses of the cyclic oligosaccharides, named cyclodextrins (CDs), in the inclusion complexation process are widely reported [1]. They are of interest in many fields, such as in pharmaceutical, cosmetics, chemical products and technologies. Moreover, the investigations on the host–guest inclusion complexation of native and modified cyclodextrins have received much attention in supramolecular chemistry and have become the fundamental basis of molecular assembly [2–4]. Different chemical modifications have been proposed to increase the solubility and to enhance the original binding ability and the molecular selectivity of parent CDs [5–9]. Besides the alkyl substituted, some CD derivatives contain the chromophoric azo group [10] or the primary basic nitrogen [11]; in the latter case the compound becomes versatile to be inserted in polymer conjugates [12]. While the azo dyes derivatives preserve their peculiarity for spectral changes upon inclusion complexation with guests, the amino CD derivative may form polymer materials containing designed functional groups [12, 13], where the change of pH can modify the cooperativity of the CD units upon the change of the macromolecular coil.

A recent strategy to improve the solubility of the β-CD and its inclusion compounds in aqueous media was the synthesis of the β-CD conjugates with biocompatible synthetic polymers having hydrophilic properties. The polymers, based on functionalized poly(amido-amine)s, PAAs, were reported for their inclusion capacity of the antiviral

(acyclovir) [13] and the antitumor (cisplatin) [12] drugs. In all cases, the compounds were obtained as copolymers including the β-CD as derived from the 6-monodeoxy-6-monoamino-β-cyclodextrin (β-CD-NH_2). The efficient method to obtain the β-CD-NH_2 allows to synthesize PAAs of different structures. As well known, the PAAs are a family of synthetic hydrophilic and biodegradable polymers containing amido and tert-amino groups regularly distributed along the macromolecular chain [14–16]. They are obtained by the Michael-type polyaddition of the primary monoamines or bis-secondary amines to bis-acrylamides, in protic solvents. Additional functions, as side chain substituents, can be easily introduced starting from the appropriate monomers. It is worth that the reaction of the β-CD-NH_2 with bis-acrylamides will introduce the β-CD in a basic or ampholytic polymer structure.

The aim of this paper is to give an insight of the protonation thermodynamics of a PAA, poly(β-CD), derived from the 1,4-bis-acryloyl-piperazine and the β-CD-NH_2 (Chart 1).

This can be considered a classical PAA whose thermodynamic functions were found to be "real", i.e., independent on the degree of protonation of the whole macromolecule [17]. Moreover, the introduction of a chromophoric amido group in the PAA allow to exhibit the appreciable spectral changes upon inclusion with guests, and thus be successfully applied as the versatile spectral probe to investigate the host–guest complexation. In the present paper, we report the synthesis of the β-CD-containing-PAA and the protonation thermodynamic properties of the tertiary amino nitrogen. These will be compared to the same properties obtained for the β-CD-NH_2 analogue. Moreover, UV-spectral data for the interaction of the PAA with L-ascorbic acid will be reported. This probe was chosen because its inclusion in β-CDs increases the stability of the guest molecule to oxidizing agents in acidic and neutral solutions [18, 19].

Experimental section

Materials

All the reagents and solvents, from Fluka and Aldrich Company, were used as received because of their analytic grade. The reagents for the polymer synthesis, the 1,4-bis-acryloyl-piperazine and the 6-monodeoxy-6-monoamino-β-cyclodextrin in the hydrochloride form, were from Sigma–Aldrich Co., as well as the L-ascorbic acid. The free reagent 6-monodeoxy-6-monoamino-β-cyclodextrin was also prepared according to a previously reported procedure [12, 13, 20]. The Amberlite IRC-50 resin (Fluka) was treated first with a 2 M HCl solution and then washed with twice-distilled water till neutrality.

Spectroscopic characterization

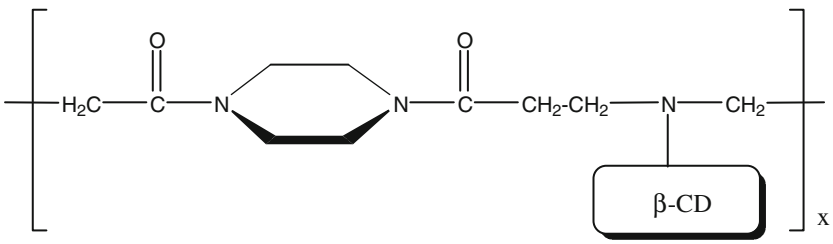
Proton NMR spectra were recorded on the 400 MHz Bruker DRX-400 Avance spectrometer using D_2O as a solvent. Infrared spectra were recorded on a Perkin–Elmer Lambda 3B spectrophotometer.

The UV spectrophotometric measurements were carried out with a Specord 210 (Analytikjena) with 10 mm quartz cuvettes.

Multi-angle light scattering (MALS)

The molecular weight and dimensions of poly(β-CD) macromolecules were measured by a multi-angle light scattering (MALS) photometer Dawn DSP-F from Wyatt (S. Barbara, CA) in 0.2 M NaCl + 0.1 M TRIS buffer pH 8.0 solvent, at room temperature in static off-line mode. The MALS photometer uses a vertically polarized laser (He–Ne) of 632.8 nm of wavelength; it measures simultaneously, by means of an array of photodiodes, the intensity of the scattered light at sixteen fixed angular locations ranging in aqueous solvent from 14.9 to 158.1°. Even though measurements were performed in static off-line mode (batch), a flow cell (K5) was preferred to reduce the scattering volume. The calibration constant that transforms the detector voltages in Rayleigh factor (R(θ)) was calculated using toluene as a standard (R(θ) = 1.406 × 10^{-5} cm^{-1}), where θ denotes the angle between the detector and the primary incident light. The angular normalization of sixteen photodiodes was carried out by means of a concentrated solution of bovine serum albumin (BSA) globular protein, assumed as isotropic scatterer. Details of the MALS hardware and software have been described elsewhere [21, 22]. The refractive index increment, dn/dc, of the polymer with respect to the solvent at

Chart 1 Monomeric structure of the poly(β-CD)



25 °C was measured by a KMX-16 differential refractometer from LDC Milton Roy (Rochester, NY, USA).

The static light scattering (LS) is a convenient method for the study of large particles in solution as macromolecules, aggregates or micelles. In a LS experiment following the treatment of Zimm [23], the reciprocal of the reduced excess of the Rayleigh Factor, R(θ), may be expressed by the equation:

$$\frac{K \cdot c}{R(\theta)} = \frac{1}{M_w \cdot P(\theta)} + 2A_2 \cdot c$$

where $K = (2\pi^2 n_0^2 (dn/dc)^2)/(\lambda_0^4 Na)$ denotes the optical constant, n_0 the refractive index of the solvent, dn/dc the refractive index increment of the solute with respect to the solvent, λ_0 the wavelength of the light in the vacuum, Na the Avogadro's number, c the concentration of the sample (g/mL), M_w the weight-average molar mass, A_2 the second virial coefficient and P(θ) the intramolecular scattering function or "form factor". Debye showed [24] that the form factor P(θ) may be approximated by:

$$P(\theta) = 1 - \frac{1}{3} \cdot \mu^2 \cdot <s^2> + \cdots$$

where, $\mu = (4\pi/\lambda)\cdot\sin(\theta/2)$ is a function of the angle (θ) and of the wavelength (λ) of the light in the medium and $<s^2>$ denotes the mean-squares radius of the molecules. As a result, from a single batch MALS experiment three important molecular data could be obtained: M_w, A_2 and the z-average-root-mean squares radius $<s^2>_z^{1/2}$, generally known as radius of gyration R_g. The refractive index increment, dn/dc, of the poly(β-CD) with respect to the solvent was found to be 0.190 mL/g. The characterization of molecular weight distribution of poly(β-CD) sample by a MALS photometer on-line to a size exclusion chromatography system (SEC-MALS) was very complex because the polymer does not elute from aqueous SEC columns. Therefore, we have characterized the poly(β-CD) polymer by off-line MALS mode (batch).

Polymer synthesis

The 6-monodeoxy-6-monoamino-β-cyclodextrin in the hydrochloride form (544.3 mg, 0.465 mmol) was dissolved in twice distilled water (2 mL) together with an excess of triethylamine (1.20 mL), under stirring. This solution was slowly added to a water solution (2 mL) containing 1,4-bis-acryloyl-piperazine (90.2 mg, 0.464 mmol) with an external ice-cooling bath, whereupon a phase separation occurred. The mixture was treated under vacuum and purged with nitrogen for three times. Then, it was allowed to react at room temperature in the dark for 3 weeks, with occasional stirring. It was noted that during the polymerization procedure the white powder dispersed in the mixture vanished and the solution became limpid after about 1 week. At the end of the reaction the mixture was treated overnight with Amberlite IRC-50 (2 g), and then filtered. The limpid solution was concentrated to a small volume and treated with methanol (5 mL) giving rise to a milk-like dispersion. Treatment with diethyl ether (100 mL) improved the separation of a finely dispersed white solid that was washed twice with fresh diethyl ether (2 × 100 mL), and finally dried in vacuo: yield, 0.45 g (71%).

Viscometric measurements

The viscometric titration was done with an AVS 310 automatic Schott–Gerate viscometer at 25 °C on a dilute polymer solution. The solution was freshly prepared by dissolving a weighed amount (35.0 mg, 0.0256 mmol) of the poly(β-CD) in 25 mL of 0.15 M NaCl containing 2.00 mL of HCl 0.0557 M. The stepwise titration was carried out with a solution of NaOH 0.0612 M delivered through a Metrohm 665 Dosimat buret.

Potentiometric measurements

Potentiometric titrations were performed at 25 °C in aqueous media following a previously reported procedure [16]. A TitraLab 90 (Radiometer Analytical) titration system was automatically controlled by the TimTalk 9, a Windows-based software. Titrations were performed in a thermostated glass cell filled with 50 mL of 0.15 M NaCl, in which a weighed amount of white powder solid (β-CD-NH_2, 0.38–1.24 mmol/L; polymer, 0.33–1.21 mmol/L) and a measured volume of standard hydrochloric acid solution were dissolved by magnetic stirring and under a presaturated nitrogen stream to avoid CO_2 contamination. Forward and backward stepwise titrations with standard 0.06 M NaOH and 0.06 M HCl solutions, respectively, showed rielable results. The basicity constants, respectively for the β-CD-NH_2 and the corresponding polymer, were evaluated with the Hyperquad [25] or with the ApparK [26] program, running on PC. For the protonation reaction of a simple ligand L: $L + H^+ = LH^+$ the basicity constant K is computed at each pH value by the well-known Henderson–Hasselbalch equation (H–H):

$$\log K = pH + \log\alpha/(1 - \alpha) \quad (1)$$

where α is the degree of protonation ($\alpha = LH^+/C_L$, being C_L the analytical concentration of the L species). For typical polyelectrolytes, the H–H equation can be modified as suggested by Katchalsky and Spitnik [27]:

$$pH = \log K^\circ + n \log(1 - \alpha)/\alpha \quad (2)$$

where $\log K^\circ = pH$ at $\alpha = 0.5$ and $n = 1$ in the case of "real" basicity constants. Summing up the Eq. 1 and the

Eq. 2 results in the quantity (n − 1): log K = log K° + (n − 1)log[(1 − α)/α] that gives a measure of the polymer deviation from the "normal" behaviour of a small molecule. The results of at least two replicates were averaged.

Calorimetric measurements

Solution calorimetric titrations were carried out at 25 °C with a Tronac calorimeter (model 1250) operating in the isothermal mode, following a previously reported procedure [16]. A weighed amount of synthetic β-CD-NH_2 in the free form (0.06–0.08 mmoL) was dissolved in a stainless steel reaction vessel containing 25 mL of aqueous 0.15 M NaCl and titrated with a standard 0.07 M HCl solution at a constant buret delivery rate (BDR) of 0.1000 mL/min through a Gilmont buret. In the case of the commercial product in the hydrochloride form (0.12–0.13 mmol) or the partially protonated polymer (0.08 mmoL), an excess of measured volume of standard 0.07 M of NaOH solution was added to free the nitrogen to be protonated. All the titrations were automatically controlled by the Thermal program (from Tronac Inc.), which was configured to operate through a NI-DAQ (National Instruments) driver software in Windows [28]. The graphical programming language LabView was used to create the application. Calibrations with tris(hydroxymethyl)aminomethane and standard sodium hydroxide solutions were made before each titration run. The enthalpy change values were evaluated with the Fith program [26], while the entropy changes were calculated by the relation ΔS° = (ΔH° − ΔG°)/T, with ΔG° = −RTlnK.

Molecular modelling

The molecular structure of a three subunits-long polymer was built with the PRODRG [29] server, and optimized with a 500 ps molecular dynamics simulation at a constant temperature of 300 K by use of the GROMACS [30] package and force field using standard parameters. The hydrogen bond between the protonated nitrogen and the cyclodextrin oxygen was modeled by imposing distance restraints. Structures were analyzed with MOLMOL [31] graphic software.

Results and discussion

Synthesis and characterization

The 6-monodeoxy-6-monoamino-β-cyclodextrin (β-CD-NH_2) was synthesized according to a three-steps procedure already reported [13, 20]. The synthetic pathway involved the mono-tosylation of the β-CD, followed by the azide formation and its catalytic hydrogenation. The yield was very low and the purity was 75 wt% as determined by potentiometric and calorimetric titrations.

The poly(β-CD) was obtained, using the commercial β-CD-NH_2 in the hydrochloride form, by the conventional Michael-type polyaddition. An excess of triethylamine was used to free the aminic function of the β-CD-NH_2, allowing its copolymerization with the 1,4-bis-acryloyl-piperazine (BAP) in water solution. When the amine solution was added to the BAP solution, a milk-like dispersion formed, even under stirring and at low temperature. The fine white dispersion progressively vanished within 1 week, improving a limpid solution for more than 2 weeks, as an evidence of occurred polymerization reaction. The final polymer was obtained as a white powder and the acid–base titrations (potentiometric and solution calorimetric) were in line with the proposed structure. Its structure was confirmed by the ^{1}H NMR and IR spectra. Figure 1 shows the ^{1}H NMR spectra of either the poly(β-CD) and the β-CD-NH_2.

The absence of the double bonds of the 1,4-bis-acryloyl-piperazine was consistent with the occurred polymer reaction, and the polymeric nature was shown by the broad lines for the backbone resonances. This was consistent with the presence of a slowly tumbling macromolecular species in solution. Unlike the clear chemical shifts of the methylene protons of the backbone [16], falling in the range 2.5–3.0 ppm, the piperazine protons, falling to 3.6 ppm, overlap the H_2 and H_4 protons of the cyclic saccharide unit [32, 33]. The infrared spectrum of the poly(β-CD) showed two more weak bands at 1626 and 1305 cm^{-1}, assigned, respectively, to the amide I and to the methylene stretching of the backbone chain that were absent in the corresponding β-CD-NH_2. The methylene piperazine ring falls to 1032 cm^{-1}, overlapping the 1027 cm^{-1} band of the β-CD-NH_2.

Figure 2 shows the Zimm Plot of the poly(β-CD) sample obtained by means of an off-line (batch) MALS characterization.

The figure shows the Zimm plot from which three important average macromolecular parameters were estimated. They were respectively: average molecular weight M_w = 13.34 kg/mol, average dimension of macromolecules R_g = 13.4 nm, second virial coefficient $A_2 = 1.17 \times 10^{-4}$ mol mL g^{-2}. The poly(β-CD) polymer concentration of the Zimm plot ranges from about 0.5 mg/mL to about 1.5 mg/mL. As a result, the poly(β-CD) showed a relatively low molecular weight sample, as usually occurs [14, 15]. Moreover, considering the relatively high R_g value with respect to the low molecular weight, we can state that the poly(β-CD) is a quite stiff polymer [34]. With respect to the dimension of the macromolecule R_g we have

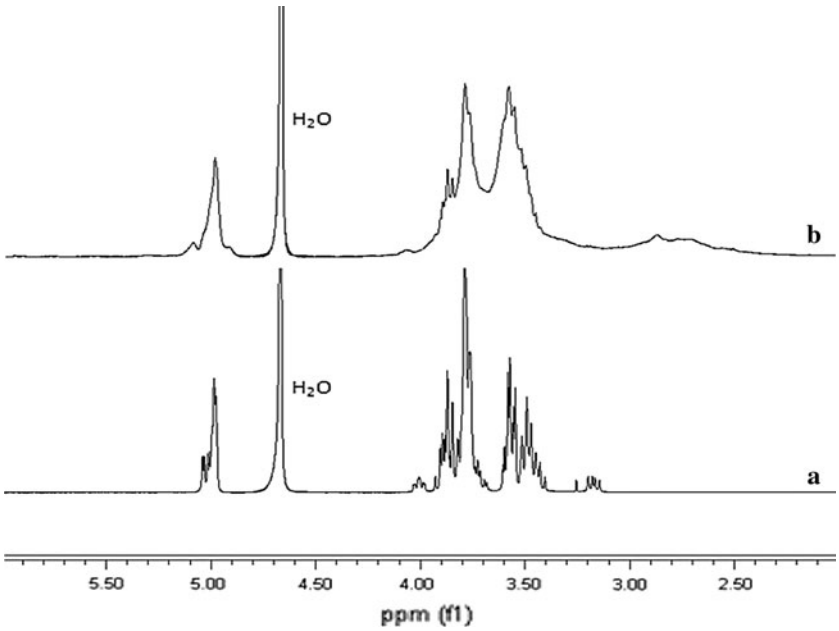


Fig. 1 Proton NMR spectra of the β-CD-NH_2 (*a*) and poly (β-CD) (*b*) in D_2O

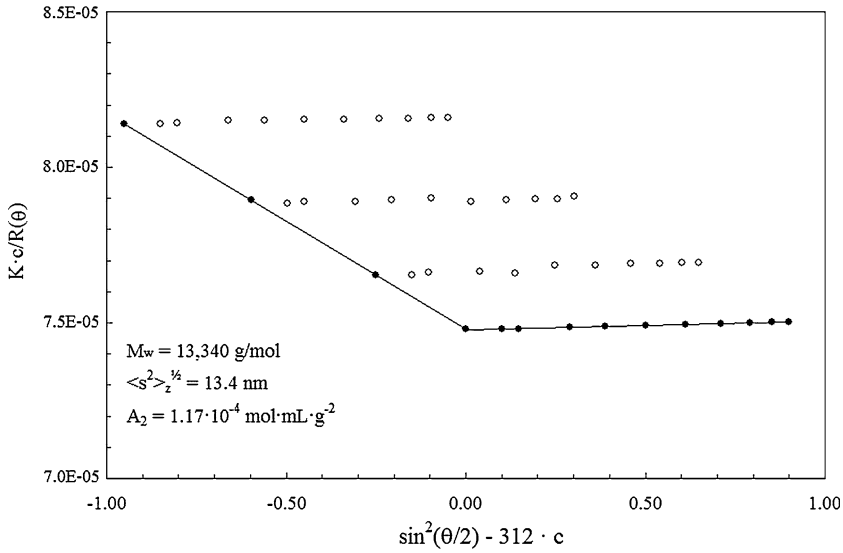


Fig. 2 Zimm Plot of poly (β-CD) sample by means of an off-line MALS characterization (*open points* experimental concentration c ranging from 0.5 to 1.5 mg/mL)

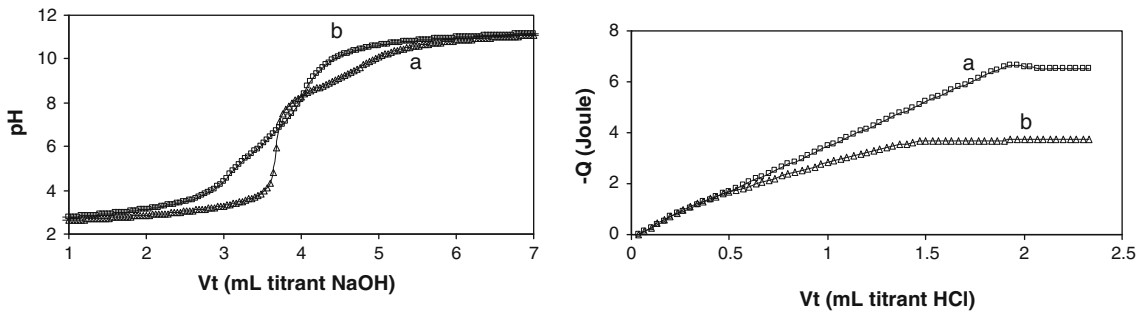


Fig. 3 Potentiometric (*left*) and calorimetric (*right*) titration curves of the β-CD-NH_2 (*a*) and the poly(β-CD) (*b*) in 0.15 M NaCl at 25 °C

to consider that the accuracy of the measure is relatively low.

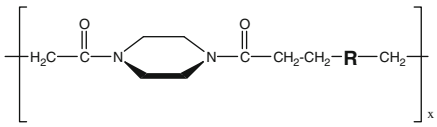
Protonation study

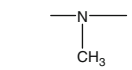
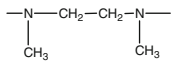
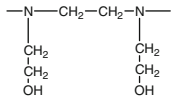
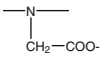
The basicity constants (log K) and the enthalpy of protonation (ΔH°) of the β-CD-NH_2 and of the corresponding polymer poly(β-CD) were evaluated, respectively, by potentiometric and calorimetric data in 0.15 M NaCl at 25 °C. In Fig. 3 is reported a typical titration plot of both the compounds showing the remarkable differences, due to the protonation of a primary or a tertiary amino group. The end-point analysis revealed a product of analytical grade for the commercial β-CD-NH_2 in the hydrochloride form, while only 75 wt% of the basic nitrogen was titrated in the synthetic β-CD-NH_2 product.

The evaluated thermodynamic data for the protonation of both the compounds, the log Ks and the enthalpy (ΔH°) and entropy (ΔS°) changes, are reported in Table 1.

As expected, the basicity of the primary amino group is higher than that of the corresponding polymer containing a tertiary amino nitrogen. The great basicity of the

Table 1 Thermodynamic values for the protonation of the β-CD-NH_2, the poly(β-CD) and some PAAs in 0.15 M NaCl at 25 °C



Compd	Reaction step	logK	n [a]	ΔG° kJ/mol	ΔH° kJ/mol	-TΔS° kJ/mol	Ref.
β-CD-NH_2	1st	**8.60** (1)	1	**-49.09** (4)	**-46.1** (4)	**-3.0** (3)	This work
poly(β-CD)	1st	**6.29** [b]	**1.75**	**-35.91** [b]	**-27.6** [b]	**-8.3** [b]	
R =							
 polyN1	1st	7.79	1	-44.47	-34.6	-9.9	44
 polyN2	1st	8.09	1	-46.18	-30.8	-15.4	44
	2nd	4.54	1	-25.92	-25.5	-0.4	
 polyN2(OH)2	1st	7.52	1	-42.93	-35.6	-7.3	45
	2nd	3.81	1	-21.75	-21.3	-0.5	
 polyN(COOH)	1st	8.30	1.07	-47.38	-31.9	-15.5	46

Values in parentheses are standard deviations

[a] $\log K = \log K^\circ + (n-1)\log(1-\alpha)/\alpha$

[b] Evaluated at $\alpha = 0.5$

β-CD-NH_2 is even lower of one or two orders with respect to the comparable primary alkyl- or substituted-alkyl amines and aminoacids [35]. This means that the many hydroxyl groups present on the β-CD exert a large inductive effect on the primary amino nitrogen in order to be protonated.

On the other hand, the polymer shows an 'apparent' log K, i.e., the protonation of the tertiary amino group linearly decreases with the degree of protonation α in a wide range (till $\alpha = 0.6$) and follows the modified Henderson–Hasselbalch (H–H) equation: $\log K = \log K^\circ + (n-1)\log(1-\alpha)/\alpha$ [26]. The greater polyelectrolyte behaviour shown by the poly(β-CD), as evidenced by the n parameter, is quite unusual in the field of the poly(amido-amine)s, PAAs, chemistry. As a rule, PAAs behave as low molecular weight compounds with thermodynamic functions being 'real', i.e., the protonation of the basic sites in the monomer unit are independent on each other [14, 17, 36]. This property was attributed to the diacylpiperazinic moiety that acts as a shielding group, improving the lack of cooperativity among the repeating units [26]. Even if n values of the modified H–H equation larger than one were found in PAAs of different structures and with carboxyl groups [37–39] in the side chain, the higher n value found for the poly(β-CD) seems to be quite anomalous. Higher n values were always associated with the hydrophilic quality of the polymer [40], and the fact that this PAA shows $n = 1.75$ should give an insight in the larger hydrophilic character of the PAA bearing the β-cyclodextrin moiety. The results were confirmed at different concentrations of the polymer, showing that the interpolymer association is negligible. In Fig. 4 is reported the trend of the log K in relation to α, fitting well the modified H–H equation in the wide α-range, at two very different concentrations of the polymer.

The linear decreasing trend of the log K/α plot may be likely related to the regular increase of the electrostatic

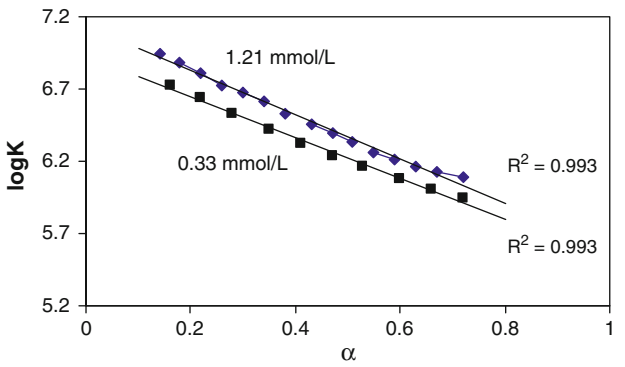


Fig. 4 Basicity constant (log K) values in relation to the degree of protonation (α) of poly(β-CD) in 0.15 M NaCl at 25 °C. The different polymer concentrations (based on the monomer unit) are reported

effect at a short range. During the protonation of the tertiary amino group the macromolecule, instead of expanding its coil dimension due to the repulsive forces between the charged onium ions, remains quite in a coil compact state. This may suggest an influence of the voluminous β-CD residues on the hydrodynamic volume of the macromolecule. A small coil dimension was suggested by the viscometric data, where the low reduced viscosity remained almost at a constant value close to 0.04 dL/g in the whole range of the pHs investigated. As a matter of fact, the PAAs are generally obtained with molecular weights of the order of magnitude of 10 kg/mol [14] and the presence of the larger β-CD is compatible with short macromolecular chains. Moreover, any attempt to measure the molecular weight of the polymer in tris buffer (pH 8) by SEC analysis failed because of the polymer retention in the gel column. These data are in line with the likely hypothesis of a packing structure, where the larger and rigid β-CD side groups of the PAA bring together with an head-to-head assembly, due to their poor hydrophilic quality [41–43]. Among the natural cyclodextrins, the solubility of β-CD in water is rather low (1.85 g/100 mL) and it may happen that the cooperation of several β-CD units enhances their interactions. This interaction may likely occur through H-bonds of the primary or the secondary hydroxyl groups in the regions of the truncated cone of the β-CD structure. During the protonation, the exposure of the basic nitrogen being protonated creates in this way an increasing electrostatic atmosphere able to decrease the log K, as reported above. The calorimetric data may well support the mentioned hypothesis. The ΔH° for the protonation of the basic nitrogen in the β-CD-NH_2 and the poly(β-CD) is reported in Table 1, along with their corresponding ΔS°, as evaluated at $\alpha = 0.5$ for the polymer. Obviously, the lower ΔH° for the polymer confirms the presence of a tertiary amino nitrogen on the macromolecule. This is in line with the trend of the thermodynamic values reported for other PAAs [44–46] (Table 1) in comparison to the corresponding simple primary or bis-secondary amines [47]. The enthalpy change, as well as the entropy contribution, of the poly(β-CD) is even lower with respect to those of the related PAAs containing additional functional groups of different electron withdrawing properties. The $-T\Delta S^\circ$ value becomes closer to that shown in the first protonation step of poly$N_2(OH)_2$ (Table 1) even though both the free energy and enthalpy changes of the latter compound are greater. As for the log K, the enthalpy and entropy changes for the protonation of the β-CD-NH_2 may be well related to those of the primary alkyl-monoamines [47]. Its ΔH° is lower of more than nine units with respect to the alkyl monoamines ($\Delta H^\circ = -55.2$ kJ/mol for methylamine), and the presence of the hydroxyl group in the

ethanolamine ($\Delta H° = -50.5$ kJ/mol) sharply reduced the difference to less than five units. In the case of the poly(β-CD), a different pattern was found because of the poly-electrolyte behaviour, the smaller heat effects being of the H-bonds order. The $\Delta H°$ for the protonation of the tertiary amino nitrogen showed a sensitive decrease up to a critical α closer to 0.5, related with a concomitant increase of the $-T\Delta S°$ (Fig. 5).

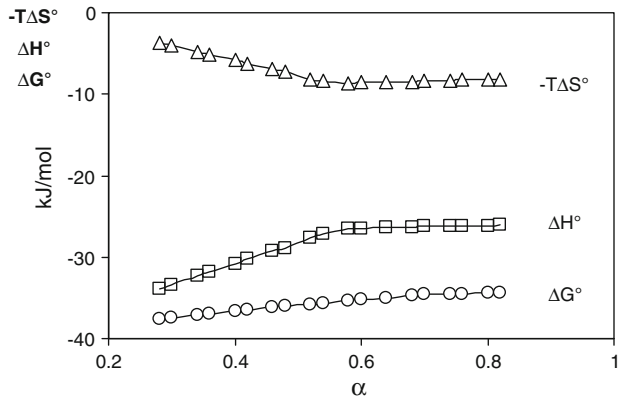
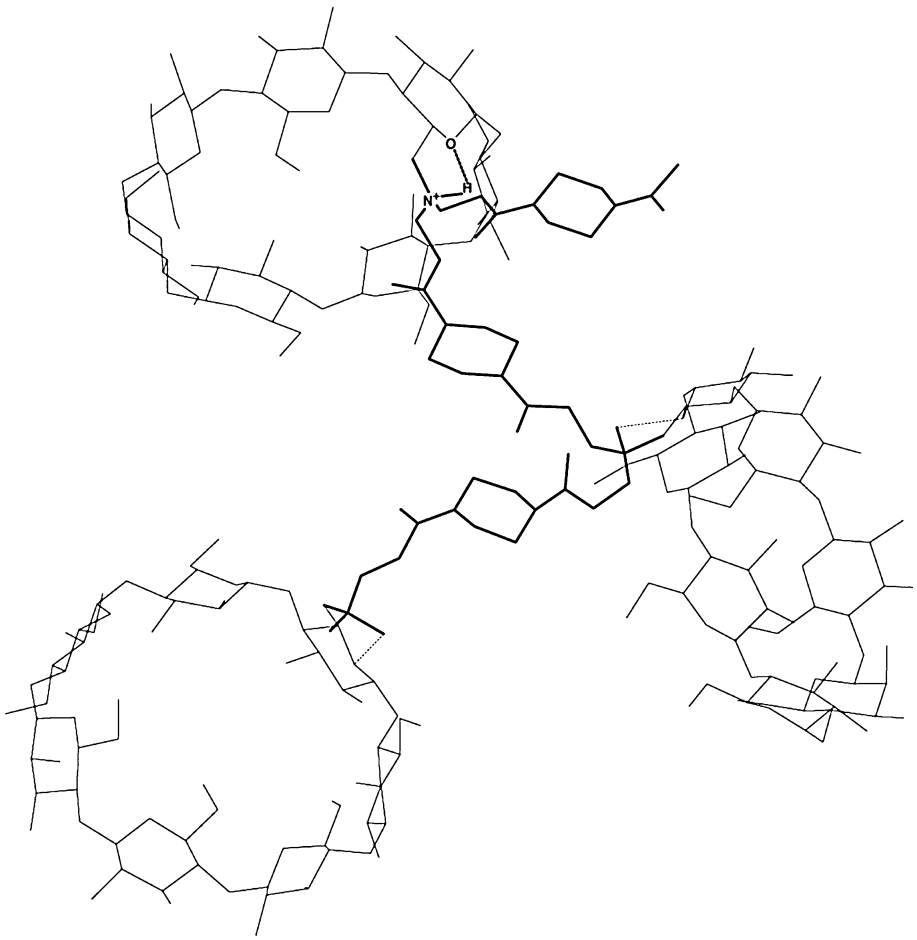


Fig. 5 Thermodynamic functions ($\Delta G°$, $\Delta H°$, and $-T\Delta S°$) in relation to the degree of protonation α for the poly(β-CD) in 0.15 M at 25 °C

After this critical α point, the enthalpy changes remained constant, while the further entropy changes followed the decrease of the $\Delta G°$. We may explain this thermodynamic trend by the likely weakening of the H-bonds between the assembled β-CD units. The protonation of the tertiary nitrogen may involve an H-bonding interaction of the onium ion with the cyclic saccharide oxygen, that is able to form a stable five-membered ring (Chart 2).

The five-membered ring is more favoured with respect to a possible six-membered one, involving the oxygen of the α-1,4-glucosidic linkage. Unlike the longer N^+–H···O distance of the latter structure (>3.9 A), the shorter one (2.2 A) of the former structure, in conjunction with the R configuration of the N^+, leads the polymer chain close to the small side of the conical cylinder, without causing steric hindrance. The protonation process may cause, along with its positive charge, some strains on the packing structure of the side chain β-CDs. The breaking of the H-bonds of the β-CD hydroxyl groups, being an

Chart 2 Molecular structure representation of a polymer chain spanning three monomer units as resulting from the molecular modelling; the hydrogen bond between the protonated nitrogen and the cyclodextrin oxygen, originating a five-membered ring, is *highlighted*



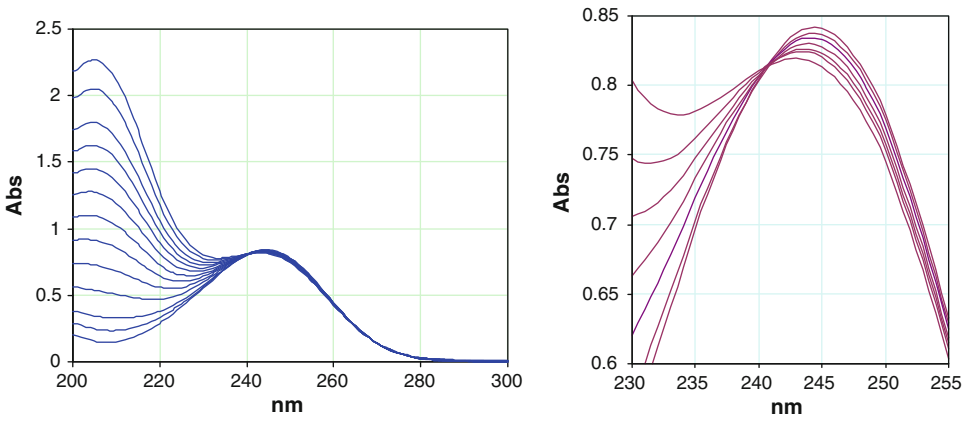


Fig. 6 UV spectra of L-ascorbic acid (H_2A, 8.8×10^{-5} M) with addition of poly(β-CD) in aqueous solution at pH 2.46. The amount of poly(β-CD) was added till a poly(β-CD/H_2A) molar ratio of 1.5 was reached

endothermic process, leads to an increase of the entropy. The process goes on till a degree of protonation α close to 0.5 is reached, i.e., when all the engaged β-CDs are in a freer state. Even if this thermodynamic protonation process has been found in several polyelectrolytes where H-bonding interactions occurred via a spatial approach [28, 48, 49], the presence of the lateral β-CD units improves the stacking model already reported for peptidocyclodextrins, that has the advantage of a self-assembling system with cooperativity between the participating CD molecules [50].

Inclusion compounds

The L-ascorbic acid (H_2A) was chosen as a molecular probe because its inclusion in the β-CD enhances its bioavailability and stability in redox processes in biological media. Manzanares [19] reported that the UV spectra for the interaction of H_2A with β-CD, unlike the α-CD, was accomplished by a decrease in the intensity of the 242 nm band of H_2A at low pH during addition of the β-CD, without any appreciable shift in λ_{max}. More recently, Terekhova [18] showed that the modified β-CD, the hydroxypropyl-β-CD (HP-β-CD), plays a different role in the inclusion of the guest molecule. The spectrophotometric results showed an increase in the intensity of the 244 nm band of the H_2A during addition of the HP-β-CD. The flexible hydroxypropyl group participates in an additional interaction with the hydrophilic part of the guest H_2A molecule.

In our investigation we found that, unlike the β-CD-NH_2, the UV spectra of the H_2A interacting with poly(β-CD) showed a slight blue-shift of the maximum peak position (from 244 to 242 nm) and a clear isosbestic point at 241 nm (Fig. 6).

The presence of the isosbestic point indicates that there is only one type of interaction and that the interaction of H_2A with the poly(β-CD) can be attributed to a more hydrophilic environment [51]. A systematic analysis will be performed for the evaluation of the complexation constant of the species involved in this system.

Conclusions

A β-cyclodextrin-containing poly(amido-amine) was synthesized for drug inclusion. The protonation thermodynamic data in 0.15 M NaCl revealed a polyelectrolyte behaviour with basicity constant (log K) greatly dependent on the degree of protonation α on a wide α-range. The log K, lower than that of the corresponding β-CD-NH_2, showed a linear decreasing trend during the protonation of the tertiary nitrogen. A similar decreasing pattern was shown by the enthalpy ($\Delta H°$) changes till α close to 0.5, after which the $\Delta H°$ remained constant. These data, in conjunction with the increasing entropy ($\Delta S°$) changes, likely supported a packing structure in which two β-CDs of the side chain interact head-to-head through hydrogen-bonds. During the protonation, the assembled structure is destroyed, making lower the $\Delta H°$ and higher the $\Delta S°$. Unlike the β-CD-NH_2, the poly(β-CD) forms complex species with the L-ascorbic acid, as shown by the blue-shift of the maximum peak position and the isosbestic point (241 nm).

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