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β-Cyclodextrin–Polyelectrolyte Interactions in Aqueous Solution: I. Poly(4-Sodium Styrenesulfonate) (NaPSS)

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Abstract. The interactions between β-cyclodextrin and a hydrosoluble polymer carrying hydrophobic residues: poly(4-sodium styrenesulfonate) have been studied. Several techniques were used: capillary viscosimetry, circular dichroïsm, and NMR spectroscopy. The results proved that the aromatic residues of NaPSS were included in the cavity of β-cyclodextrin, and that, in addition, a network of hydrogen bonding between sulfonic acid residues and the hydroxyl groups of β-cyclodextrin occured.

Key words: β-Cyclodextrin, inclusion complexes, polymer, viscosimetry, circular dichroïsm, NMR spectroscopy, thermogravimetric analyses.

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six (α-CD), seven (β-CD), or eight (γ-CD) D-(+)-glucopyranose units linked by α-(1,4) bonds. The existence of a central cavity with hydrophobic properties allows these molecules to form inclusion complexes with organic or inorganic substrates. This fact opened the way to applications in many different fields, such as pharmacology, food science, analytical chemistry, chemical synthesis and catalysis [1–5].

Cyclodextrins form inclusion complexes with a wide variety of low molecular weight compounds [3], which can easily fit in the cavity of the CD host. Inclusion complex formation has been studied by a variety of methods, such as fluorescence spectroscopy [6–11], circular dichroism (CD) spectroscopy or optical rotatory dispersion (ORD) [8,10,12–17], ultraviolet (UV) and visible spectroscopy [7,9–11,17–19], proton nuclear magnetic resonance (^{1}H–NMR) spectroscopy [10–11,17,20–26], thermogravimetric analysis [27–28], and calorimetry [14,29]. Among these methods, ^{1}H-NMR spectroscopy, UV-visible spectroscopy, and the CD methods are the most widely used.

In most cases, the guest molecules are small and there are only a few literature references to the interactions between β-cyclodextrin and macromolecules in aqueous solution. Harada *et al.* [30] found that cyclodextrins form complexes with

polymers, such as poly(ethylene glycol) in the crystalline state. Wenz *et al.* [31] studied mixtures of cyclodextrin and polyethyleneimine in water. In our laboratory we have studied covalent and non-covalent cyclodextrin–polyvinylamine systems in aqueous medium [32].

Interactions between cyclodextrin and a polymer in aqueous medium should be favoured in the case where the former has an amphiphilic character. Most such polymers exhibit special conformational properties (compact or extended structure). Therefore, this characteristic should be modified upon addition of cyclodextrin, because of the possible inclusion of the hydrophobic groups of the macromolecule. We have recently carried out a systematic study of several amphiphilic polymers and we have investigated whether they are able to interact with cyclodextrin. Indirect clues, such as the viscosity changes, but also direct methods, such as NMR, have been used. Among the polymers studied, poly (4-sodium styrenesulfonate) (NaPSS) has been considered. In fact, Seo *et al.* [33] have already reported that cyclodextrin may be included inside the hydrophobic microdomain of this polymer. These results were obtained from a catalytic study of the cleavage of nitrophenyl esters. In this paper, we confirm the previous findings and introduce additional knowledge about what happens when cyclodextrin is mixed with NaPSS in water.

The interactions between β-cyclodextrin and NaPSS were investigated by measuring the changes of the circular dichroism (CD) spectra. The internal cavity of cyclodextrin provides an asymmetric field which perturbs the electronic state of the included guest and induces optical activity [34–35]. The induced CD spectra have been investigated to elucidate the structure of host–guest interaction in the cyclodextrin complexes, especially in the complexes with optically inactive guests [13].

The possible structure of the inclusion complex was also studied by ^{1}H-NMR spectroscopy. NMR studies of cyclodextrin complexes with aromatic compounds have been conducted by many researchers [20–26]. In the structure of β-cyclodextrin, only the H_3 and H_5 protons are located inside the cavity, forming a ring around the larger and the smaller opening of the cavity, respectively. It has been found that when an aromatic moiety of the guest molecule is included in the cavity of β-cyclodextrin, only these protons are sensitive to anisotropic shielding of the aromatic moiety.

Furthermore, the viscosity changes induced by the formation of the inclusion complex were also investigated. Viscosimetry studies have frequently been conducted by many researchers in order to estimate changes in the hydrodynamic size of polymers [36–40]. In general, such studies are used to describe the conformational changes induced in polymers by pH or ionic strength. The best known polymer, which shows a marked pH-induced conformational transition, is poly(methacrylic acid). In the case of NaPSS, which is fully expanded in water at neutral pH and low ionic strength, the conformational changes are expected to result from the interaction with β-cyclodextrin by inclusion and/or hydrogen bonding.

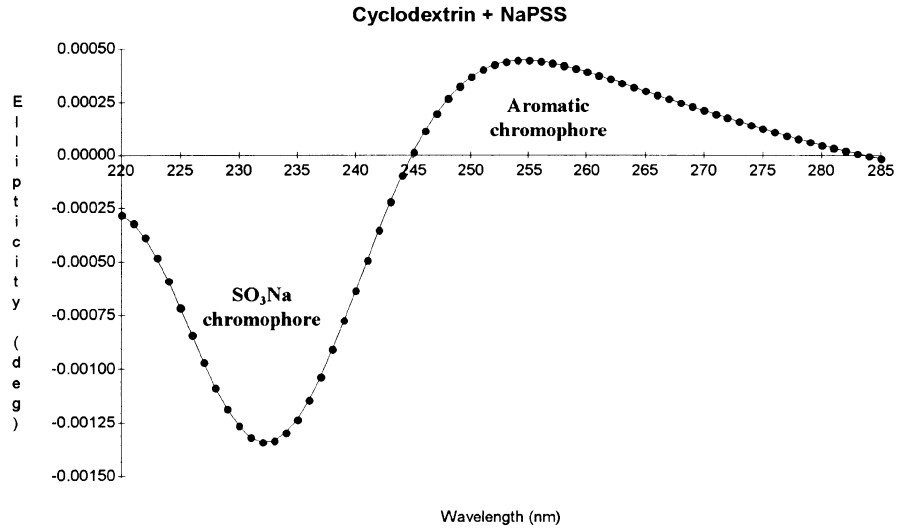

Figure 1. Induced circular dichroism spectrum of NaPSS + β-cyclodextrin.

2. Experimental

Reagent grade β-cyclodextrin (Kleptose) was supplied by Roquette. Poly(4-sodium styrenesulfonate) (NaPSS) was supplied by Acros and had an average molecular weight of 70 000.

The CD spectra were obtained on a Jobin-Yvon Mark IV spectropolarimeter at 25 °C under nitrogen. Stoppered quartz cells with an optical pathlength of 1.00 cm were used. Viscosimetric studies were carried out using a Schott Geräte AVS 400 capillary viscosimeter at 25 °C. All measurements were carried out in water, the natural pH of the solution was 6.5. The change of pH upon addition of β-cyclodextrin is negligible.

Thermogravimetric analyses were performed on a Shimadzu TGA 51 system at a heating rate of 10 °C/min in air. The βCD-NaPSS complex was prepared by freeze-drying an aqueous solution containing both βCD and NaPSS with a tenfold molar excess of NaPSS. The physical mixture was prepared by simple mixing of the two components with the same molar excess of NaPSS.

The ^{1}H-NMR spectra were recorded in deuterium oxide on a Brüker AC 300 spectrometer (300 MHz) with 32 transients and by irradiating the peak of the solvent. NOESY spectra were recorded in deuterium oxide on a Brüker AMX 300 spectrometer (300 MHz) with the phase sensitive mode method (512 increments and 64 transients for each increment). The temperature was 25 °C and mixing time was 0.2 s.

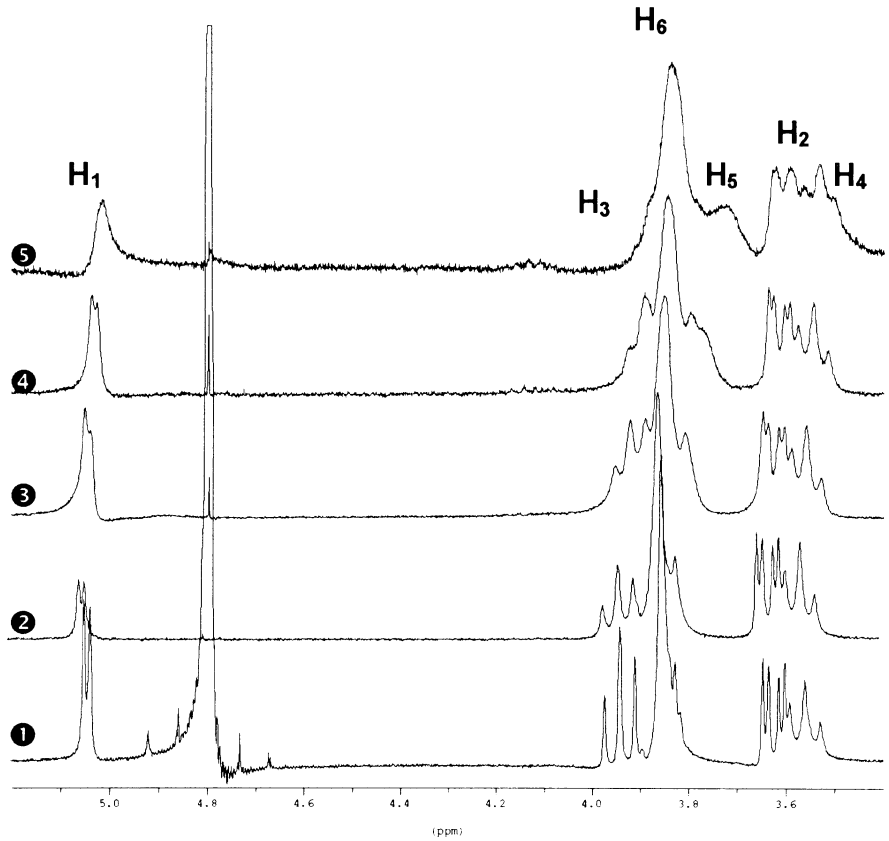

Figure 2. The upfield region (3.4–5.2 ppm) of the ^{1}H-NMR spectra of 1.0×10^{-2} M β-cyclodextrin and increasing concentrations of NaPSS in D_2O. ❶ 0 (β-cyclodextrin alone), ❷ 6.06×10^{-3} M, ❸ 1.75×10^{-2} M, ❹ 2.86×10^{-2} M, ❺ 4.0×10^{-2} M NaPSS.

3. Results and Discussion

In the presence of β-cyclodextrin, an induced CD signal for NaPSS appears, as seen in Figure 1. This CD signal was not seen when D-glucose, which is the constituent unit making up β-cyclodextrin, was added in place of β-cyclodextrin. Therefore, the induced CD spectrum can be attributed to the induced optical activity of NaPSS by formation of an inclusion complex with β-cyclodextrin. The CD spectrum shows a negative band at 232 nm and a positive band at 254 nm. The latter transition corresponds to the aromatic chromophore whereas the former may be attributed to the sulfonate group on the basis of the UV absorption spectrum.

The ^{1}H-NMR spectrum of free β-cyclodextrin in D_2O is shown in Figure 2 ❶. The spectrum of free β-cyclodextrin was initially assigned by Demarco and Thakker [41]. The assignments have been confirmed in the present work. The free β-cyclodextrin resonance positions at 25 °C relative to the solvent line (D_2O, 4.79

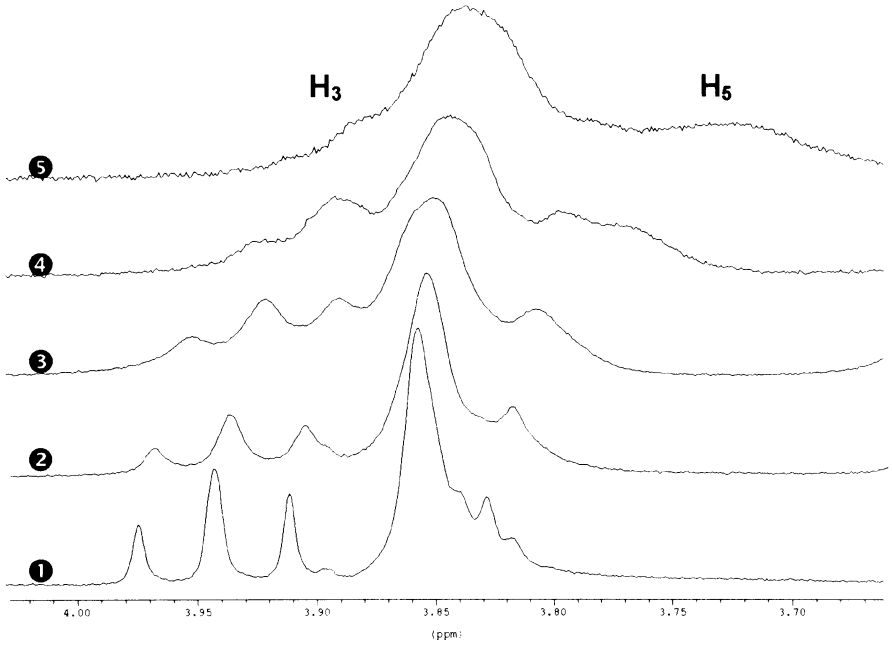

Figure 3. The region (3.7–4.0 ppm) of Figure 2.

ppm) are H_1 at 5.047 ppm (doublet), H_2 at 3.626 ppm (doublet of doublets), H_3 at 3.944 ppm (triplet), H_4 at 3.561 ppm (triplet), and H_5 at 3.829 ppm (doublet of triplets), and both H_6 protons nearly overlap at 3.858 ppm. In D_2O solution only resonances from the nonchanging hydrogens attached to the carbons are detected. The resonances for the active H_2, H_3, and H_6 hydroxylic protons were not observed.

The effects of NaPSS on the spectrum of β-cyclodextrin were investigated by holding the concentration of β-cyclodextrin constant at 10^{-2} M, and changing the molar ratios of β-cyclodextrin to NaPSS from 0 to 1.65. Parts ❷–❺ of Figure 2 show the effect of NaPSS on the ^{1}H-NMR spectrum of β-cyclodextrin. An expansion of the region (3.7–4.0 ppm) is shown in Figure 3. As expected, the lower field triplet assigned to the H_3 protons' resonance is progressively shifted to higher field with increasing concentration of NaPSS in the solution. An upfield shift is also observed for the H_5 protons' resonance which was originally superimposed on the H_6 protons' signals. No significant chemical shift or line broadening is observed for the H_2, H_4, and H_6 protons. The strong molar ratio dependance of the $\Delta\delta$ values for the H_3 and H_5 protons is the direct evidence for the inclusion complex formation between β-cyclodextrin and the phenyl group of NaPSS, since only when the phenyl group on the NaPSS includes into the cavity can the strong anisotropic shielding of the aromatic ring become accessible to the H_3 and H_5 protons. The $\Delta\delta$ value for the H_3 protons, which form a ring around the larger opening of the cavity, is related to the stability of the inclusion complex [20], while the $\Delta\delta$ value for the

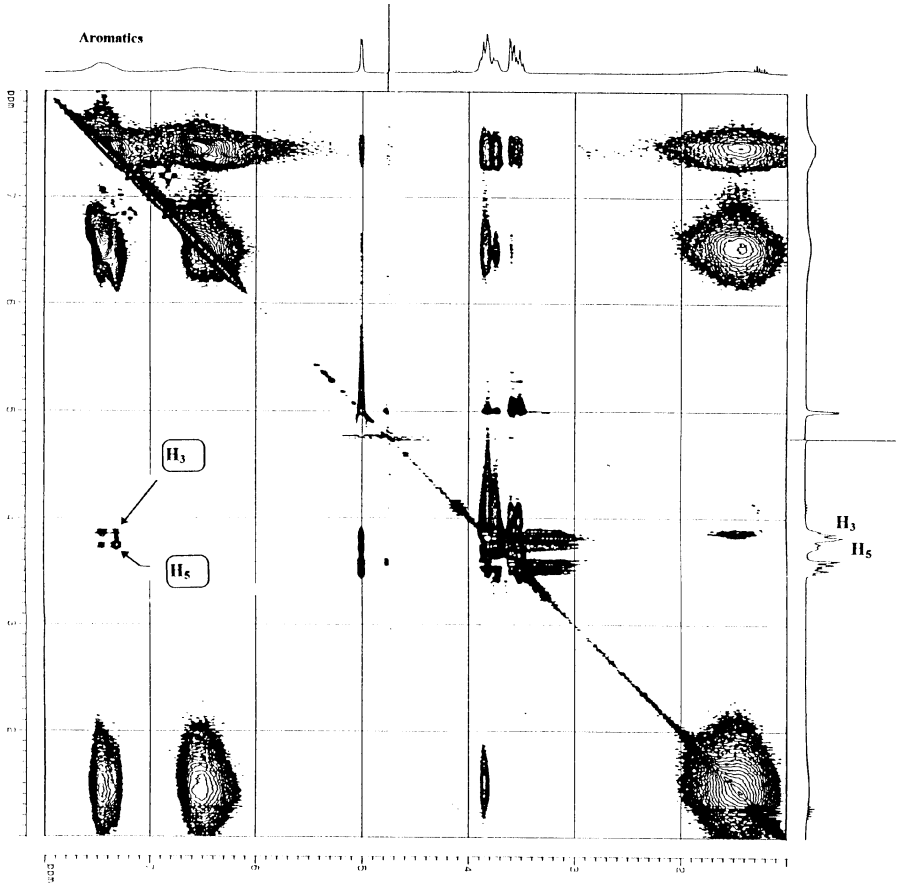

Figure 4. NOESY spectrum of solution containing NaPSS (4×10^{-2} M) and β-cyclodextrin (10^{-2} M) in deuterium oxide.

H_5 protons which form a ring near the smaller opening of the cavity can be taken as an indicator for the penetration depth of the aromatic group of the NaPSS. The $\Delta\delta$ values for the H_3 and H_5 protons observed in the presence of NaPSS suggest that NaPSS forms a stable inclusion complex with β-cyclodextrin, and the phenyl group of NaPSS penetrates into the cavity deeply enough to allow the sulfonate group (hydrophilic group) to protrude (at least partly) from the cavity (hydrophobic) of β-cyclodextrin.

To confirm the structure of the inclusion complex, the NOESY spectrum of a solution containing β-cyclodextrin (10^{-2} M) and NaPSS (4×10^{-2} M) was measured in deuterium oxide (Figure 4). The cross peaks were observed between the H_5 protons of β-cyclodextrin and the aromatic protons of NaPSS, and also between the H_3 protons of β-cyclodextrin and the aromatic protons of NaPSS. Therefore, the structure of the inclusion complex was confirmed.

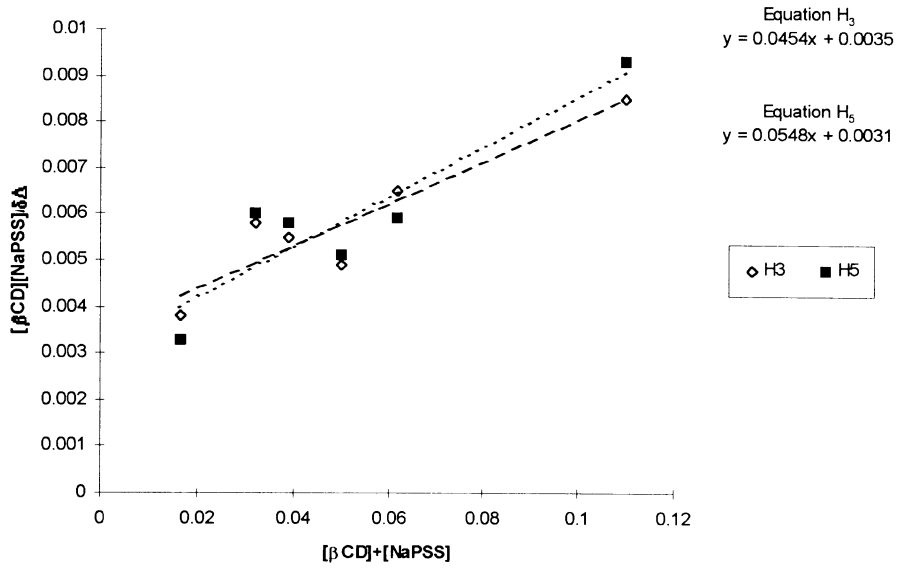

Figure 5. Determination of the dissociation constant K_d by the Bergeron equation.

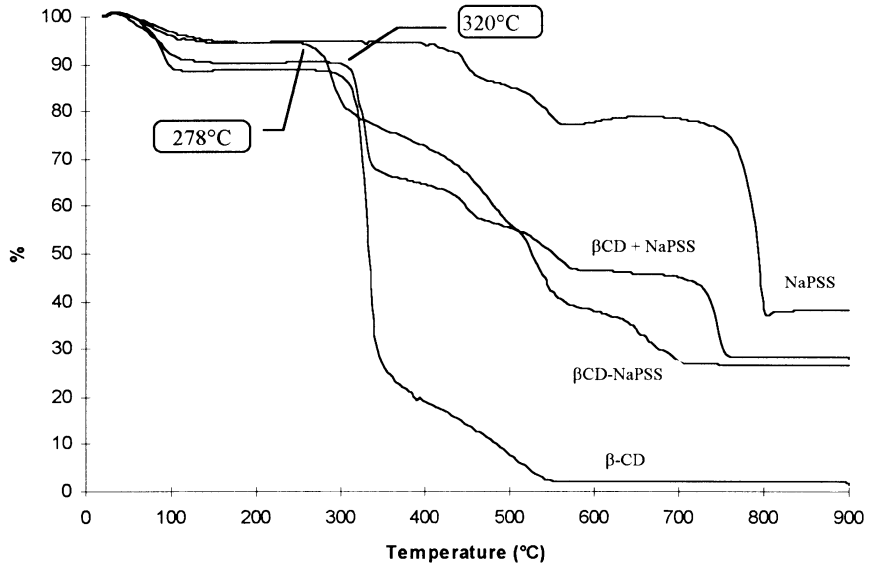

Figure 6. Thermogravimetric analysis of β-cyclodextrin, NaPSS, physical mixture NaPSS + β-cyclodextrin, and the NaPSS-β-cyclodextrin complex.

The association constant was determined by using the Bergeron equation [42].

$$[\beta\text{CD}][\text{NaPSS}]/\Delta\delta = K_d/Q + ([\text{NaPSS}] + [\beta\text{CD}])/Q$$

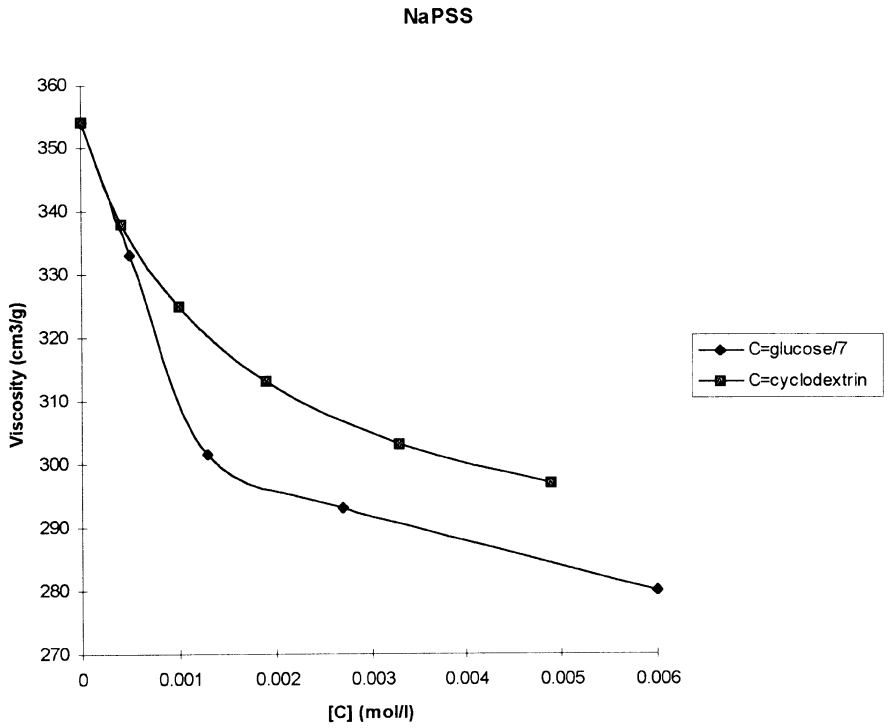

Figure 7. Reduced viscosity measurements as a function of the concentration of D-glucose/7 and β-cyclodextrin. Polymer concentration: 0.02 g/dL.

where K_d is the dissociation constant of the complex, $\Delta\delta$ is the change of chemical shift of the H_3 or H_5 protons, and $Q = (\delta_C - \delta_H)/[\text{NaPSS}]$. [$\beta$CD][NaPSS]/$\Delta\delta$ was plotted against ([NaPSS] + [βCD]) (Figure 5). The dissociation constant was obtained by dividing the intercept by the slope. We have found a K_d value of 0.067 M (i.e. K_s = 15 M^{-1}) by this method. This is compatible with data for similar systems, such as benzoic acid (K_s = 10–20 M^{-1} [43]). Seo has found that the dissociation constant of the substrate–cyclodextrin complex is practically unaffected by the presence of NaPSS. So this result shows that the association constant of the NaPSS–cyclodextrin complex is relatively weak. Our work has confirmed this interpretation.

Thermogravimetric analyses were carried out on β-cyclodextrin, NaPSS, a physical mixture of NaPSS + β-cyclodextrin, and the NaPSS–β-cyclodextrin complex (Figure 6). The experiments show that βCD decomposes at a lower temperature than NaPSS. A dehydration process occurs around 80 °C and the decomposition occurs at 320 °C, as already reported in the literature [28]. On the other hand, NaPSS has a first decomposition step around 450 °C, a second around 520 °C, and a third one around 800 °C. The decomposition temperature of βCD remains unchanged when it is physically mixed with the polymer, whereas it is lower (278

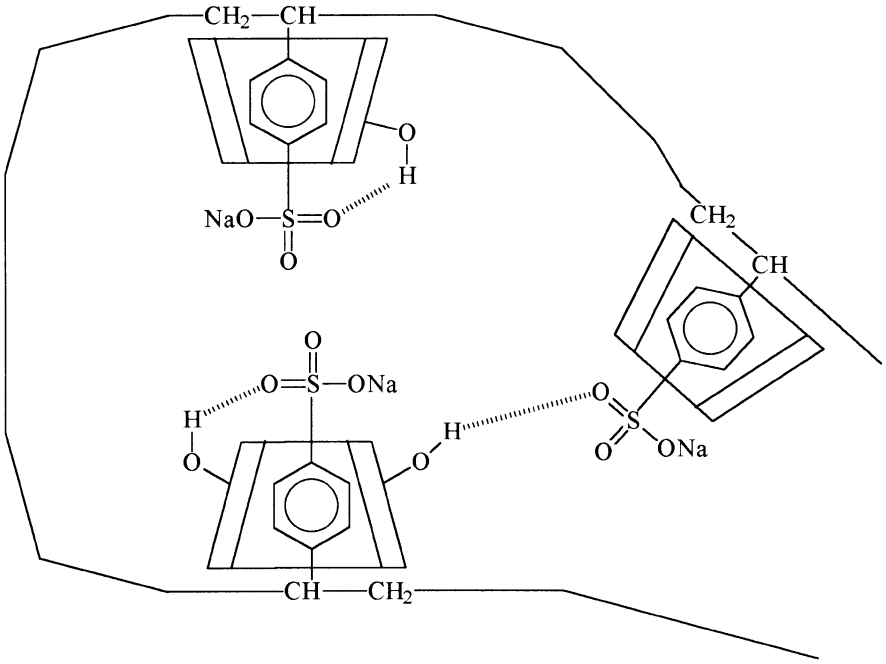

Figure 8. Nature of the NaPSS-β-cyclodextrin interactions (inclusion and hydrogen bonding).

°C) with complex formation. This difference of about 40 °C is sufficient to confirm the formation of an inclusion complex.

Capillary viscosimetry studies were carried out in order to describe the conformational changes induced in the polymer NaPSS by the addition of β-cyclodextrin. Under the experimental conditions used, the polymer NaPSS was always ionised. In the same way, experiments were carried out with D-glucose, as a model compound of β-cyclodextrin. The inclusion of aromatic groups of NaPSS by β-cyclodextrin is expected to induce an increase in the hydrodynamic size of the polymer by increasing the apparent molecular weight, and therefore an increase of the reduced viscosity. Figure 7 shows the reduced viscosity (η/c) curves of NaPSS in the presence of β-cyclodextrin and D-glucose. The same behaviour (a decrease in the reduced viscosity) was observed. As D-glucose cannot enclose molecules, the only explanation is the formation of a network of intramolecular hydrogen bonding between the sulfonate groups of NaPSS and the hydroxyl groups of D-glucose or β-cyclodextrin leading to a collapse of the polymer chain. The decrease in η/c is less marked in the presence of β-cyclodextrin because it is partly balanced by the increase of the apparent molecular weight of the repeat unit cited above. NMR data have shown that the sulfonate group protrudes from the cavity of β-cyclodextrin and is available for such interactions. Intramolecular hydrogen bonding induces a decrease in the hydrodynamic size of the polymer, and therefore a decrease in the reduced viscosity.

4. Conclusion

In summary, it can be concluded that induced circular dichroism, ^{1}H-NMR spectroscopy, and viscosimetry can provide valuable information about the nature of cyclodextrin complexation. Judging from investigation by the CPK model, the structure for the inclusion complex is that the entire benzene ring of NaPSS is enclosed inside the cavity of β-cyclodextrin, the sulfonate group being partly on the exterior of the cavity.

In fact, the results show that the inclusion of aromatic groups of NaPSS by the cavity of β-cyclodextrin is accompanied by the formation of a network of intramolecular hydrogen bonding between the sulfonate groups of NaPSS and the hydroxyl groups of β-cyclodextrin (Figure 8).

Acknowledgements

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