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^{1}H NMR relaxation study of a chitosan–cyclodextrin network

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Abstract

The results of measurements of longitudinal and transverse proton relaxation times for a chemical network obtained by reacting chitosan with oxidized β-cyclodextrin (β-cyclodextrin polyaldehyde) are presented. The network was characterized by a 'two-component' transverse relaxation mechanism relative to structurally different environments experienced by water molecules. Different environments were also indicated by the temperature of the spin–spin relaxation times (T_2) studied in the range 4–50 °C. Between 4 and 18 °C, proton exchange between the matrix and water prevails on the inter- and intra-molecular dipolar interactions of the water confined in the meshes of the network, resulting in a marked change in the slope of T_2 with temperature. Stiffness of the matrix and reduced mobility of water in the gel meshes are prerequisites for observing such relaxation phenomena. Possible mechanisms contributing to the activation energy in the case of chitosan–cyclodextrin networks are discussed. The behaviour of the chitosan–cyclodextrin hydrogel is compared with that of a gellan gel. © 1997 Elsevier Science Ltd.

Keywords: Hydrogels; Chitosan; β-Cyclodextrin; Pulsed low-resolution ^{1}H NMR spectroscopy; Proton exchange

1. Introduction

The attempt to formulate new biocompatible and biodegradable hydrogels for a wide variety of purposes has produced a number of studies in the last decade. In particular, polymer networks with a high affinity for water as suitable materials in the pharmaceutical, medical, and cosmetics fields have been investigated. A number of polysaccharides are principal components of these polymer networks because of their hydrophilicity, availability in nature, and biocompatibility. In addition, carbohydrate polymer hydrogels (physical gels) can form spontaneously in dilute aqueous media as a consequence of disorder-to-order transitions, gellan gum being a well-known example in this context. To understand how water interacts with the network chains is a very demanding task, because many of the conventional techniques used in polymer science do not give information about the gel state.

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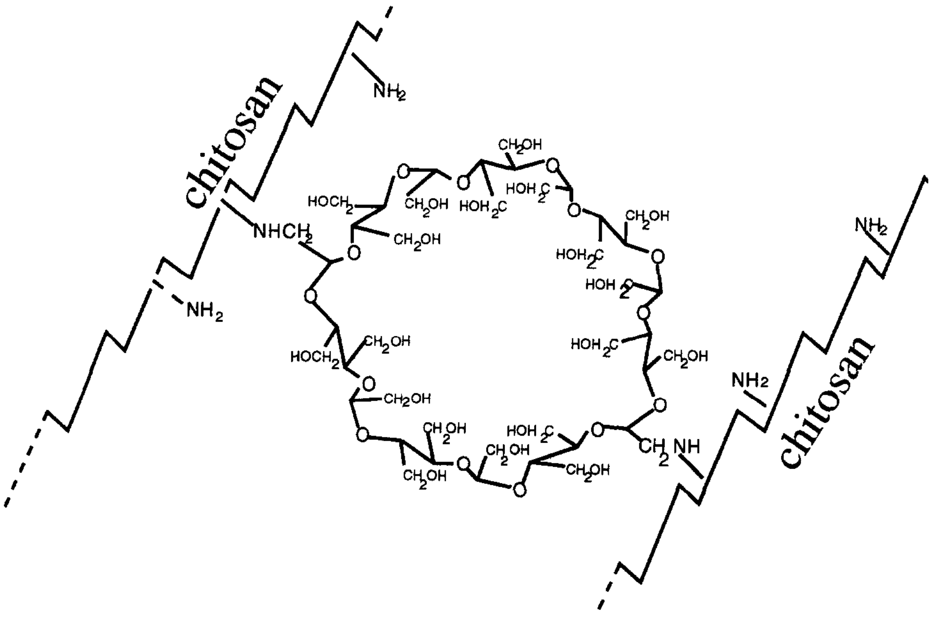

Scheme 1.

Proton NMR relaxometry studies on hydrogels [1] as well as on several dietary fibers [2] have been reported in recent literature. We have recently applied NMR relaxometry to some new polysaccharide-based networks [3,4] and shown that a study of the properties of water involved in the gel matrix can afford comparative information on some relevant system properties such as water–network interactions. We present here the results of an NMR study of the relaxation behaviour of water molecules inside a chemical network based on chitosan and oxidized β-cyclodextrin (Scheme 1). For comparison, similar data concerning a gellan aqueous physical gel are briefly outlined.

2. Experimental

Materials.—β-Cyclodextrin from Sigma (USA) Lot #51H3521 was used without further purification. Oxidized β-cyclodextrin was obtained by reacting the seven C-2–C-3 vicinal diols present in the molecule with an excess of $NaIO_4$ (Carlo Erba, Italy). The reaction was carried out in aq medium in the dark at 4 °C for 24 h. Unreacted periodate as well as iodate formed during the reaction were precipitated with $BaCl_2$. After filtration, oxidized β-cyclodextrin was recovered as a freeze-dried powder and used within a few days. Chitosan from Sigma (USA) (degree of deacetylation: 0.66) was used without further purification. The degree of deacetylation was determined by CP/MAS solid-state NMR according to the method described by Raymond et al. [5]. Determination of the molecular weight of chitosan was made by viscosity measurements at 25 °C using as solvent 0.3 M CH_3COOH/0.2 M NaOAc (pH 4.5). In these conditions the molecular weight was equal to 2.4×10^5 (Mark–Houwink parameters: $k = 0.07$, $a = 0.77$ [6]).

The preparation of the chitosan-oxidized β-cyclodextrin hydrogel network consisted of the reductive alkylation of chitosan by the procedure outlined in the literature by Yalpani and Hall [7]. Oxidized β-cyclodextrin (polyaldehyde cross-linking agent) was added under stirring to aq chitosan (3% w/v) in 2% v/v CH_3COOH (pH = 5.0, 25 °C) in a molar ratio of cross-linking agent–amine groups equal to 0.1. A wall-to-wall gel (formation of Schiff bases) was obtained in a few hours. The gel, left overnight without stirring, was transferred to aq CH_3COOH (2% v/v, pH 4) in the presence of excess $NaBH_3CN$. The resulting gel, extensively washed against distilled water for two weeks, was finally freeze-dried and stored in this form.

All measurements were carried out on the same gel preparation for which the stoichiometric molar ratio is assumed to be representative of the actual degree of cross-linking.

The gellan sample (Kelco–Merck trade name "Gelrite") was a kind gift of Prof. D.A. Brant. It was purified as previously described [8]. Gellan gel was prepared starting with a 10% (w/v) dispersion of the polysaccharide in water and raising the temperature to 100 °C. After stirring for 1 h, concd NaCl was added to the mixture in order to obtain a final 0.2 M NaCl. Two annealing processes at 100 °C were performed in order to have a well-structured, macroscopically homogeneous gel.

Methods.—^{13}C NMR spectra were obtained in the solid state by a CP/MAS NMR double bearing probe on a Bruker AM-400 (Germany) spectrometer operating at 100.63 MHz. The rotor was spun at 3800–4600 Hz and a contact time of 1–2 ms was applied to obtain polarization transfer. Chemical shifts were referred to TMS. T_1 and T_2 relaxation time measurements were carried out with a Bruker WP80, operating at 80 MHz. Longitudinal and transverse relaxation times were calculated from inversion recovery and CPMG pulse experiments, respectively. The CPMG method was also employed for studying the temperature dependence of T_2. Transverse proton relaxation times at different temperatures were determined by a CPMG pulse sequence using a pulse separation, τ, ranging from 0.1 to 0.5 ms for the chitosan–cyclodextrin chemical gel and from 1 to 9 ms for gellan physical gel. For these measurements, a water-to-network weight ratio, R, of 3 and 9 for chitosan–cyclodextrin and gellan was employed, respectively. Temperature control was within ± 1 °C.

For the NMR measurements as a function of swelling, weighted amounts of the samples were swollen in known volumes of water. The water contents in the hydrogels is given here as the ratio, R, of grams of water per gram of network. In this study, R ranged from 0.1 to 30, depending on the system under study. All samples were equilibrated for two days before NMR measurements. MilliQ water (Millipore) with a conductivity lower than 0.05 μS at 25 °C was used for the preparation of the samples.

In order to derive the relevant parameters for the characterization of the state of water molecules in hydrogels with different R, a starting hypothesis, which took into account the characteristics of the instrument used, had to be formulated. We have limited the maximum number of components contributing to the relaxation process to two since the time windows explored were always larger than 1 ms and spin–spin relaxations relative to non-exchangeable protons of the matrix are typically of the order of 0.1 ms or less. Inversion recovery experiments for the evaluation of T_1 gave a monoexponential relaxation for all the systems examined. For T_1 and for T_2 measurements we used as fitting equations the following relationships, respectively:

$$M_z = M_0\left[1 - 2\exp\left(-\frac{t}{T_1}\right)\right] \tag{1}$$

and

$$I_{\mathrm{echo}} = \alpha_{2,\mathrm{short}} \exp\left(-\frac{t}{T_{2,\mathrm{short}}}\right) + \alpha_{2,\mathrm{long}} \exp\left(-\frac{t}{T_{2,\mathrm{long}}}\right) + \mathrm{offset} \tag{2}$$

where M_0 represents the magnetization at time $t = 0$, $\alpha_{2,\mathrm{short}}$ and $\alpha_{2,\mathrm{long}}$ are the respective fractional amplitudes of the fast and slow components of the transverse relaxation, and 'offset' is a constant accounting for instrumental offset. In order to avoid any contribution to the resonances due to the protons of the matrix, in all the experiments pulse separations at least larger than one order of magnitude with respect to the relaxation times of the non-exchangeable protons of the saccharidic moiety were used. Transverse relaxation time measurements as a function of temperature were made on heating. After heating, few measurements on cooling were performed. Both hydrogels show large hysteresis, but a systematic investigation of this effect was beyond the scope of this work.

T_1, short and long T_2 relaxation times along with their relative intensities were determined by a non-linear least-square fitting procedure based on the Marquardt algorithm. The relative error for the relaxation times was always less than 5% and the relative error on the fractional intensities of the transverse relaxation behaviour was less than 25%. Variations of the fractional intensities of transverse relaxations as a function of the temperature were within the given errors.

3. Results and discussion

Several factors are important in changing the dynamic properties of water confined inside hydrogels: hydrophobicity of the matrix; flexibility of the chains; degree of cross-linking defining the mesh size of the networks; charge density on the chains. In the model proposed by Watanabe et al. [1], the state of water in the networks can be described in terms of at least two average water ensembles which relax with different time constants. Motionally different water molecules are indicative of different domains in the network. In the case of two distinct relaxation processes, a domain will comprise a set of water molecules more tightly bound in those parts of the gel in which network chains overlap with each other for physical entanglements or by chemical cross-linking. The second domain will be related to water molecules confined, in their diffusive motion, in the meshes of the gels. If the two ensembles are not coupled, or if their time constants differ by orders of magnitude, their relaxing modes are well separated and a bimodal or biexponential decay of the longitudinal magnetization is observed.

This picture, although quite schematic, allows a direct correlation of the NMR relaxation measurements with the structural properties of the gels under study. T_1 can be considered as an empirical parameter which reflects the state of the solvent in the matrix. An asymptotic value of $T_1 \sim 2.5$ s at room temperature is characteristic of bulk water. Any value of T_1 lower than the asymptotic one is indicative of a heterogeneity in the environment in which water is confined. In Fig. 1, longitudinal relaxations are reported for gellan and for chitosan–cyclodextrin gels, at different R values. In order to have a comparative overview of the swelling behaviour of the gels prepared in this study, in the same figure are shown results obtained from proton relaxation NMR measurements of these gels. Data for Sephadex G100, a 'reference' gel matrix as far as the control of the pore size and the backbone of the network is concerned, are also reported.

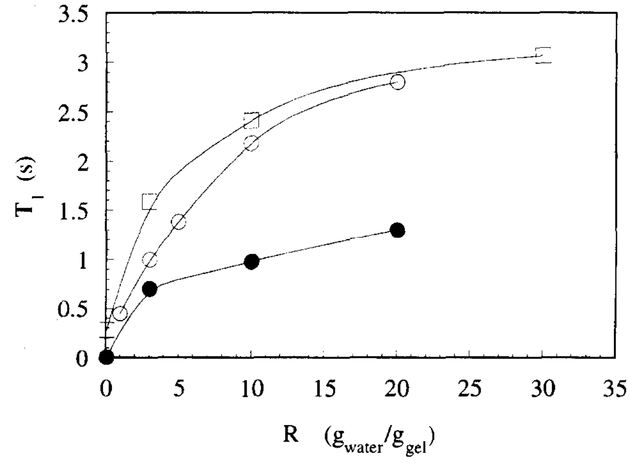

Fig. 1. Longitudinal relaxation times, T_1, as a function of the water content, R: Sephadex G100 (□), Gellan (○), Chitosan–cyclodextrin (●). The points at $R = 0$ have a pictorial purpose.

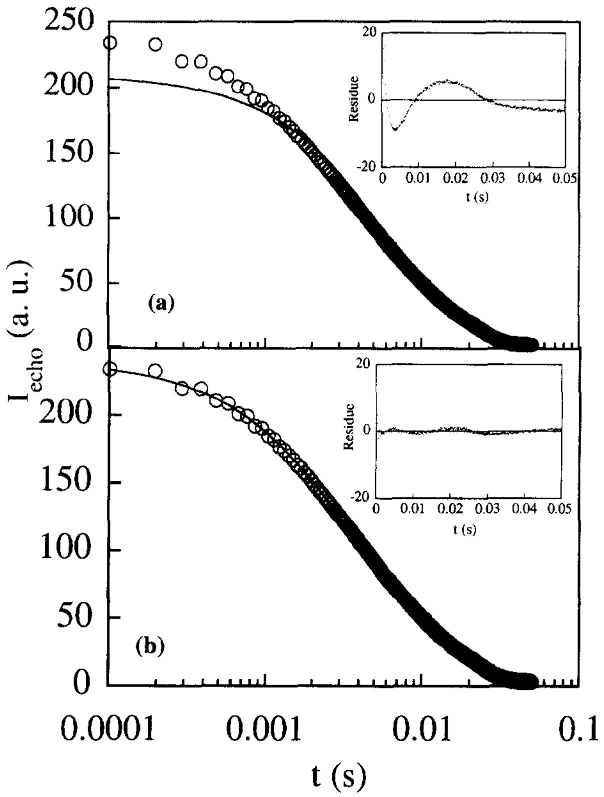

Fig. 2. Typical CPMG experiment for chitosan–cyclodextrin at room temperature ($R = 3$): (a) monoexponential fit; (b) biexponential fit.

Fig. 1 shows the differences in the T_1 of water in gellan and in cyclodextrin–chitosan hydrogels. For the latter network, the chemical cross-links and the topology of the cross-linker are more efficient in lowering the T_1 compared to the physical junction zones of gellan. Transverse relaxation, T_2, measurements offer a more detailed analysis in terms of nuclear transfer processes. Although the individuation of the components of the transverse decay of the magnetization is a matter to be established for each single case, in the chitosan–cyclodextrin network two definite transverse relaxation modes were detected. Multiexponential analysis of the experimental data was done on the basis of the criteria established by Clayden and Hesler [9]. To test the correctness of this approach we processed the data by both one exponential and two exponential least-square fittings. For a typical CPMG experiment, both a mono- and a bi-exponential least-square fit was attempted (Fig. 2a, b) with the latter suggested by comparison of the correlation coefficients and of the residuals (see inserts, Fig. 2). The same analysis was performed for gellan hydrogels. In this case, since a qualitative improvement of the results was not achieved with a biexponential fitting, all CPMG experiments were monoexponentially fitted. Variation of the content of water, i.e. of R, in the chitosan–cyclodextrin gel always led to the presence of two transverse relaxation times (Fig. 3).

The study of the temperature dependence of T_2 disclosed, for hydrogels with suitable structural characteristics, a chemical exchange process potentially contributing to the decay mechanisms. The solvent–matrix proton transfer can be separated from the dipolar interaction process operating in the solvent because of the opposite temperature dependence of the chemical exchange and of the dipolar relaxation mechanism. On this basis, we studied the behaviour of T_2 as a function of temperature for chitosan–cyclodextrin hydrogel and for gellan at $R = 3$ and 9, respectively. The different water contents adopted for the two systems were dictated by the characteristics of the two networks. In the case of chitosan–cyclodextrin gel, a higher water content would cause an increase of the value of the long T_2 component (see Fig. 3). On the other hand, a gellan sample with a lower content of water than that used in our experiment would shift the order–disorder transition temperature out of the range accessible to the temperature control unit of the spectrometer. In any event, due to the great differences of the two networks in the chemical and conformational arrangement of the chains, the same water content value would not lead to a comparable state of water in the hydrogels.

Data for the chitosan–cyclodextrin hydrogel in the temperature range 5–35 °C reveals the presence of a nuclear transfer mechanism between exchangeable protons of the hydroxylic groups of the matrix and water. The T_2 of free diffusing water protons is influenced by dipolar interactions, and its value is expected to increase with increasing temperature. Protons of water molecules in the network are motionally restricted and show lower T_2 values, reflecting a smaller diffusional average distance. In this situation, the T_2 of protons may have an opposite temperature dependence with respect to the case of free bulk water. This is due to the chemical exchange between protons of the matrix and of the solvent. The activation energy of the process is responsible for the inversion in the temperature dependence of the measured T_2. As is shown in Fig. 4a and b and in Fig. 5, this holds true for chitosan–cyclodextrin hydrogel and gellan gel, respectively.

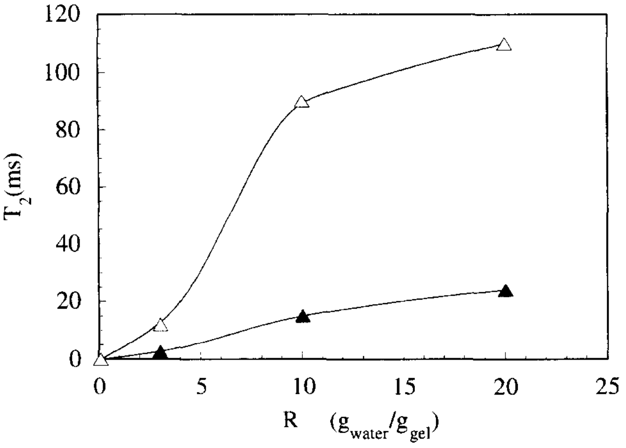

Fig. 3. Transverse relaxation times, T_2, as a function of water content, R, for chitosan–cyclodextrin: short component of T_2 (▲); long component of T_2 (△). The points at $R = 0$ have a pictorial purpose.

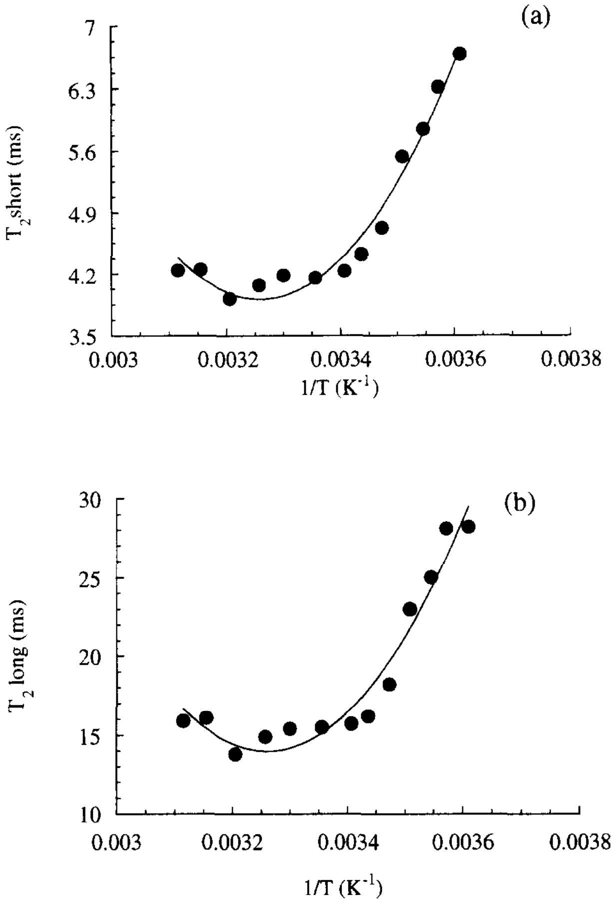

Fig. 4. Temperature dependence of T_2 short and long components for chitosan–cyclodextrin hydrogel ($R = 3$).

However, some distinctive features, between the two systems, are evident, in addition to the longer and monoexponential decay of the transverse magnetization of water evidenced in gellan gels. In the case of cyclodextrin–chitosan network, an inversion of T_2 was observed at about 40 °C. For gellan gel the

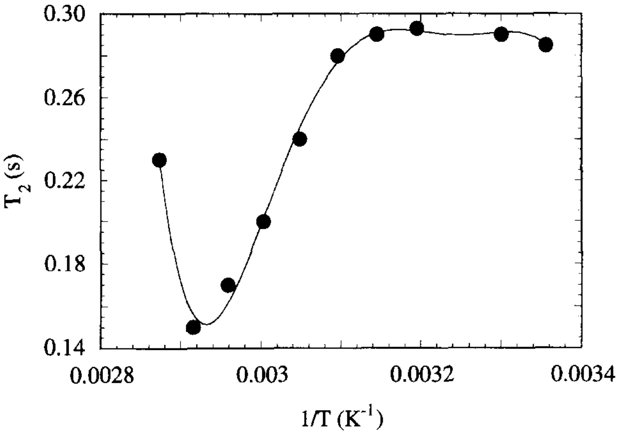

Fig. 5. Temperature dependence of T_2 for gellan hydrogel ($R = 9$).

proton-exchange process was coupled to a disorder–order conformational transition, resulting in the sharp increase in T_2 values occurring above 70 °C at an ionic strength of 0.2. This melting process is well known for gellan [10,11]. This temperature corresponds to a partial melting of the gel 'junction zones' with a consequent increase in chains mobility. Analogous coupling of the proton-exchange phenomenon with a conformational transition had already been observed for carrageenan gels [12].

Data for the chitosan–cyclodextrin sample ($R = 3$) reveals that in the range 5–35 °C a proton-exchange mechanism was present for both components. The small values of the two transverse relaxation times in the entire temperature range investigated (see Fig. 4a, b), is indicative of the fact that water was spatially constrained in the network compartments. The fractional populations of the two classes of water are reported in Table 1. On the basis of these considerations, we hypothesize that two water populations were active in the decay of the magnetization for the chitosan–cyclodextrin hydrogel. They were qualitatively recognizable and related to two environments with different stiffness in network constituents, in which the zones of cross-linking were probably the stiffer regions. Using a model for nuclear exchange processes introduced by Woessner [13], it is possible to write:

$$\frac{1}{T_{2,\mathrm{obs}}} = \frac{1}{T_2} + \left(\frac{p_{2,\mathrm{matrix}}}{p_2}\right)\left[T_{2,\mathrm{matrix}} + \frac{1}{C_\mathrm{b}}\right]^{-1} \quad (3)$$

in which the observed T_2, $T_{2,\mathrm{obs}}$, is described in terms of the relaxation time, T_2, relative to the dipolar exchange, the molar ratio of the exchangeable protons of the matrix and of the compartmentalized water, ($p_{2,\mathrm{matrix}}/p_2$), the transverse relaxation time of the protons (not exchangeable) of the matrix, $T_{2,\mathrm{matrix}}$, and the parameter C_b, i.e. the rate of exchange between the protons of the matrix.

C_b is a key parameter for the study of the behaviour of water interacting with the polymeric matrix, since the temperature dependence of this rate is opposite to that of dipolar interaction, T_2. As is shown in Fig. 4a and b, when the water–gel system is in the interval of temperatures where proton exchange takes place, it is possible to determine the value of C_b from the $T_{2,\mathrm{obs}}$, using Eq. (3). In this approach, it is taken into account that (i) the contribution of $1/T_2$ is negligible with respect to the chemical exchange rate, and that (ii) $T_{2,\mathrm{matrix}}$ is of the order of 0.1 ms [13]. The ratio ($p_{2,\mathrm{matrix}}/p_2$) is evaluated

Table 1
Fractional population of the two classes of water in the chitosan–cyclodextrin hydrogel, $R = 3$

Temperature (°C)	T^{-1} (K^{-1}) · 10^3	Relative population of short component	Relative population of long component
2	3.63	0.8	0.2
5	3.60	0.8	0.2
8	3.57	0.8	0.2
11	3.52	0.8	0.2
14	3.48	0.7	0.3
17	3.45	0.7	0.3
20	3.41	0.7	0.3
25	3.35	0.7	0.3
30	3.30	0.7	0.3
35	3.25	0.7	0.3
40	3.19	0.6	0.4
45	3.14	0.7	0.3
50	3.09	0.7	0.3

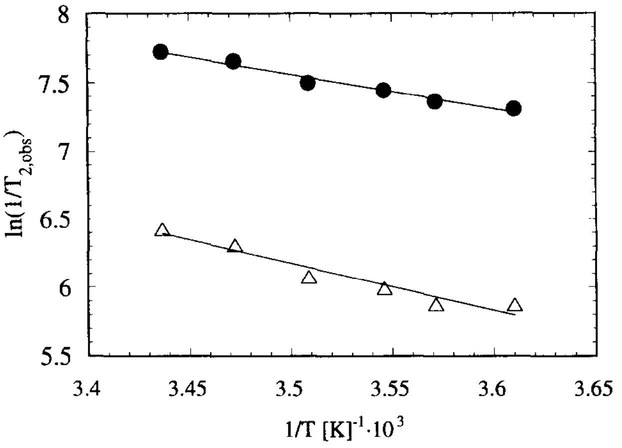

Fig. 6. Arrhenius plot for T_2 short (●) and long (△) components of chitosan–cyclodextrin hydrogel ($R = 3$). Activation energy from the slope: 5 (●) and 7 kcal/mol (△).

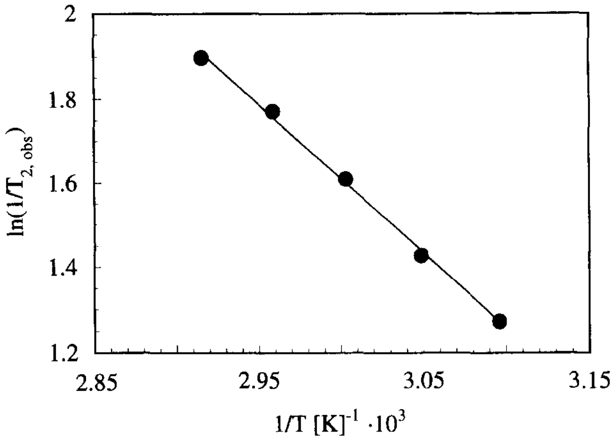

Fig. 7. Arrhenius plot for T_2 of gellan hydrogel ($R = 9$). Activation energy from the slope: 7 kcal/mol.

from the molar fractions of protons belonging to the cyclodextrin and the chitosan, respectively, and from the fractional populations worked out from the biexponential fitting. The correctness of this approach is confirmed by the linear behaviour of the Arrhenius plot of the $1/T_{2,\mathrm{obs}}$ values (see Fig. 6).

The chemical rates, C_b, evaluated in this way and reported in Table 2, indicate that the dynamics of the proton transfer differs markedly in the two classes of water. If we consider the proton exchange to be an elementary reactive step between the matrix and the water tightly bound to it, an activation energy value of 5–7 kcal/mol can be determined from the Arrhenius plot (see Fig. 6), confirming that for both components the proton-exchange mechanism involved the breaking of hydrogen bonds between water and exchangeable protons of the matrix. For gellan an activation energy of about 7 kcal/mol was found (Fig. 7).

In conclusion, NMR relaxometry measurements on water have been used to characterize polysaccharidic gels. T_2 measurements yield information on gel–solvent interactions. In the chitosan–cyclodextrin gels used in this study, which are gels formed by covalent cross-linking, two types of water with different relaxation modes are present in the network. Both types of water undergo proton exchange with the matrix, and the energetics of this process were regulated by hydrogen-bond interactions. In gellan gels, which are physical gels, the relaxation properties of water reflect both the characteristics of the matrix and the presence, in the melting temperature range, of a conformational transition. Despite different origins of the stiffness characterizing these two networks, both systems present the same activation energy for the proton exchange, confirming the validity of the general picture used for studying the water relaxation process in hydrogels.

Table 2
Temperature dependence of the proton-exchange rate, C_b, for the chitosan–cyclodextrin network ($R = 3$)

T (°C)	C_b (s^{-1})·10^{-3}	τ_b (ms)	C_b (s^{-1})	τ_b (ms)
4	3.98	0.25	425	2.35
7	4.31	0.23	427	2.34
9	4.83	0.21	482	2.07
12	5.23	0.19	500	2.00
15	6.76	0.15	674	1.48
18	7.52	0.13	764	1.31

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