

Collaborations:

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Introduction :

In recent years, great attention has been devoted to biopolymer gels because of their biocompatibility and biological functions, and consequently, potential applications in the biomedical and pharmaceutical fields. The chitosan gel has been widely studied as drug carriers [1,2]. Chitosan is produced by deacetylation of chitin (figure 1), a polysaccharide of widespread abundance in the nature after cellulose. The shells of crustaceans are common sources of chitin.

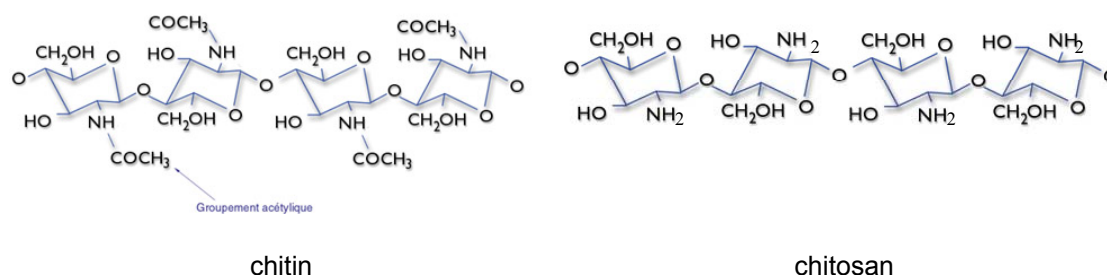
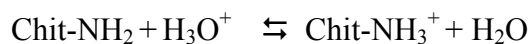


Figure 1: Formula of chitin and chitosan

Chitosan can be dissolved only in acid aqueous solution by protonation of amino groups following:



Chemically cross-linked gels can be formed via covalent association of chitosan amino groups with dialdehydes.

The physicochemical properties of chitosan are interesting to produce controlled drug delivery systems [3]. There are several works [4,5] which have shown that it is possible to improve these properties by forming semi-interpenetrating polymer network: chitosan-poly(ethylene glycol). Indeed the introduction of the polyethylene glycol induces a reinforcement of the mechanical properties and higher pH-dependent swelling properties.

Nevertheless very few data are available on the study of the gel formation (kinetics and structure in particular for semi interpenetrating networks) although this information is fundamental for an optimization of these systems, a better knowledge and a prediction of their properties. In the same manner the diffusion is very often studied from a chitosan gel to an aqueous solution [6] but not the self-diffusion within the gel.

Problem

The aim of this paper is to investigate the viscoelastic properties during the sol-gel transition of chemically cross-linked chitosan hydrogels and the mass diffusion within the chitosan gels of dextran polymers. The gelation time and structural parameters were determined from the power law frequency dependence of the elastic and loss moduli measured by mechanical spectroscopy for different physico-chemical parameters. The self-diffusion data are extracted from fringe pattern photobleaching of dextran polymer with labelled FITC. Brownian diffusion coefficients in the chitosan gels were determined for dextran polymers of different molecular weights and for different chitosan weight fractions.

Methods**-Materials**

Chitosan was prepared industrially by Aldrich from chitin. We have determined the average molecular weight from capillary viscosimetric measurements and from the Mark-Houwink equation:

$$[\eta] = K.M_w^a \quad (1)$$

where $K = 1.81.10^{-3}$ dL/g and $a = 0.93$ for chitosan solutions in acetic acid 0.1M/NaCl 0.2M at 20°C [7].

The degree of deacetylation (82%) was determined by infrared spectroscopy [8].

The entanglement concentration of chitosane ($C_e = 0,7\%$ w:w), was estimated by determination of the evolution of low shear viscosity as function of chitosan concentration [9].

A chitosan stock solution (2% w:w) was prepared by dissolving the proper amount of chitosan in 0.1 mol/L acetic acid.

This stock solution was diluted in 0.1 mol/L acetic acid to obtain chitosan weight fractions Φ_c ranging between 1.5 and 2.0 %. To form the semi-interpenetrating networks, POE (purchased from Aldrich with a average molecular weight of 1000000 g/mol) was dissolved in water to obtain POE weight fractions Φ_p ranging between 0.8 and 3%. Then these solutions of POE were added to the chitosan stock solution (and completed with water if required) to have a solution with 1.5% of chitosan and POE weight fractions ranging between 0 and 2.5%.

FITC-dextran was supplied by Sigma. The values of molecular weights of FITC-dextran (given by Sigma) were 4400, 19500, 40000, 77000 and 282000 g/mol. For diffusion measurement, FITC-dextran was added either to the chitosan solution or to water in order to obtain a solution with 0.1% (w:w) of dextran and chitosan at a given weight fraction Φ_c .

Cross-linking of the chitosan was achieved by adding 20 μ L of glyoxal (dialdehyde purchased from Riedel-De-Haen) to 5 g of polymer solution.

-Analytical techniques.

Because the gelation is a gradual transition from a viscoelastic liquid to viscoelastic solid, the measurements of rheological properties are very useful [10].

Small amplitude oscillatory shear allows the investigation of the sol-gel transition by probing the monotonously changing of the viscoelastic properties which occurs during gelation without extrapolation and shear modification of molecular structure. Initially the elastic G' and viscous G'' moduli increase with time while G' remains lower than G'' (sol state). Consequently, there is a critical time where G' becomes greater than G'' . Gelation time has been often relied on simple criteria $G' = G''$ [11]. However, Winter et al. [12] have shown that this determination of gelation time is only valid when both G' and G'' moduli are proportional to the square root of the frequency. The authors proposed a mechanical definition of the gel point by assuming that a critical gel (intermediate state between liquid and solid at the gel point) has a self-similar relaxation time spectrum. The stress relaxation modulus $G(t)$ could then be represented by a power law as such:

$$G(t) = S t^{-\Delta} \lambda_0 < t < \infty \quad (2)$$

where S is called the gel stiffness, Δ is the relaxation exponent and λ_0 is a lower power law limit depending on molecular structure. It corresponds to the crossover to the glass transition or to the entanglement region respectively for low and high molecular weight precursor materials [13].

The values of the relaxation exponent are restricted between 0 and 1 in order to ensure the zero value of the equilibrium shear modulus and the divergence of the steady shear viscosity at the gelation time.

The power law is a consequence of the coupling of the relaxation modes leading to a self-similar behaviour of the critical gel which has no characteristic length or time scale. A Fourier transform from the time to the frequency domain leads also to power laws for the frequency dependence of the elastic G' and viscous G'' shear moduli [14]. It can shown that the ratio of the two moduli is independent of frequency:

$$\tan\delta = G''/G' = \tan(\Delta\pi/2) \quad (3)$$

The main consequence of this result is that all the curves G''/G' versus time at different frequencies present an intersection point allowing an easy determination of the gelation time t_g and the relaxation exponent Δ .

The rheological measurements were performed using a Haake RS 150 rheometer (figure 2) with a cone-plate geometry (diameter: 60 mm, cone angle: 1°). The oscillatory measurements consisted of frequency sweeps between 0.25 and 1 Hz at constant shear amplitude (0.1 Pa). A solvent trap was used to avoid solvent evaporation. The temperature was controlled using

water circulation from a thermostated bath.

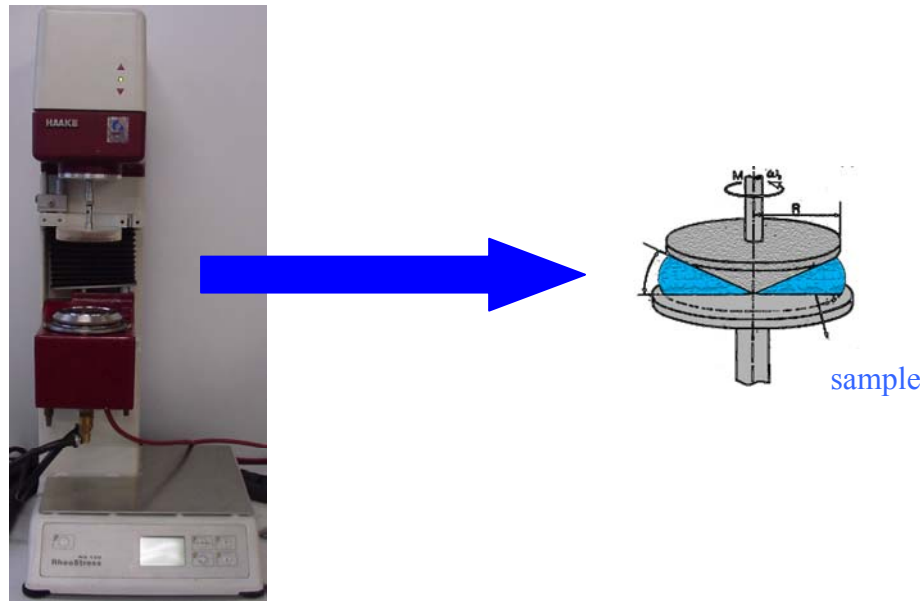


Figure 2: Experimental apparatus and geometry

Fluorescence recovery after photobleaching by a fringe pattern was used to study the mobility of FITC-dextran molecules within the chitosan hydrogels. This experiment consists in forming a fringe pattern with interfringe spacing i at the crossing point of two coherent laser beams [15]. First a fluorescence concentration profile is created in the sample by illumination with a short pulse of high intensity. Then the beams are attenuated and the fluorescence recovery is monitored while the spatial position of the fringes is modulated at frequency $\omega/2\pi$. From the fluorescence recovery signal due to the progressive blurring out of the concentration profile through diffusion of the photobleached probes, one can extract the diffusion coefficient. The fluorescence signal can be decomposed into a harmonic serie with respect to the fundamental modulating frequency of the sinusoidal modulation of the phase. The second harmonic component is a monoexponential decay of time with a characteristic time τ from which the lateral diffusion coefficient can be deduced according to:

$$D = i^2/4\pi^2 \tau \quad (4)$$

Results and discussion

Figure 3 displays a typical plot of G''/G' around the sol-gel transition as a function of time for different frequencies obtained with a chitosan hydrogel $\Phi_c = 1.8\%$. The gelation time defined as the time at which G''/G' is independent of the frequency (crossing at the curves: $t_g = 1430s$). From the value of G''/G' at the gelation time and the viscoelasticity equations, the relaxation exponent ($\Delta = 0.56$) and the gel stiffness ($S = 1.69 \text{ Pa}\cdot\text{s}^\Delta$) could be extracted.

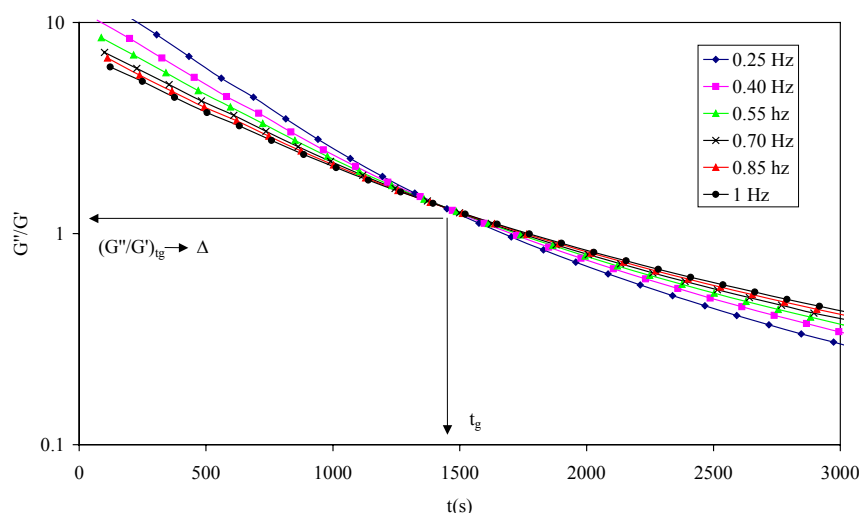


Figure 3:
Variation of
 G''/G' versus
time for a
chitosan
hydrogel
 $\Phi_c = 1.8\%$

The viscoelastic properties have been determined as a function of the chitosan weight fraction Φ_c ($1.5\% < \Phi_c < 1.85\%$). The different corresponding parameters are gathered in table 1.

Φ (%)	t_g (s)	Δ	S (Pa.s $^\Delta$)
1.5	6600	0.68	0.63
1.6	4660	0.61	1.1
1.7	2460	0.60	1.38
1.8	1430	0.59	1.69
1.85	790	0.57	2.35

Table 1. Gelation times, relaxation exponents and gel stiffness values for different chitosan weight fractions at 25°C.

Figure 4 shows the variation of the gelation time as a function of Φ_c .

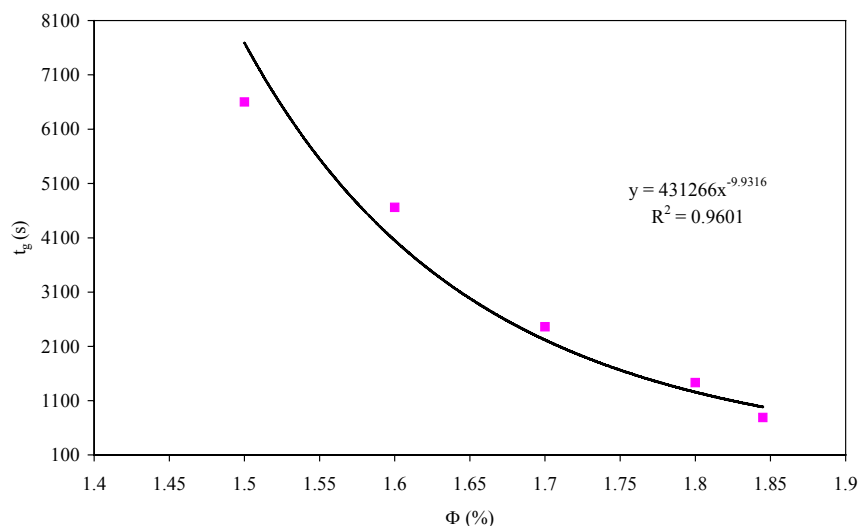


Figure 4: Variation of gelation time t_g versus chitosan weight fractions.

Chitosan weight fraction dependence of the gelation is found well described by a power law. This result is similar to the one in protein gels for which the gelation time is inversely proportional to the polymer concentration [16].

In the same manner an increase of the chitosan weight fraction induces a decrease of the relaxation exponent and an increase of the gel stiffness i.e. an increase of the elasticity of the critical gel.

The enhanced elasticity by the addition of the POE chains in the chemical crosslinked chitosan gel is illustrated in figure 5.

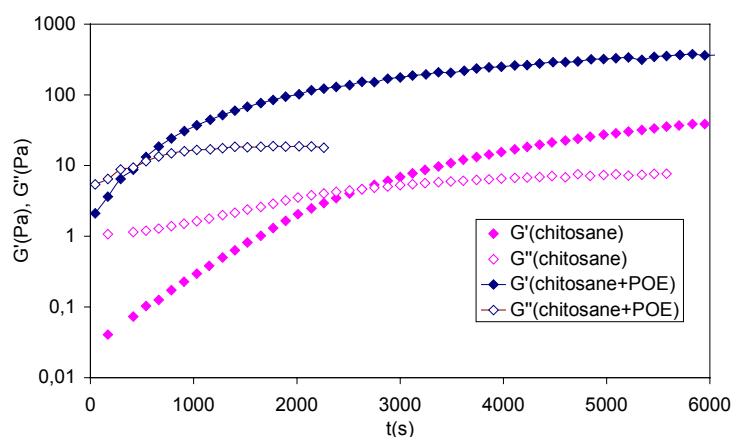


Figure 5: Elastic G' and viscous G'' moduli versus time for a 1.5 % chitosan gel and 1.5% chitosan gel + POE (0.4% w:w)

The viscoelastic properties for semi-interpenetrating networks (chitosan-poly(ethylene glycol)) have been determined as a function of the POE weight fraction Φ_p ($0.5\% < \Phi_p < 2.5\%$). The different corresponding parameters are gathered in table 2.

POE(%)	t_g (s)	Δ	S (Pa.s $^\Delta$)
0	1350	0.60	1.14
0.5	1250	0.59	1.07
0.8	1640	0.58	1.91
1	1650	0.55	2.38
1.6	1370	0.54	3.21
2	1660	0.52	4.12
2.5	1680	0.50	5.38

Table 2 : Gelation times, relaxation exponents and gel stiffness values for different POE weight fractions at 25°C.

The evolutions of the relaxation exponent Δ and the gel stiffness S are respectively given in Figure 6 and 7.

The gelation time decreases (the gelation kinetic is speed up) and the network is more and more elastic (Δ decreases and S increases) when the POE weight fraction increases. This means that the POE chains take part in the formation of the three-dimensionnal structure.

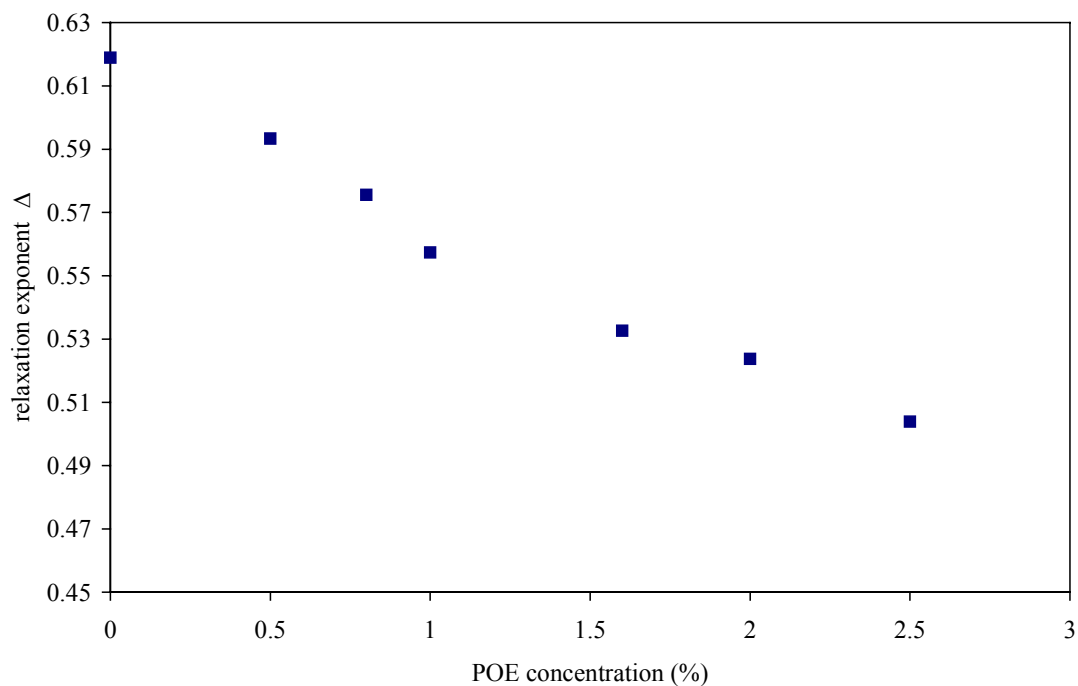


Figure 6 : Variation of the relaxation exponent Δ versus POE concentration

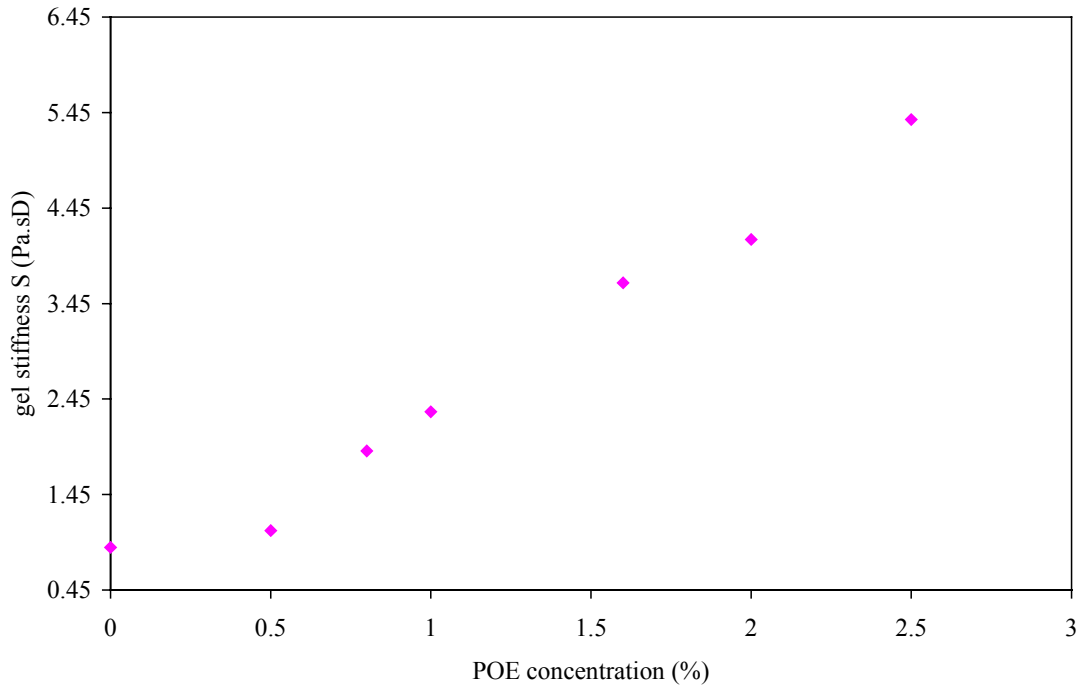


Figure 7 : Variation of the gel stiffness S versus POE concentration

After the rheological characterization, the results on diffusion are presented below.

A typical variation of the second harmonic modulated fluorescence recovery signal for FITC-dextran in a chitosan gel is given in figure 8. This plot corresponds to the diffusion of 19500 g/mol FITC-dextran. The exponential decay is well defined and has a characteristic relaxation time of 0.39 s.

2nd harmonic

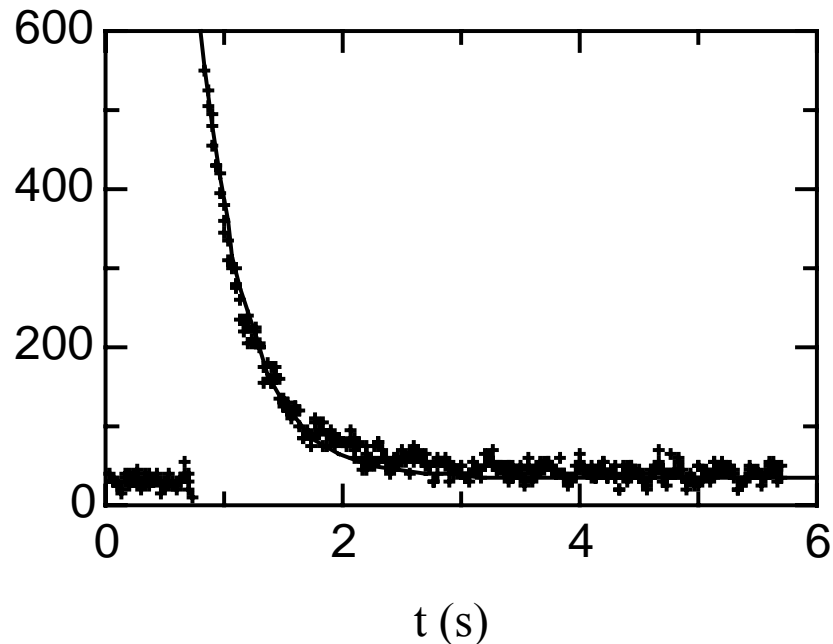


Figure 8: Recording of the second harmonic modulated fluorescence recovery signal for a chitosan hydrogel ($\phi = 1.5\%$) loaded with FITC dextran (0.1%) $M_w = 19500$ g/mol. The interfrange spacing i is $21.51 \mu\text{m}$.

In figure 9, the relaxation times are plotted as a function of i^2 for four molecular weights of

FITC-dextran (19500, 40000, 77000, 282000 g/mol) in 1.5% chitosan gel. The relaxation times are found to be linear with respect to i^2 and the linear regression line crossed the origin. This linearity is characteristic of a single diffusion of irreversibly bleached fluorophores and has been verified for all studied experimental conditions (dextran diffusion in water or in gel versus molecular weights of FITC-dextran and chitosan weight fractions).

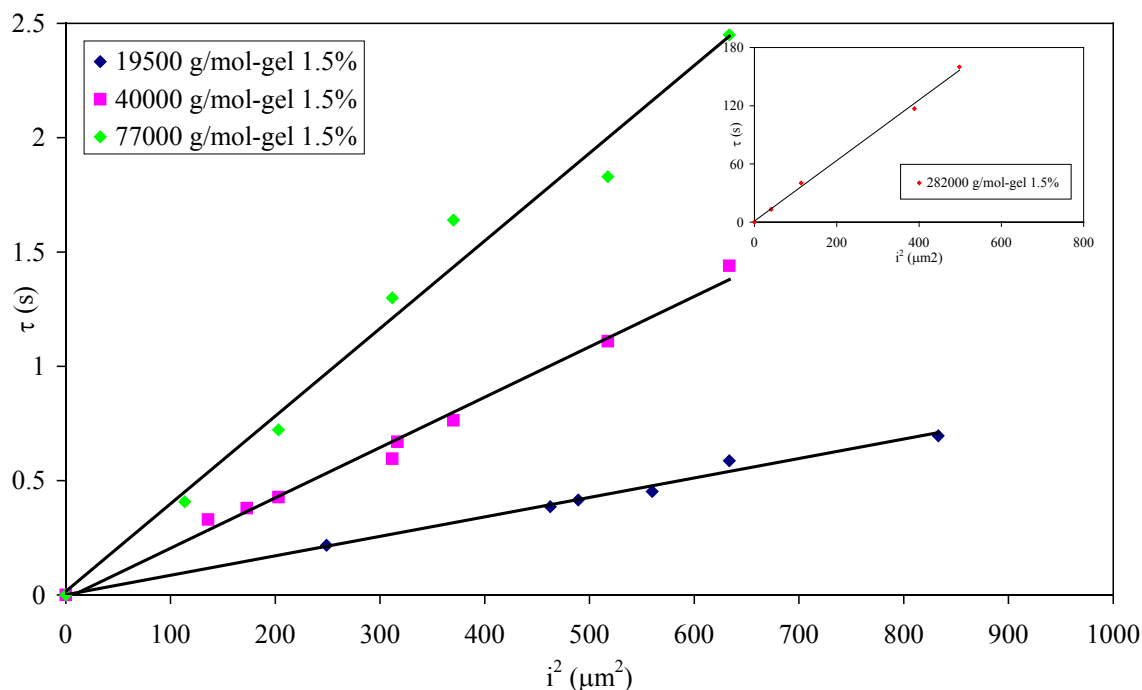


Figure 9 : Relaxation times versus i^2 for three molecular weights of FITC-dextran in chitosan hydrogel ($\Phi = 1.5\%$). In the inset the results are presented for the highest molecular weight of FITC-dextran (282000 g/mol).

The values of the diffusion coefficients in 1.5% chitosan gel and in water for the studied molecular weights of FITC-dextran are reported in table 3.

M_w FITC (g/mol)	$D_{\text{eau}} * 10^6$ (cm^2/s)	$D_{\text{gel}} * 10^6$ (cm^2/s)
19500	1.414	0.298
40000	1.013	0.115
77000	0.740	0.066
282000	0.334	0.001

Table 3 : Diffusion coefficients for different molecular weight of dextran in water and in 1.5% chitosan gel.

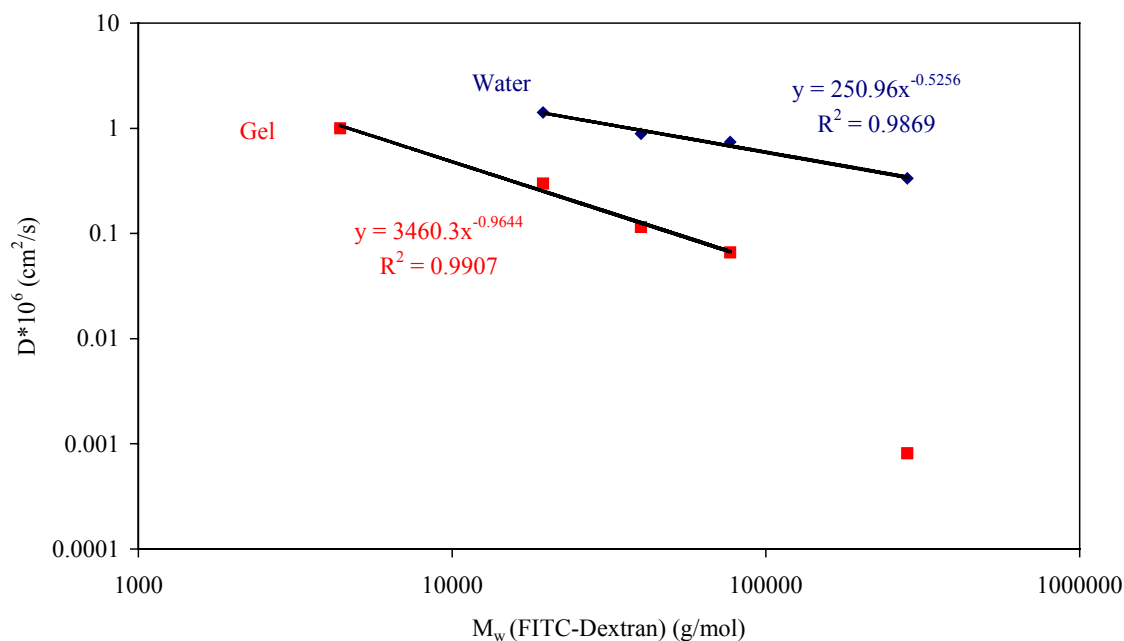


Figure 10 : Diffusion coefficients versus FITC-dextran molecular weight in water and in a chitosan gel ($\Phi = 1.5\%$).

The evolution of diffusion coefficients in water (Figure 10) is in a good agreement with the results summarized by Smit et al [17] and Bu et al [18]. The power law exponent value (-0.53) describing the dependence of the diffusion coefficient in water on molecular weight of FITC-dextran is consistent with a linear or randomly branched chain in good solvent.

When the diffusion takes place in chitosan hydrogel, we obtained an exponent close to -1 (Figure 10) ignoring the highest molecular weight. The difference between the two exponents underlines the slow down mass diffusion in the gel.

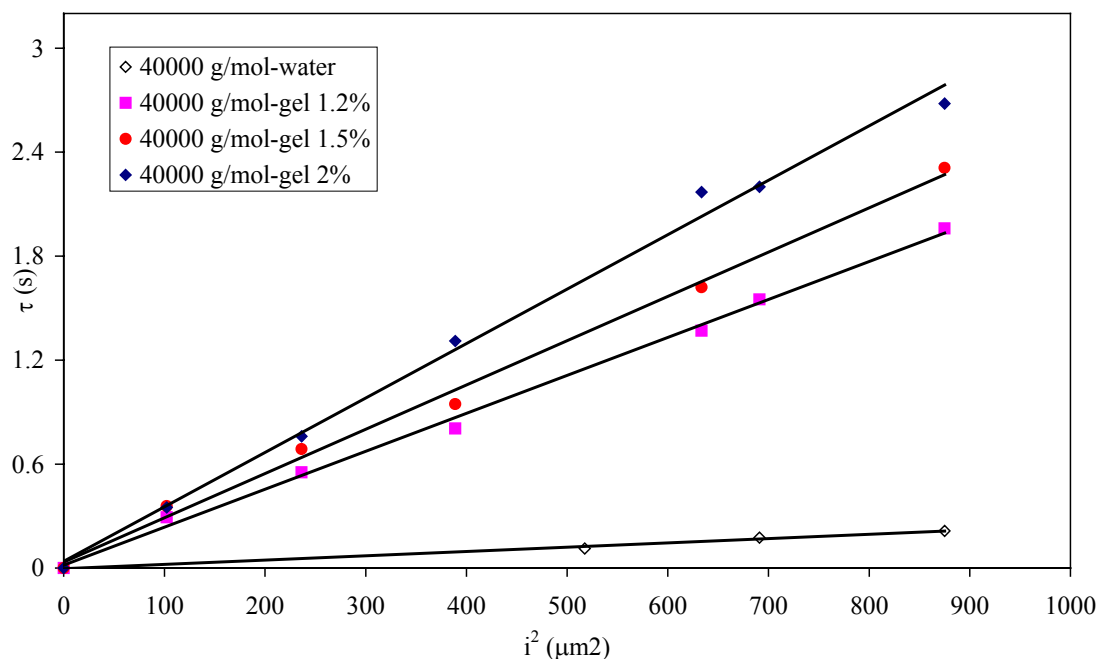


Figure 11 : Relaxation times versus i^2 for dextran with 40000 g/mol in water and in a chitosan gel with different weight fractions Φ_c .

Figure 11 illustrates the effect of the weight fraction of chitosan on diffusivity. The values of the diffusion coefficient in chitosan gel D_{gel} (and the ones D_{sol} in uncross-linked chitosan solutions for comparison) for different weight fractions of chitosan Φ_c are gathered in Table 4. The decrease of D_{gel} with an increase of Φ shows that the weight fraction of chitosan is a relevant chemical parameter to control the mass diffusion. This result is in a good agreement with the rheological data. When the chitosan weight fraction increases, the network becomes more elastic (relaxation exponent lower and gel stiffness higher). As a consequence the diffusion of the probe is slowed down.

$\Phi_c(\%)$	$D_{\text{gel}} \cdot 10^6$ (cm^2/s)	$D_{\text{sol}} \cdot 10^6$ (cm^2/s)
1.2	0.116	0.136
1.5	0.099	0.113
2	0.081	0.093

Table 4 : Diffusion coefficients for dextran with $M_w = 40000$ g/mol in uncrosslinked chitosan solution and in chitosan gel as function of the weight fractions of chitosan Φ_c .

It should be noted that the difference between the diffusion in gel and in uncross-linked solutions is not very important for the studied molecular weight of FITC-dextran ($M_w = 40000$ g/mol). Measurements for the higher molecular weights ($M_w = 77000$ g/mol, $M_w = 282000$ g/mol) are underway.

Conclusion

In this paper we presented the investigation of the sol-gel transition in chemically cross-linked chitosan hydrogels and semi-interpenetrating chitosan hydrogels for the first time. The characterization of the viscoelastic properties was achieved by mechanical spectroscopy. The gelation time and the structure of the critical gels determined from the power law frequency dependence of the viscoelastic moduli characteristic of a distribution of the relaxation times were found to depend on the weight fraction of chitosan. Concerning the semi-interpenetrating networks, the effect of the addition of POE chains was clearly demonstrated. An increase of POE concentration led to an enhanced elasticity of the critical gel (decrease of the relaxation exponent and increase of the gel stiffness). An improved method based on the fringe pattern photobleaching performed up to now in solutions was successfully used to study the diffusion properties in these biogels of controlled structure. We were thus able to determine Brownian diffusion coefficients of FITC-dextran of different molecular weights in water and in chitosan hydrogels. We also studied the effect of the weight fraction of chitosan for a given molecular weight of FITC-dextran. The slow down of diffusion with an increase of the weight fraction of chitosan shows that it is possible to control the diffusion in these biogels. More experimental and theoretical studies should be done to complete these first results and to quantitatively link viscoelasticity and diffusion. For example, we would study the effect of adding POE on the diffusion in order to complete the data concerning the semi-interpenetrating network that could be useful to produce controlled drug delivery systems such as pharmaceutical patches.

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