New Supramolecular Assemblies of a Cyclodextrin-Grafted Chitosan through Specific Complexation

Rachel Auzély-Velty* and Marguerite Rinaudo

Centre de Recherches sur les Macromolécules Végétales (CNRS), Université Joseph Fourier de Grenoble, BP 53, 38041 Grenoble Cedex, France

Received April 30, 2002; Revised Manuscript Received August 1, 2002

ABSTRACT: New supramolecular assemblies based on a chitosan bearing pendant cyclodextrins were prepared. For this purpose, adamantyl groups which can selectively be included in the cyclodextrin cavity were grafted on chitosan and various poly(ethylene glycol)s (M_n = 3400, 6000, 20000) affording guest macromolecules with different structural features. The specific interaction of the latter with CD−chitosan through inclusion complex formation was examined by rheological measurements. Physical cross-linking between CD−chitosan chains was shown to occur, leading to large increase of the viscosity or gellike behavior, depending on the guest.

Introduction

Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides with six, seven or eight α-1,4-linked D-glucopyranose units (α-, *β*-, and *γ*-cyclodextrins, respectively) which can selectively include a wide range of guest molecules into their hydrophobic cavity.[1] Beside their use as carriers, CDs have been described as supramolecular assemblies for the creation of novel molecular materials. The representatives are rotaxanes or polyrotaxanes in which one or several cyclic molecules are threaded onto a polymeric chain end-capped with bulky groups.[2] Such polymer inclusion complexes have been used to develop molecular shuttles in which one or several rings can move reversibly back and forth in response to external stimuli.[3,4] The noncovalent bonding interactions of CDs with the side chain guest groups attached to a polymer chain represent another interesting kind of macromolecular recognition. CDs were shown to recognize guests on a polymer backbone more specifically than low molecular weight guests.[5] It was suggested that this might be due to the fact that introduction of the guest in the CD cavity can take place from only one direction. Furthermore, CDs were shown to be able to suppress the hydrophobic association of several hydrophobized polymers leading to changes in solution properties.[6−8] More recently, supramolecular-structured hydrogels have been demontrated using inclusion complexation between α-CDs and poly(ethylene glycol)-grafted dextrans.[9] Such biodegradable hydrogels exhibiting a thermoreversible gel−sol transition would be very useful in many biomedical applications. It was also shown that solution viscosity enhancements could be obtained by host−guest interactions between branched or linear cyclodextrin synthetic polymers and hydrophobically modified polymers.[10,11] In the present paper, we describe new supramolecular assemblies based on inclusion complex formation between a cyclodextrin-grafted chitosan (CD−chitosan) and various guest macromolecules. The effect of the guest molecular structure on the rheological behavior of CD−chitosan solutions is examined.

* Corresponding author.

Experimental Section

Materials. The chitosan used has a weight-average molecular weight M_w of 195000; it is a commercial sample from Pronova (Norway) with a degree of N-acetylation equal to 0.12. It was purified by solubilization in aqueous CH_3COOH and reprecipitation by NaOH at neutral pH. The polysaccharide was finally washed with deionized water and ethanol and then dried. The *β*-cyclodextrin was kindly supplied by Roquette Frères (Lestrem, France). CD−chitosan was synthesized in the laboratory as described previously.[12] Poly(ethylene glycol) (number-average molecular weight M_n = 6000), diaminopoly(ethylene glycol)s (M_n = 3400 and 20000), 1-adamantaneacetic acid, 1-adamantaneethanol, and all other chemicals were purchased from Fluka (Buchs, Switzerland).

Mass Spectrometry. Electrospray mass spectra were measured in the positive mode on a ZabSpec TOF (Micromass) mass spectrometer. PEG derivatives were dissolved in methanol/water (1:1 v/v) at a concentration of 0.1 mg.mL^{-1} and infused into the electrospray ion source. The capillary voltage was set to 4 kV.

NMR Spectroscopy. ^{1}H NMR experiments were performed using Bruker DRX500, DRX400, and AC300 spectrometers operating at 500, 400, and 300 MHz, respectively. 1D NMR spectra were collected using 16K data points. 2D T-ROESY and TOCSY experiments were acquired using 2K data points and 256 time increments. The phase sensitive TPPI was used and processing resulted in a 1K × 1K (real−real) matrix. Chemical shifts are given relative to external tetramethylsilane (TMS = 0 ppm) and calibration was performed using the signal of the residual protons of the solvent as a secondary reference. Deuterium oxide and deutered chloroform were obtained from SDS (Vitry, France). Details concerning experimental conditions are given in the figure captions. Association constants for the formation of 1:1 complexes were determined using the following equation:[13]

$$[B]_t = \Delta\delta_{Aobs}/\Delta\delta_{Ac}\,([A]_t + (K_a(1 - \Delta\delta_{Aobs}/\Delta\delta_{Ac})) - 1) \quad (1)$$

where $[A]_t$ and $[B]_t$ are the total concentrations of the host (A) and guest (B) molecules, respectively. $\Delta\delta_{Aobs}$ represents the chemical shift difference (for a given proton) between free A (obtained in the absence of B) and the observed value in the presence of B, whereas $\Delta\delta_{Ac}$ represents the chemical shift difference between free A and the pure complex. The experimental data (corresponding to $[A]_t$, $[B]_t$ and $\Delta\delta_{Aobs}$) were processed using a multiparameter iterative fitting procedure contained in the SIMPLEX algorithm.[14]

10.1021/ma020664o CCC: $22.00
Published on Web 09/11/2002

Fluorescence Spectroscopy. Pyrene emission spectra were measured on a Perkin-Elmer LS 50B spectrofluorometer between 360 and 500 nm. Pyrene solubilized in ethanol was added up to a concentration of 10^{-7} M in the polymer solution and excited at 334 nm. The III/I ratio of the intensities of the third and the first peaks of fluorescence spectrum of pyrene was used to study the formation of hydrophobic microdomains resulting from the association of amphiphilic molecules.[15]

Dynamic Light Scattering. Dynamic light-scattering measurements were performed on an AMTEC SM200 goniometer fitted with an ionized argon laser source (Spectra Physics 2016) operating at a wavelength $\lambda = 514.5$ nm and a BI30 correlator. The polymer solutions were investigated in the range of concentration from 0.1 to 5 g/L at the temperature $T = 25$ °C in pure water.

Dilute Solution Viscometry. The intrinsic viscosities were determined by measuring viscosity of polymer solutions at low concentrations (<1 g/L) with an Ubbelohde capillary viscometer ($\phi = 0.58$ mm) and extrapolating to infinite dilution using the Huggins equation[16] as described below:

$$\eta_{sp}/C = [\eta] + k'[\eta]^2 C \qquad (2)$$

Here, η_{sp} is the specific viscosity, C is the polymer concentration, and k', is the Huggins constant. A low shear viscometer (LS30 from Contraves) was used for polymer solutions in the range of concentration from 1 to 5 g/L.

Rheological Experiments. The steady shear flow and dynamic properties of polymer solutions at high concentrations were measured using a cone plate rheometer (AR1000 from TA Instruments). The cone used has a diameter of 4 cm and an angle of 3° 59′, and it was equipped with a cap to avoid vaporization. All the dynamic rheological data were checked as a function of strain amplitude to ensure that the measurements were performed in the linear viscoelastic region. CD−chitosan, PEG−diadamantane, and adamantane-grafted chitosan solutions were prepared separately in 0.3 M CH_3COOH /0.03 M CH_3COONa. The dissolution time was at least 1 day at room temperature. CD−chitosan and PEG−diadamantane solutions or CD−chitosan and adamantane-grafted chitosan solutions were then mixed. Gellike behavior is obtained upon mixing, then the samples were vigorously stirred and allowed to rest for at least 1 h. We checked that the rheological properties of the samples did not change with time (from 1 to 24 h).

Synthesis. PEG−Diadamantane 5a,b. To a solution of diaminopoly(ethylene glycol) ($M_n = 3400$ or 20000) (0.10 mmol) in dry DMF (6 mM)) were added 1-adamantaneacetic acid (0.42 mmol), diisopropylcarbodiimide (DIC) (0.84 mmol) and hydroxybenzotriazole (HOBt) (0.20 mmol) successively. The mixture was stirred under nitrogen at room temperature for 24 h. The reaction was stopped by addition of water (∼ 0.5 mL). After evaporation of most of the solvent, the residual oil was poured into diethyl ether (Et_2O). The white precipitate was filtered, washed with Et_2O and dried to give pure **5a** or **5b** (80−90%).

^{1}H NMR (D_2O, 500 MHz), δ/ppm: 3.58 (s, $(OCH_2CH_2)_n$), 3.26 (t, CH_2NHCO), 1.88 (s, CH_2CO), 1.83 and 1.61−1.42 (2 m, H adamantane).

PEG—Diadamantane 7. To a solution of poly(ethylene glycol) 6000 (1 g, 0.167 mmol) and 1-adamantaneacetic acid (0.13 g, 0.67 mmol) in dry dichloromethane (CH_2Cl_2, 40 mL) were added DIC (0.206 mL, 1.33 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.040 g, 0.33 mmol) successively. The mixture was stirred under nitrogen at room temperature for 24 h. The reaction was stopped by addition of water (∼0.5 mL). After evaporation of most of the solvent, the residual oil was poured into Et_2O. The white precipitate was filtered, washed with Et_2O, and dried to give pure **7** (85%).

^{1}H NMR (D_2O, 500 MHz), δ/ppm: 4.15 (m, CH_2OCO) 3.58 (s, $(OCH_2CH_2)_n$), 2.04 (s, CH_2CO), 1.84 and 1.62−1.44 (2 m, H adamantane).

1-Adamantaneacetaldehyde 8. To a solution of 1-adamantaneethanol (0.5 g, 2.77 mmol) in dry CH_2Cl_2 (20 mL) was added pyridinium dichromate (2.087 g, 5.55 mmol). The resulting mixture was stirred under nitrogen at room temperature for 24 h. The mixture was then applied onto a small silica gel pad with a 10 cm layer of Et_2O on the top of the gel in order to precipitate the chromium compounds. The latter were removed by filtration (elution with Et_2O), and the filtrate was concentrated under reduced pressure to give an oil which contained almost pure **8** (0.4 g, 84%).

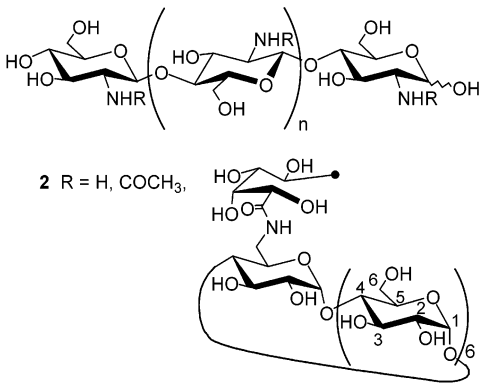
^{1}H NMR ($CDCl_3$, 300 MHz), δ/ppm: 9.85 (t, CHO), 2.10, 1.97 and 1.74−1.59 (d and 2 m, H adamantane).

Adamantane-Grafted Chitosan 9. To a solution of chitosan (0.3 g) in a mixture of 0.2 M aqueous CH_3CO_2H (20 mL) and EtOH (12 mL) was added a 0.1 M aqueous NaOH solution in order to adjust the pH to 5. Compound **8** (0.0225 g, 0.126 mmol) dissolved in EtOH (3 mL) was then added. After the mixture was stirred at room temperature for 7 h, a solution of $NaCNBH_3$ (0.237 g, 3.78 mmol) in water (2 mL) was added, and the resulting mixture was stirred overnight. The polymer was then precipitated by addition of 0.5 M aqueous NaOH and EtOH (75 mL). The precipitate was successively washed with 6:4, 7:3, 9:1 water−EtOH and then EtOH and dried to give adamantane-grafted chitosan **9** (0.28 g, 88%).

^{1}H NMR (D_2O, 500 MHz), δ/ppm: 4.96−4.65 (m, H-1 of N-deacetylated and N-alkylated glucosamine units), 4.46 (m, H-1 of N-acetylated glucosamine units), 4−3.35 (m, H-2 of N-acetylated glucosamine units, H-3,H-4, H-5, H-6,6′), 3.07 (m, H-2 of N-acetylated glucosamine units), 1.93, 1.81 and 1.65−1.33 (s and 2 m, H adamantane).

Results and Discussion

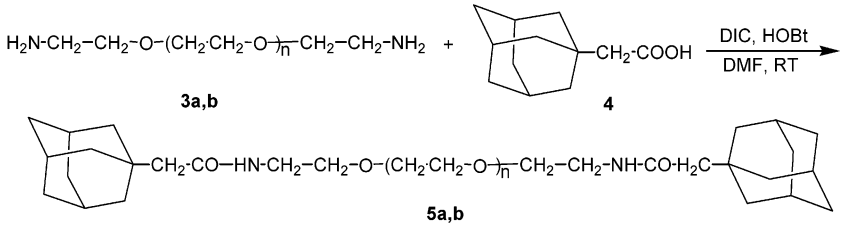
1. Synthesis and Characterization of the Host and Guest Molecules. (a) Host. CD−chitosan **2** was

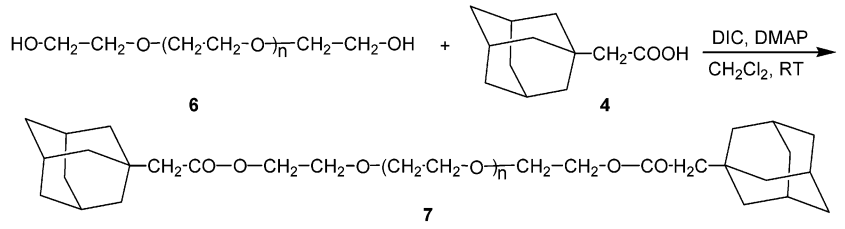


synthesized by the use of a reductive amination reaction performed in homogeneous solution between chitosan **1** with a low degree of acetylation DA (DA = 0.12) and a monosubstituted β-cyclodextrin derivative possessing a reducing sugar on the primary face.[12] The average degree of substitution (DS) of this chitosan derivative having pendant CD cavities regularly distributed along the chain was found to be 0.10 (i.e., on average one CD every 10 glucosamine units) by ^{1}H NMR analysis. It was demonstrated by NMR spectroscopy that the grafted β-CDs retain their abilities to form inclusion complexes with small hydrophobic guest molecules.

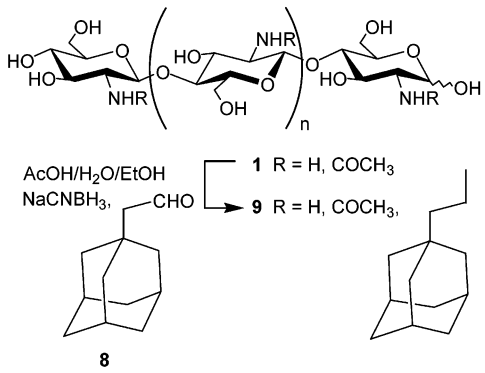
(b) Guests. A wide variety of guest molecules can be accommodated in cyclodextrins. The association constants K_a range from 10 M^{-1} to 3×10^4 M^{-1} and are critically dependent on the size-matching between guest and host.[17] In the case of β-CD, deep and snug-fitting complexes are formed with adamantyl derivatives leading to very high association constants ($K_a \sim (1-3) \times 10^4$ M^{-1}). Adamantyl groups were thus introduced on polymer backbones in order to obtain guest macromol-

Scheme 1. Synthetic Routes to PEG−Diadamantane Guests





Scheme 2. Synthesis of Adamantane-Grafted Chitosan



ecules exhibiting a high affinity for cyclodextrin-grafted chitosan. We chose end-capped poly(ethylene glycol) and randomly grafted chitosan as guest macromolecules to interact with CD−chitosan as different rheological properties depending on the structural features of the guest polymers were expected. The synthetic routes to the targeted guests are indicated in Schemes 1 and 2.

Adamantyl groups were introduced on PEGs with molecular weights ranging from 3400 to 20000 either through amide bond formation or ester formation. 1-Adamantaneacetic acid (**4**) was reacted with diaminopoly(ethylene glycol) 3400 (**3a**) and with diaminopoly(ethylene glycol) 20000 (**3b**) under standard peptide coupling conditions (*N*,*N*′-diisopropylcarbodiimide (DIC), hydroxybenzotriazole (HOBt)) to yield PEG−diadamantane 3400 (**5a**) and PEG−diadamantane 20000 (**5b**), respectively. The quantitative coupling of adamantane derivative **4** with the amine functions of **3a** and **3b** was clearly demonstrated by ^{1}H NMR. However, an important feature for **5a** and **5b** concerns the adamantane content per chain which straightforwardly results from the amine content in starting compound **3a** and **3b**. The average number of amines per polyether chain in **3a** was derived from the number-average molecular weight M_n estimated by electrospray ionization mass spectrometry and the number of chain-ends per gram of polymer obtained by ^{1}H NMR titration using an external reference (sodium 4-*tert*-butylbenzoate). The average number of amines per chain was found to be 1.8 instead of the theoritical 2 value indicating that commercial diaminopoly(ethylene glycol) **3a** and product **5a** likely contain a few monosubstituted chains. It was assumed that for commercial PEG derivative **3b** and **5b**, the functionality was in the same order.

Table 1. Characteristics of CD−Chitosan and Initial Chitosan in Dilute Solutions

compound	$[\eta]$ (mL/g)	k'	C^* (g/L)
chitosan **1**	1100 ± 150	0.34	0.9 ± 0.2
CD−chitosan **2**	860 ± 130	0.60	1.16 ± 0.2

Reaction of poly(ethylene glycol) 6000 (**6**) with 1-adamantaneacetic acid (**4**) in dichloromethane in the presence of DIC and 4-(dimethylamino)pyridine (DMAP) afforded compound **7**. At end, it was shown that no hydrolysis can be detected by ^{1}H NMR after 72 h at room temperature in 0.3 M CH_3COOH /0.03 M CH_3COONa which indicates the hydrolytic stability of the ester bonds in **7**.

Adamantane-grafted chitosan **9** was prepared by a reductive amination reaction between chitosan **1** and 1-adamantaneacetaldehyde (**8**). Compound **8** was readily produced by oxidation of the primary hydroxyl group of 1-adamantaneethanol with pyridinium dichromate (PDC). ^{1}H NMR analysis performed in D_2O/DCl (pD 4) demonstrated that **9** was free of any byproduct. Digital integration of the NMR signals arising from the anomeric protons of chitosan and the protons of adamantane gave a substitution degree of approximately 0.07 (i.e., seven adamantyl groups per every hundred glucosamine units).

2. Solution Properties of Host and Guest Macromolecules Alone. (a) CD—Chitosan. The reduced viscosity of CD−chitosan **2** (DS = 0.10) was determined as a function of concentration in 0.3 M CH_3COOH /0.03 M CH_3COONa and compared with that of initial chitosan. From the dependence of the reduced viscosity on concentration, the intrinsic viscosity $[\eta]$ and the Huggins constant k' could be derived using the Huggins eq 2. The results are summarized in Table 1. Viscosities in the Newtonian plateau and their dependence on shear rate were determined on the two polymers at different concentrations; a few experimental results are shown in Figure 1. In the 1−5 g/L concentration range, reduction in viscosity with increasing shear rate is

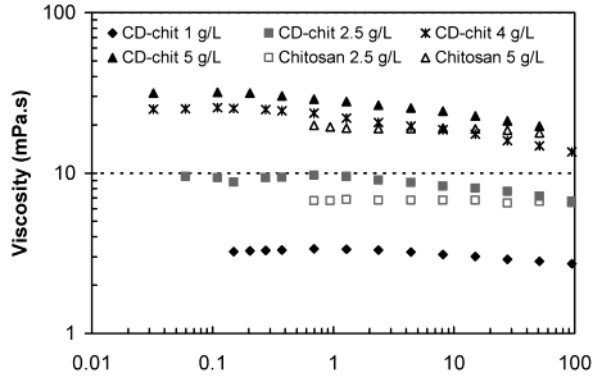


Figure 1. Viscosity dependence on shear rate for chitosan and CD−chitosan solutions (0.3 M CH_3COOH/0.03 M CH_3COONa, 25 °C) in the range of concentration from 1 to 5 g/L.

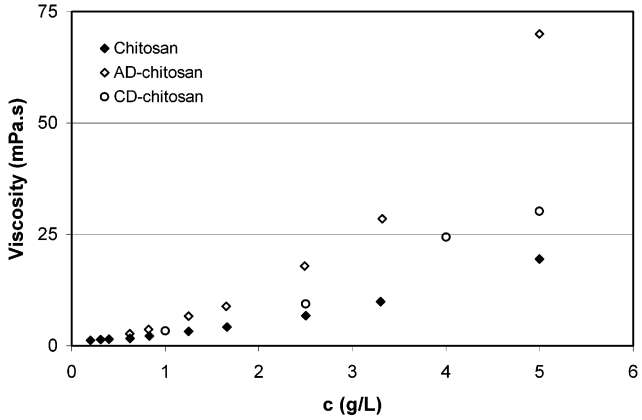


Figure 2. Variation of the viscosity of solutions of adamantane−chitosan, cyclodextrin−chitosan and unmodified chitosan with the concentration in 0.3 M CH_3COOH /0.03 M CH_3COONa at 25 °C.

relatively minor for initial chitosan whereas shear-thinning is much larger for solutions of chitosan bearing pendant CDs. This may arise from interchain interactions promoted by enhanced hydrogen bonding between pendant cyclodextrins and evidenced by a larger value of k'. Moreover, at a given concentration, the viscosity of CD−chitosan is larger than that of initial chitosan which confirms the presence of additional interchain interactions induced by the grafted cyclodextrin cavities as shown by Figure 2.

One of the crucial parameters for interchain interaction is the critical concentration C^* at which polymer molecules in solution start to overlap, favoring contacts. The values of C^* for CD−chitosan and initial chitosan were deduced from the intrinsic viscosity assuming that $C^*[\eta]$ is about unity (see Table 1).[18]

(b) PEG—Diadamantane and Adamantane-Grafted Chitosan. Guest macromolecules, i.e., adamantane-terminated poly(ethylene glycol)s and adamantane-grafted chitosan, were shown to be soluble in the solvent used for CD−chitosan (0.3 M CH_3COOH /0.03 M CH_3COONa). However, despite the bulky structure of adamantyl groups which should minimize inter and/or intramolecular hydrophobic interactions, it was important to check if self-associating behaviors were promoted by these hydrophobic moieties as it is usually the case for alkyl groups.[19,20]

The aggregation behavior of PEG−diadamantane **5a** was first examined using the peak III/I ratio of pyrene monomer fluorescence which is a measure of the hydrophobicity of pyrene's environment.[15] The III/I ratio

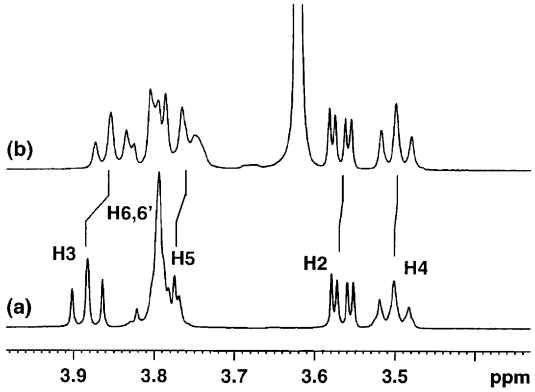


Figure 3. Partial ^1H NMR spectra (400 MHz, 25 °C, D_2O) of (a) β-CD (2 mM) and (b) a mixture of β-CD (1.5 mM) and PEG−diadamantane **5a** ([Ad] = 0.5 mM).

of 0.60 at a PEG−diadamantane concentration in water of 0.1 g/L was very similar to that observed in pure aqueous solution. Increasing the PEG−diadamantane concentration up to 20 g/L only increased the III/I ratio to 0.64 which does not support self-association of **5a**. This was further confirmed by dynamic light scattering (DLS) experiments performed on aqueous solutions of **5a** in the range of concentration from 0.1 to 5 g/L. Whatever the concentration, the hydrodynamic radius of **5a** was found to be very small (≈1 nm) indicating that no micellar aggregates are formed from **5a**. Since strong interactions between adamantyl moieties appear to be hindered by steric hindrance effects, other PEG−diadamantane derivatives **5b** and **7** were assumed to have a behavior similar to that of **5a**.

Figure 2 shows the variation of the viscosity of adamantane−chitosan, cyclodextrin−chitosan and initial chitosan solutions as a function of the concentration in 0.3 M CH_3COOH /0.03 M CH_3COONa. From these curves, it appears that some interchain interactions are promoted by the hydrophobic adamantyl moieties. These interactions are stronger than those between CD−chitosan chains.

3. Binding Properties of Guest Macromolecules with Natural β-Cyclodextrin. The formation of inclusion complexes between free β-cyclodextrin and adamantyl moieties in **5a** and **9** was first examined using ^1H NMR in order to show evidence of specific interactions between the CD cavity and grafted adamantyl moieties. Figure 3 shows the variation of the proton NMR spectra of β-CD upon addition of **5a**. Large variations of the chemical shifts of both H-3 and H-5 protons are observed. This indicates the formation of an inclusion complex since these protons are located in the hydrophobic cavity of CD and are hence expected to be the most prone to variations of chemical shifts if an inclusion complex is formed.[21] A modification of the signals of the H-6 protons can also be observed. More direct evidence for the formation of inclusion complexes can be derived from the observation of dipolar interactions between protons of the adamantyl groups and cyclodextrin. This can be achieved by 2D T-ROESY experiments[22] dedicated to evidence nuclear Overhauser effects. The corresponding contour plot is displayed in Figure 4. The presence of strong cross-peaks between the protons of the adamantyl moiety and the H-3, H-5 and H-6 protons of cyclodextrin fully supports the formation of inclusion complexes. A more detailed analysis of the binding properties was performed by measuring the apparent association constant K_a. How-

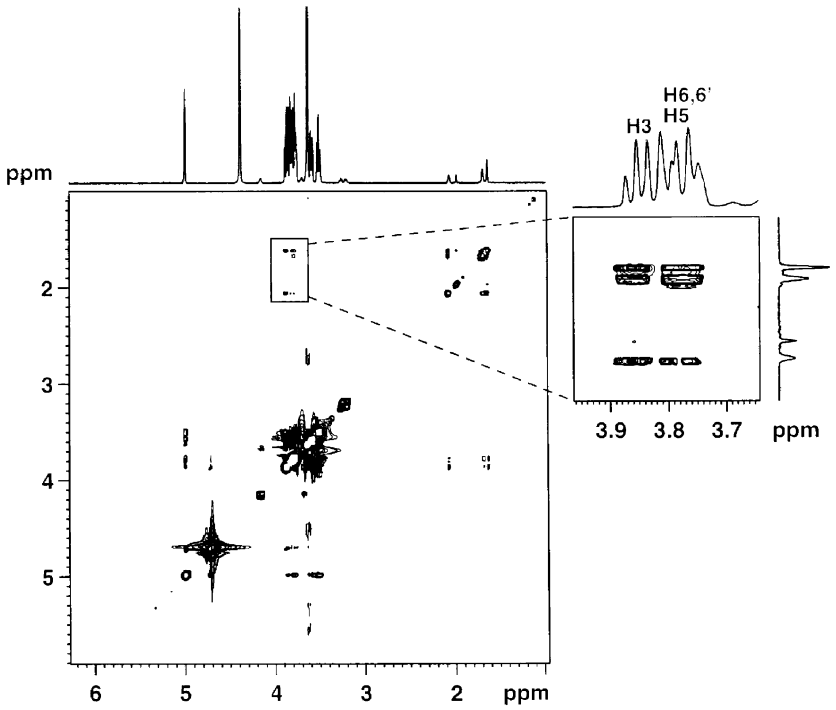


Figure 4. T-ROESY experiment (500 MHz, 25 °C, D_2O, 350 ms spin-lock time at 16 dB attenuation) performed on a sample containing β-CD (1.5 mM) and PEG−diadamantane **5a** ([Ad] = 0.5 mM).

Table 2. Thermodynamic Parameters for Inclusion Complex Formation of PEG−Diadamantane with β-CD

T (°C)	K_a (M^{-1})	$\Delta G°$ (kJ/mol)	$\Delta H°$ (kJ/mol)	$T\Delta S°$ (kJ/mol)
25	$(1.8 \pm 0.09) \times 10^4$	-24.3 ± 0.1	-25.5 ± 0.5	1.1 ± 1
35	$(1.37 \pm 0.07) \times 10^4$	-24.4 ± 0.1		
45	$(9.98 \pm 0.5) \times 10^3$	-24.3 ± 0.1		
55	$(7 \pm 0.4) \times 10^3$	-24.1 ± 0.1		

ever, any precise determination of the association constant implies that the stoichiometry of the interaction process is unambiguously determined. Both the stoichiometry and the binding constant could be derived by observing the changes in chemical shifts of the H-3 CD protons. Determination of the stoichiometry of the complex was performed using the continuous variation method. In this procedure, the total concentration of β-CD and adamantane is kept constant, the molar ratio r of each component being varied from 0 to 1. The concentration of adamantane was determined by ^{1}H NMR integration using an external reference (sodium 4-*tert*-butylbenzoate). A total concentration (2 mM) was used. The stoichiometry of the complex was found to be 1:1 which implies that the inclusion complex can be considered as a first-order system. The average value for K_a derived from a numerical simulation of the experimental data[13] was found to be $(1.8 \pm 0.09) \times 10^4$ M^{-1} at 25 °C. This K_a value is similar to that found for inclusion of 1-adamantanecarboxylate in β-CD (pH 8.5) using microcalorimetric titration.[23] Moreover the values for K_a at 35, 45, and 55 °C were also determined in order to estimate the thermodynamic parameters for inclusion complexation (Table 2). A large negative ΔH and a near zero ΔS are found for the complex suggesting that inclusion of the adamantyl moiety in β-CD is an enthalpy-driven process and that K_a decreases when the temperature increases. These data thus provide evidence of specific interactions between grafted adamantane groups and β-CD with no influence of the polyether chain in the inclusion process. This implies that other

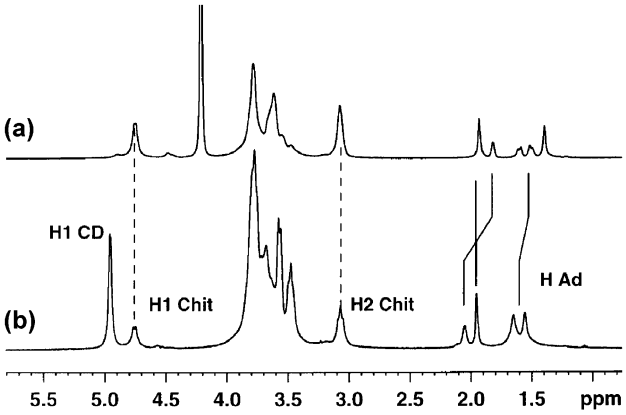


Figure 5. Partial ^{1}H NMR spectra (400 MHz, 25 °C, D_2O+DCl, pD 4) of (a) adamantane−chitosan (6 mg mL^{-1}) and (b) a mixture of adamantane−chitosan (6 mg mL^{-1}) and free β-CD (2 mM).

PEG−diadamantane derivatives **5b** and **7** should present the same binding properties.

When AD−chitosan **9** is considered in the presence of free β-CD, ^{1}H NMR spectra clearly indicate a specific interaction with the grafted adamantane groups, not prevented by loose auto-association of the amphiphilic polymer (see Figure 5). Moreover, 2D TOCSY experiments,[24] based upon stepwise magnetization transfers from anomeric protons, allowed the observation of the large shifts experienced by the H-3 and H-5 cavity protons of cyclodextrin which are hidden in the 1D ^{1}H NMR spectrum by the proton signals of AD−chitosan (see Figure 6). This confirmed the selective interaction between the pendant hydrophobic adamantyl groups and β-cyclodextrin.

4. Rheological Behavior of CD—Chitosan in the Presence of PEG—Diadamantane Samples or Adamantane-Grafted Chitosan. (a) CD—Chitosan/PEG—Diadamantane Mixtures. Bridging of CD−chitosan chains through complex formation with PEG−

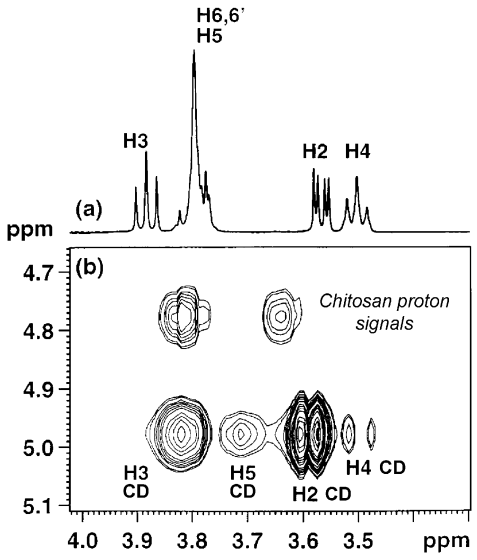


Figure 6. Partial ^{1}H NMR spectrum (400 MHz, 25 °C, D_2O+DCl, pD 4) of β-CD (2 mM) alone (a) and partial contour plot of a 2D TOCSY experiment (400 MHz, 25 °C, D_2O + DCl, pD 4, 120 ms mixing time) performed on β-CD in the presence of adamantane−chitosan (6 mg mL^{-1}) (b). Comparison of the chemical shifts of the H-3 and H-5 protons of β-CD alone and in the presence of adamantane−chitosan shows significant shifts proving inclusion of adamantane in the CD cavity.

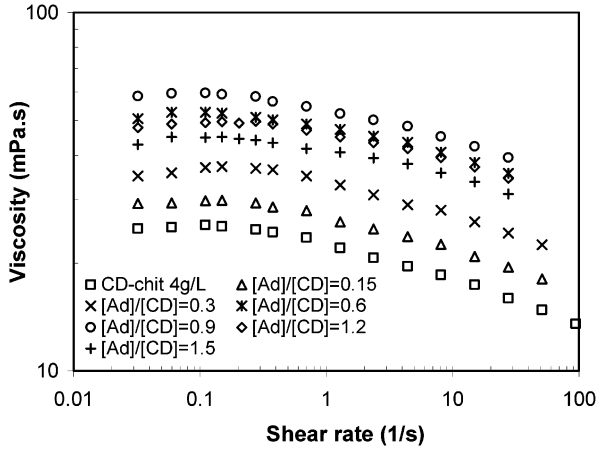


Figure 7. Viscosity dependence on shear rate for solutions of CD−chitosan (C = 4 g/L, 0.3 M CH_3COOH /0.03 M CH_3COONa, 25 °C) in the absence and in the presence of increasing PEG−diadamantane **5a**.

diadamantane 3400 (**5a**) was first examined in the range of CD−chitosan concentration from 1 to 30 g/L. Figure 7 shows the steady shear viscosities of solutions of CD−chitosan (C = 4 g/L) alone and in the presence of increasing PEG−diadamantane content. From these flow curves, the zero shear viscosity was extracted and plotted as a function of the [Ad]/[CD] ratio, the cyclodextrin concentration being kept constant (Figure 8). Upon addition of **5**, solution viscosities are observed to increase up to an optimum adamantane concentration (0.9/1 ratio). Above this concentration, solution viscosities decrease. Viscosity enhancements can be attributed to cross-linking of CD−chitosan chains through complexation of PEG−diadamantane with grafted CDs. However, when more PEG−diadamantane is added, the probability of effective interchain cross-links decreases as a result of increasing PEG−diadamantane monocomplexation, which leads to a decrease in viscosity. Moreover viscosity enhancements are only clearly ob-

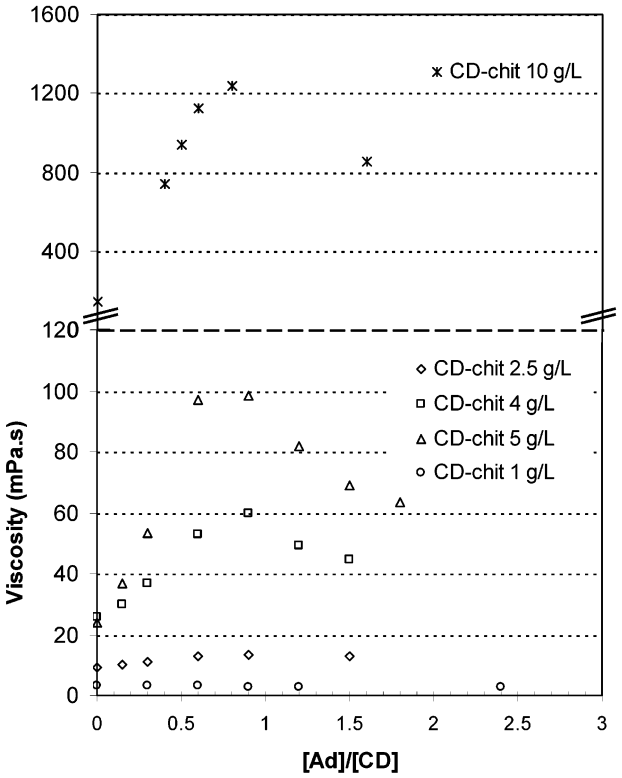


Figure 8. Variation of the viscosity of solutions of CD−chitosan in the range of concentration from 1 to 10 g/L (0.3 M CH_3COOH /0.03 M CH_3COONa, 25 °C) with the [Ad]/[CD] ratio (i.e., with increasing PEG−diadamantane **5a** concentration).

served for CD−chitosan concentrations higher than 1 g/L which means that interchain bridging can only be effective in the range of the overlap concentration C^*. Above this concentration, the thickening effect increases largely when the PEG−diadamantane and CD−chitosan concentrations increase. It can be noticed that similar trends can be observed when hydrophobically modified water-soluble polymers are mixed with surfactants or amylose.[25−27] The viscosity of such mixtures goes through a maximum with increasing concentration of surfactant or amylose for a given polymer concentration. This behavior has been attributed to the formation of mixed micelles of surfactants and polymer hydrophobic groups or to the formation of helical inclusion complexes between amylose and polymer hydrophobic side chains, respectively, leading in both cases to the cross-linking of the polymer chains.

We further investigated the influence of the polyether chain length on the thickening properties. Figure 9 shows the flow curves of a CD−chitosan solution at a concentration of 5 g/L in the presence of PEG−diadamantane 3400, 6000, and 20000 respectively, at a fixed [Ad]/[CD] ratio ([Ad]/[CD] = 0.9). From these data, it is observed that gain in viscosity increases with the molecular mass of PEG−diamantane, i.e., the length of the polyether chain separating the adamantyl groups. It must be mentioned that the contribution of the PEG derivatives to the viscosity is negligible. So, it can be concluded that the increase in the polymer chain length results in an increase in the effective connectivity of the system favoring network formation.

Dynamic rheological measurements were performed on the 30 g/L solution of CD−chitosan in the absence and in the presence of PEG−diadamantane 3400 ([Ad]/

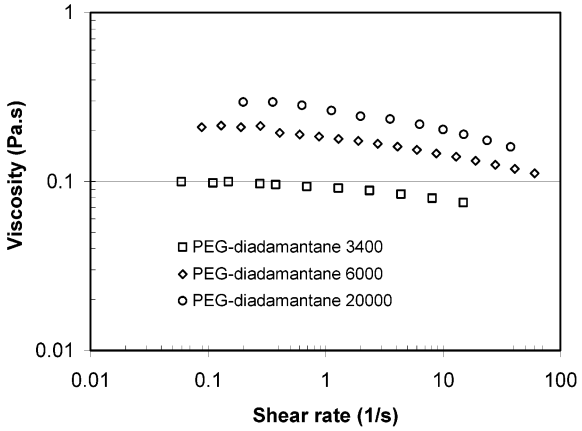


Figure 9. Viscosity dependence on shear rate for solutions of CD−chitosan (C = 4 g/L, 0.3 M CH_3COOH /0.03 M CH_3COONa, 25 °C) in the presence of PEG−diadamantane guests **5a**, **5b**, and **7** ([Ad]/[CD] = 0.9).

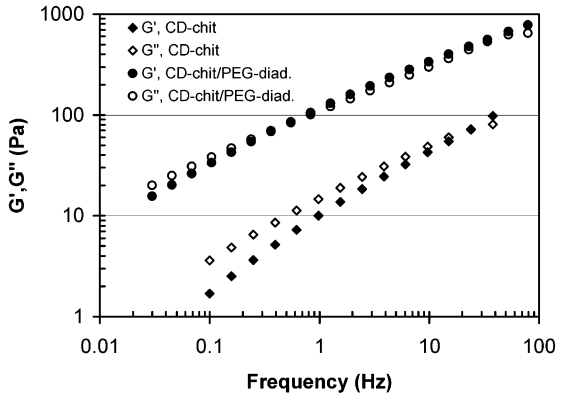


Figure 10. Storage and loss moduli dependence on frequency for a solution of CD−chitosan (C = 30 g/L, 0.3 M CH_3COOH /0.03 M CH_3COONa, 10 °C) in the absence and in the presence of PEG−diadamantane **5a** ([Ad]/[CD] = 0.7).

[CD] = 0.7). The dynamic storage modulus ($G'(\omega)$) and the loss modulus ($G''(\omega)$) are shown as a function of the frequency (ω) in Figure 10. Upon addition of PEG−diadamantane, the G' and G'' moduli increase as a result of interchain bridging through cyclodextrin−adamantane complexation. Moreover G' and G'' cross at a frequency ω_p lower than that observed for CD−chitosan alone. However, no gelification is observed which is in agreement with the flow experiments. Addition of PEG−diadamantane to CD−chitosan solution leads to high viscosity enhancements but not to a stable gel formation. The absence of network structure might be related to the fast exchange regime of the CD/PEG−diadamantane complexes. The fast exchange between the free and bound states of the included adamantyl moieties of PEG−diadamantane guest cannot maintain long-range connectivity.

(b) CD—Chitosan/Adamantane—Chitosan Mixtures. A very different rheological behavior is observed when adamantane−chitosan guest is added to CD−chitosan. Figure 11 compares the dynamic rheological moduli of CD−chitosan/adamantane−chitosan mixtures at different [Ad]/[CD] ratios, the total concentration of CD−chitosan and adamantane−chitosan being approximately 4 g/L. The interaction of adamantane-grafted chitosan with CD−chitosan appears to be much larger if one compares the values of G' at 1 Hz for the CD−chitosan/PEG−diadamantane **5a** mixture ([CD−chit] = 30 g/L, $G' \sim 100$ Pa) and the CD−chitosan/adamantane−chitosan mixture ([CD−chit] ∼ 2 g/L, $G' \sim 75$ Pa with [Ad]/[CD] = 1.2). Moreover G' is larger than G'' within a very large range of frequency, reflecting a gellike behavior for the CD−chitosan/adamantane−chitosan mixture. However G' and G'' are not independent of the applied frequency; they decrease with decreasing frequency, indicating that the network relaxes as a result of the breaking and re-forming of the cross-links (i.e., the CD−adamantane complexes); this system reflects the behavior of the reversible networks described by Leibler et al.[28] Finally, although the stoichiometry of the CD−adamantane complex is the same for both mixtures, the [Ad]/[CD] ratio for which interchain bridging is maximum seems to be higher for the CD−chitosan/adamantane−chitosan system ([Ad]/[CD] ∼ 1.2 whereas [Ad]/[CD] ∼ 0.9 for PEG-based systems).

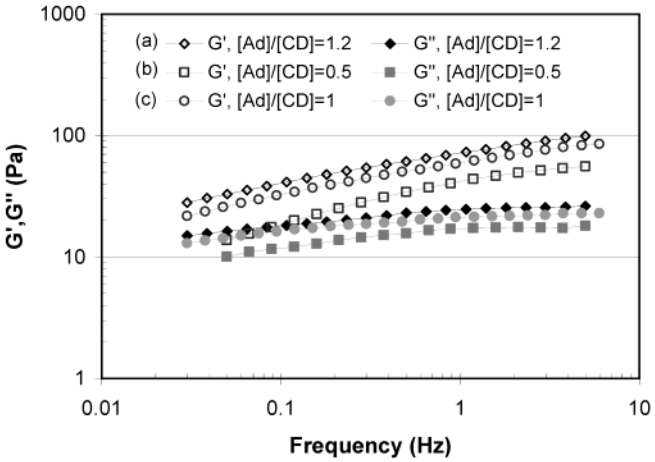


Figure 11. Storage and loss moduli dependence on frequency for CD−chitosan/AD−chitosan mixtures (0.3 M CH_3COOH /0.03 M CH_3COONa, 25 °C): (a) [CD−chit] = 1.94 g/L, [AD−chit] = 2 g/L; (b) [CD−chit] = 3.03 g/L, [AD−chit] = 1.29 g/L; (c) [CD−chit] = 2.17 g/L, [AD−chit] = 1.85 g/L.

In conclusion, new supramolecular assemblies were prepared in aqueous solution by complex formation between CDs grafted on chitosan and adamantyl groups attached to chitosan or poly(ethylene glycol). Very different rheological behaviors depending on the guest macromolecule were evidenced. High viscosity enhancements were observed upon addition of PEG end-capped with adamantyl moieties to CD−chitosan solutions whereas network structures were obtained upon addition of multiadamantane-grafted chitosan. More experiments are in progress to analyze the mechanism of formation of these chitosan based complexes.

Acknowledgment. We gratefully acknowledge P. Guenot and P. Jéhan for the mass spectrometry measurements. We thank S. Désert for the light scattering measurements.

References and Notes

(1) (a) Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 803. (b) Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743.
(2) Nepogodiev, S. A.; Stoddart, J. F. *Chem. Rev.* **1998**, *98*, 1959.
(3) Ikeda, T.; Ooya, T.; Yui, N. *Polym. J.* **1999**, *31*, 658.
(4) Fujita, H.; Ooya, T.; Yui, N. *Macromolecules* **1999**, *32*, 2534.
(5) Harada, A.; Adachi, H.; Kawaguchi, Y.; Kamachi, M. *Macromolecules* **1997**, *30*, 5181.
(6) Zhang, H.; Hogen-Esch, T. E.; Boschet, F.; Margaillan, A. *Langmuir* **1998**, *14*, 4972.
(7) Islam, M. F.; Jenkins, R. D.; Bassett, D. R.; Lau, W.; Ou-Yang, H. D. *Macromolecules* **2000**, *33*, 2480.

(8) Akiyoshi, K.; Ueminami, A.; Kurumada, S.; Nomura, Y. *Macromolecules* **2000**, *33*, 6752.
(9) Huh, K. M.; Ooya, T.; Lee, W. K.; Sasaki, S.; Kwon, I. C.; Jeong, S. Y.; Yui, N. *Macromolecules* **2001**, *34*, 8657.
(10) (a) Amiel, C.; Sebille, B. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1996**, *25*, 61. (b) Sandier, A.; Brown, W.; Mays, H.; Amiel, C. *Langmuir* **2000**, *16*, 1634.
(11) Weickenmeier, M.; Wenz, G.; Huff, *J. Macromol. Rapid Commun.* **1997**, *18*, 1117.
(12) Auzély-Velty, R.; Rinaudo, M. *Macromolecules* **2001**, *34*, 3574.
(13) Djedaïni, F. Ph.D. Dissertation, University of Paris XI, France, **1991.**
(14) Nedler, M. *Einführung in die Optimierung*, Verlag Chemie, Weinheim, Germany, 1971.
(15) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039.
(16) Huggins, M. L. *J. Am. Chem. Soc.* **1942**, *64*, 2716.
(17) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875.
(18) Macosko, C. W. G. In *Rheology: Principles, Measurements and Applications*; Macosko, C. W., Ed.; VCH Publishers: Weinheim, Germany, 1994.
(19) Alami, E.; Almgren, M.; Brown, W.; François, J. *Macromolecules* **1996**, *29*, 2229.
(20) Desbrières, J.; Rinaudo, M.; Chtcheglova, L. *Macromol. Symp.* **1997**, *113*, 135.
(21) Djedaïni, F.; Perly, B. *J. Mol. Struct.* **1990**, *239*, 161.
(22) Hwang, T. L.; Shaka, A. J. *J. Magn. Reson., Ser. B* **1993**, *102*, 155.
(23) Eftink, M. R.; Andy, M. L.; Bystrom, K.; Perlmutter, H. D.; Kristol, D. S. *J. Am. Chem. Soc.* **1989**, *111*, 6765.
(24) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355.
(25) Jiménez-Regalado, E.; Selb, J.; Candau, F. *Langmuir* **2000**, *16*, 8611.
(26) Gruber, J. V.; Konish, P. N. *Macromolecules* **1997**, *30*, 5361.
(27) Chronakis, I. S.; Egermayer, M.; Piculell, L. *Macromolecules* **2002**, *35*, 4113.
(28) Leibler, L.; Rubinstein, M.; Colby, R. H. *Macromolecules* **1991**, *24*, 4701.

MA020664O