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Dynamo-mechanical and rheological characterization of guar gum hydrogels

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Abstract

Polymeric systems gained great interest in the last decades as specialties in the field of drug delivery, being very attractive for their peculiar physico-chemical characteristics. In this paper we report the characterization of a chemical network based on guar gum (GG) cross-linked with glutaraldehyde (Ga). The system was studied at three different temperatures, 7, 25 and 37 °C and at different ageing times in order to evaluate the influence of these two parameters on its stability. The experiments were carried out with a Texture Analyzer thus hardness, cohesion and Young modulus of the hydrogel were evaluated in the different conditions. Furthermore, relaxation experiments were performed and their interpretation, according to the Maxwell generalized model, allowed to describe the mechanical behaviour in terms of materials viscoelasticity theory. The kinetics of the chemical cross-linking was followed at 37 °C by means of rheological measurements, i.e. recording the mechanical spectra of the gelling system. The storage and loss moduli followed a power law, expression of the mechanical and structural self-similarity which evolves during the cross-linking reaction. The critical exponent at the gel point was evaluated, together with the critical time corresponding to the infinity connectivity of the system and the phase angle between stress and strain. Furthermore, also the fractal dimension was evaluated assuming a complete screening of the excluded volume of the chains at the critical point. Information about the release behaviour of the hydrogel, used as a matrix for modified drug delivery of model molecules with different steric hindrance, is also given.

Keywords: Guar gum; Hydrogel; Texture analysis; Gel-point; Fractal dimension

1. Introduction

Guar gum (GG) belongs to the wide family of galactomannans, polymers with a backbone built up by D-mannopyranosyl residues (M) linked β-(1 → 4) and usually bearing, in different extent, side chains of a single galactopyranosyl units (G) linked α-(1 → 6). Galactomannans occur in nature especially in the endosperm of leguminous seeds plants where they act as food reserve materials for germination. It is known that there are various galactomannans with a different M/G ratio, a different substitution pattern of side-chain units and different

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molecular weights [1–5]. All these properties are responsible, for this class of polysaccharides, of their peculiar chemical–physical characteristics in solutions whose knowledge can result very useful for their application in several technological fields [6–9]. In particular GG extracted from the seeds of *Cyamopsis tetragonolobus* possesses a high level of G substitution along the mannan backbone (approximately 40%). On the average, there are between 1.5 and 2 mannose residues for every galactose residue, with few, if any non-substituted regions [10]. When dissolved in aqueous solutions, GG assumes a flexible coil conformation as evidenced by viscometric experiments that gave for the Mark–Houwink–Sakurada exponent α a value of 0.74 [11,12]. However, the rheological behaviour of GG solutions is still object of debate and incompletely understood, due to the complex associations of the chains that cannot be related to a single specific model [13]. GG was cross-linked with glutaraldehyde (Ga), and it was proposed for specific colon drug delivery [14]. Recently [15] the GG/Ga hydrogel was tested as a matrix for oral solid dosage forms. The chemical reaction between an excess of cross-linker and the polymeric chains leads to the formation of a network whose dynamo-mechanical properties are time and temperature dependent, as reported in the present paper.

According to the chemical structure of the repeating units of GG, and to the fact that the reaction of Ga occurs only with the vicinal diols, the network formed in the presence of an excess of Ga will show a high degree of cross-linking. In fact, also from the macroscopic point of view, the macromolecular network yielded a gel capable to maintain its shape in a "test-tube inverting method" (i.e., self-sustaining gel) [16]. Furthermore, since the polysaccharidic hydrogels in the last decades aroused increasing interest in the biopharmaceutical field for the design of modified drug release formulations, it is even more important to study the behaviour of these systems as it is well known that the bioavailability of many drugs is deeply dependent on the performance of the carrier system [17–19].

In the present work a detailed study on the strength of the network, in terms of hardness, cohesiveness, and Young modulus of the hydrogel is reported at three different temperatures, exploring a wide interval from 7 to 37 °C, and for a period of time long enough to allow the samples to start to degrade, as evidenced by the loss of their mechanical properties.

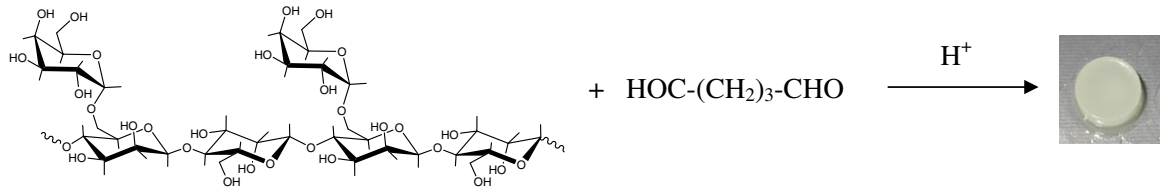
The gel samples were tested, always at three temperatures, 7, 25 and 37 °C, and at different time intervals, also by means of relaxation experiments. The obtained spectra were analysed in terms of the generalized Maxwell model that allowed to obtain the mechanical spectra for all samples studied in the various experimental conditions. Thus, the modelling gave the possibility to acquire important information on the viscoelastic behaviour and, if present, on the elastic residual component of the networks.

Furthermore, the details of the chemical cross-linking kinetics between GG and Ga were followed, at 37 °C, by means of rheological measurements. The mechanical spectra of the gelling system were recorded in the linear viscoelastic interval as a function of ageing time; a power law of the storage and loss moduli was detected after a certain time from the addition of the cross-linker indicating the critical sol–gel transition. Thus, it was possible to give an estimation of the power law exponent near the gel point, n, and its value was compared with literature data.

2. Experimental

2.1. Materials

Guar gum (GG) was a gift of Lamberti s.p.a. (Plant and Thecnological Centre of Albizzate, Italy); it was provided with a molecular weight $M_w = 2.7 \times 10^6$ g/mol (from GPC and viscometric



Scheme 1. Representation of the chemical reaction between GG and Ga together with a photograph of the obtained hydrogel.

measurements) and with a ratio mannose/galactose ~ 1.5 (Scheme 1) [20].

Theophylline (TPH) and Ga were Carlo Erba products (Italy) while Myoglobin (MGB) was purchased from Fluka (Germany). All other products and reagents were of analytical grade. For the preparation of the samples distilled water was always used.

2.2. Polymer purification

A given amount of GG was dissolved in distilled water, and then kept under magnetic and mechanical stirring for 24 h. The obtained solution was exhaustively dialyzed at 7 °C against distilled water with dialysis membranes of a cut-off 12,000–14,000, and then freeze-dried. The lyophilized product was stored in a desiccator until use.

2.3. Hydrogel preparation

The hydrogel was prepared according to the procedure previously described [15]. A given amount of GG (100 mg for the release experiments from the gels and 180 mg for the gels to be used for the preparation of tablets) at the appropriate concentration ($c_p = 1.5\%$ (w/v)) was kept, to obtain a complete dissolution of the polymer, under magnetic and mechanical stirring in distilled water at 60 °C for 24 h and at room temperature for 24 additional hours.

A few drops of concentrated sulphuric acid were then added (to protonate the hydroxyl groups of the polymer) to the solution that was kept under magnetic stirring for 30 min. An appropriate amount of Ga was added (Scheme 1) corresponding to an r value = 4.0, where r represents the ratio between the cross-linker moles and the moles of repeating units of the polymer: r = (cross-linker moles)/(moles of repeating units of polymer).

The reaction mixtures were then stirred for 30 min and kept in a thermostatted bath at 30 °C for 48 additional hours for gel setting. The samples were dialysed, using dialysis membranes with a cut-off 12,000–14,000, at 7 °C until the unreacted Ga disappeared from the dialysis solvent. The presence of polymeric Ga was detected at 235 nm and the monomeric Ga at 280 nm. Due to the presence of various forms of Ga in solution (free aldehyde, mono- and dihydrated monomeric glutaraldehyde, monomeric and polymeric cyclic hemiacetals and various α,β-unsaturated polymers) [21,22] it is not possible to give an exact description of the resulting network. Nevertheless Ga has been frequently used as a cross-linking agent since it is not expensive, it is readily available and it is highly soluble in aqueous solution.

The obtained self-sustaining gels, settled in a 10 ml beaker, showed the geometry of a cylinder having a diameter of 22 mm and a height of 18 mm. The hydrogels were then used to study the release of TPH and MGB or lyophilized for the preparation of tablets. As reported below, a different procedure to load the hydrogels with the different molecules was performed.

2.4. Hydrogel loading, release experiments from gels, preparation of tablets and dissolution experiments from tablets

The dialysed hydrogels (obtained starting from ca. 100 mg of polymer) were freeze-dried and then soaked in a saturated solution of TPH for 48 h. The samples were then washed with distilled water to remove the excess of imbibed solution and then tested for the release of TPH. In the case of MGB, drug loading was carried out by directly inserting with a needle a given amount of an aqueous MGB saturated solution in the freeze-dried samples until the matrices were completely soaked up and homogeneously imbibed, as evidenced by the uniform brown colour acquired by the samples.

Release experiments were carried out by immersion of the gel in distilled water ($V_r = 200\ cm^3$; $T = 37$ °C) at a fixed distance from the vessel bottom by means of a thin web hosting the gels. Release medium was gently magnetically stirred; 3 ml samples, at appropriate time intervals, were withdrawn from the solution and replaced with the same amount of fresh solvent. TPH and MGB concentrations were detected, respectively, at 272 nm and 409 nm by means of a spectrophotometer (Perkin–Elmer, lambda 3a, UV–Vis spectrometer) equipped with 1.0 cm path-length quartz cells.

Tablets were prepared by compressing with an IR die (Perkin–Elmer hydraulic press; compression force = 5.0 kN for 30 s) the freeze-dried hydrogel (obtained starting from ca. 180 mg of polymer) containing the model drug.

Reference tablets, i.e. tablets made up of not-cross-linked polymer, were prepared by freeze-drying aqueous solutions containing the polymer and the model drug followed by compression at the

same conditions above reported to obtain the final dosage forms.

All tablets had a weight of 190 ± 10 mg, a diameter of 13.00 ± 0.05 mm, and a thickness of 1.50 ± 0.10 mm.

Dissolution experiments from tablets were carried out according to Ph. Eur. 5th, using the rotating basket apparatus at 37.0 ± 0.1 °C, 100 rpm and distilled water (500 ml, pH = 5.4) as a dissolution medium. Aliquots of the dissolution medium (5 ml) were taken at fixed time intervals, the same amount of fresh solvent was added in the apparatus, and TPH or MGB concentration was spectrophotometrically measured at 272 nm and 409 nm, respectively. Both, release and dissolution experiments, were carried out in triplicate ($N = 3$). The values reported in the present paper represent the mean values and lay within 10% of the mean.

2.5. Mechanical characterization: texture analysis

A software-controlled dynamometer, TA-XT2*i* Texture Analyzer (Stable Micro Systems, UK), with a 5 kg load cell, a force measurements accuracy of 0.0025% and a distance resolution of 0.0025 mm (according to the instrument specifications), was used for the mechanical characterization of the gel samples [23–26]. The gels, prepared without loading of model molecules and without performing the dialysis, where kept in thermostatted baths at three different temperatures, 7, 25 and 37 °C. After different intervals of time the systems where tested with penetration/withdrawal experiments and with relaxation experiments.

The hydrogel resistance to penetration and withdrawal of an ebonite cylindrical probe with a diameter of 10 mm (P10) was measured. The pre-test speed was set up at 2.0 mm/s, the test speed and the post-test speed at 1.0 m/s and the penetration depth was variable, being imposed a fixed deformation of 20% with an acquisition rate of 200 points/s.

Also for the relaxation experiments a 20% deformation was imposed to the samples and then the relaxation of the systems was recorded until a baseline was reached. A cylinder probe with a diameter of 35 mm (P35) was used, a pre-test of 2.0 mm/s, a test speed of 4.0 mm/s and a post-test speed of 1.0 mm/s were applied for the analysis.

Syneresis was determined by the evaluation of the weight loss with time of the samples at the different tested temperatures and is expressed as the corresponding percentage increase of c_p.

All measurements were performed in triplicate ($N = 3$). Before performing both kinds of experiments the range of linear viscoelasticity was monitored and the deformation to be applied was consequently assessed.

2.6. Rheological measurements

The rheological characterization of the GG/Ga gels was performed by means of a controlled stress rheometer (Haake Rheo-Stress RS300; Thermo Haake DC50 water bath); a grained plate-plate device (Haake PP35 TI: diameter = 35 mm; gap between plates = 1 mm) was used in order to reduce the wall slippage phenomena extent [27]. Rheological properties were studied in oscillatory experiments; mechanical spectra were recorded in the frequency range 0.001–10 Hz. The linear viscoelastic region was assessed, at 1 Hz, by stress sweep experiments; a constant deformation of $\gamma = 0.1$ for GG and GG/Ga hydrogels was used.

The samples of GG were analysed, at three different temperatures, 7, 25 and 37 °C, as simple solutions, after the addition of sulphuric acid, and after the addition of Ga.

The mechanical spectra of the GG/Ga system for the gel point detection were recorded, on the same sample, in a stepwise sequence during the reaction progress; a deformation of $\gamma = 0.01$ was applied in order to influence at the minimum possible level the gel forming process.

3. Results and discussion

3.1. Release experiments

In order to investigate the role of the GG/Ga network on the delivery of drug molecules of different size the release of TPH (MW = 198.18, radius of van der Waals = 3.7 Å) and MGB (MW = 17,800, radius of van der Waals = 21.0 Å) was followed [15]. As reported in Table 1 the diffusion of TPH out of the gel occurs without serious constrains and after 8 h almost all the drug is delivered (96.4%). On the other side the molecule with higher steric hindrance (MGB) is released only in a reduced extent even after 24 h (33.8%). When the tablets are tested the release of TPH from GG is slightly smaller at 8 h than that from GG/Ga. This peculiar result is due to the formation of a very viscous entangled system in the case of GG tablets that hinders the diffusion of TPH more than in the case of

Table 1
Relative delivery, $(M_t/M_\infty) \times 100$, of TPH and MGB obtained with GG/Ga hydrogels, with tablets of GG and with tablets prepared with the GG/Ga hydrogels ($N = 3$)

Drug	Hydrogel		Tablets			
	GG/Ga		GG		GG/Ga	
	$t = 8$	$t = 24$ h	$t = 8$ h	$t = 24$ h	$t = 8$ h	$t = 24$ h
TPH	96.4 ± 2.0	100.0 ± 2.2	78.0 ± 3.0	100.0 ± 2.0	97.1 ± 3.1	96.8 ± 3.0
MGB	28.3 ± 1.2	33.8 ± 0.6	7.0 ± 0.8	41.7 ± 1.9	1.0 ± 0.4	1.0 ± 0.2

GG/Ga tablets where the presence of the cross-linker leads to the formation of mesh-size big enough to have free diffusion of the guest molecule. After 24 h, in the case of GG a complete release is observed due to the partial erosion of the matrix while in the case of GG/Ga the release is constant due to the presence of the chemical network. When MGB was loaded in the plain tablets of GG a lower delivery is observed than in the case of the gel. In fact the imbibition process represents a critical step that interferes negatively with the diffusion of MGB. As observed with TPH, also with MGB at 24 h an increased delivery is detected due to the erosion of the tablets. The presence of the network, together with the imbibition process, is so effective that in the case of GG/Ga tablets a negligible MGB release is obtained within the investigated time interval.

3.2. Penetration experiments

Texture analysis is a penetrometry technique that in the past has been especially employed in the mechanical characterization of food materials [28] while nowadays its application to the pharmaceutical gels analysis [25,26,29] is remarkably increasing. From a penetration/withdrawal experiments several parameters can be derived: the system hardness, i.e. the maximum positive force registered while attaining the imposed deformation, F_{max}; the work of cohesion (cohesiveness), proportional to the positive area under the force–time curve from zero to the maximum deformation imposed, the work of adhesion (adhesiveness), proportional to the negative area under the force–time curve and the Young modulus E (obtained from the initial slope of the stress–strain curve). Fig. 1 represents, as an example, the profile obtained from a penetration experiment carried out on GG/Ga samples, kept at 7 °C, at different ageing time. Both, F_{max} and cohesiveness, increase with time and they reach their maximum values at the 175th day. After this time a decrease in the hardness of the system is observed

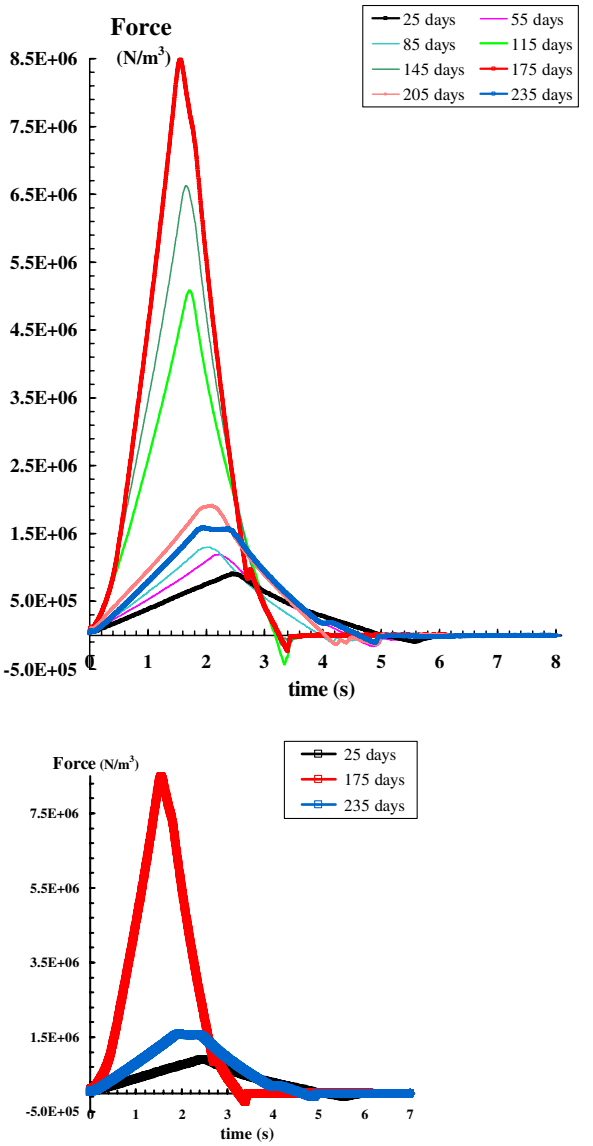


Fig. 1. Penetration profiles obtained on hydrogel samples of GG/Ga kept at 7 °C for different periods of time. The data obtained after 25 days (first measurement), after 175 days (maximum registered values) and after 235 days (last measurement) are reported below. $c_p = 1.5\%$ (w/V); $r = 4.0$; $N = 3$.

indicating the prevalence of the degradation process of the network.

In Fig. 2 the F_{max} values for the system cured at 7 °C are reported as a function of time together with the values registered for the samples kept at 25 and 37 °C. It is interesting to note how different are the profiles of the samples kept at different temperatures: in particular, those maintained at 7 °C were more stable and measurements could be performed even after 235 days. As above pointed out, the maximum hardness was reached after 175 days, i.e. the time corresponding to the equilibrium between the hardening and the degradation processes. After that time, the erosion of the matrix became predominant and the cohesiveness decreased progressively. When the samples were kept at 25 °C a rather different profile was obtained. In this case, the maximum hardness was reached after 80 days and then only a slight decrease of F_{max} could be monitored. At 25 °C the systems were always harder than those studied at 7 °C; this can be due not only to a kinetic factor, but mainly to a different rearrangement of the chains within the network. The effect is even more pronounced in the case of the samples kept at 37 °C. Again, the increase of F_{max} is faster than that found at 25 and 7 °C; in fact, at 37 °C, the maximum value is reached after only 21 days and then the degradation process predominates reducing dramatically the hardness of the system. Furthermore, the maximum values detected at 7 and 37 °C are comparable while there is a difference of a factor ten with those obtained at 25 °C. In Table 2 the most significative parameters, evaluated at the three tested temperatures, are given at three specific times (i.e. the first measurements after the addition of the cross-linker, when F_{max} reached the highest values and at the end of the experiment). It can be immediately observed that the value of hardness found after 175 days at 7 °C is quite similar to that obtained after only 21 days at 37 °C (9.17 and 13.51×10^6 N/m^3 respectively); while at 25 °C the maximum hardness value, reached after 80 days, was significantly higher (59.53×10^6 N/m^3) and then slowly decreased. Obviously, also the Young modulus follows a similar trend in the deformation interval that was explored (see Table 2). Significative, to this respect, appear to be also the data reported in Table 3 that refer to the syneresis observed, although at a different extent, in correspondence to the maximum values of F_{max}, with all the samples. The lowest syneresis effect was found for the samples kept at 7 °C, followed by those kept at 37 °C and then by those kept at 25 °C. The maximum percentage increase in hardness and cohesiveness was registered for the samples with the highest syneresis. Comparing the values of Tables 2 and 3 it can be evidenced that the systems kept at 7 °C are the most stable; such samples showed the lowest

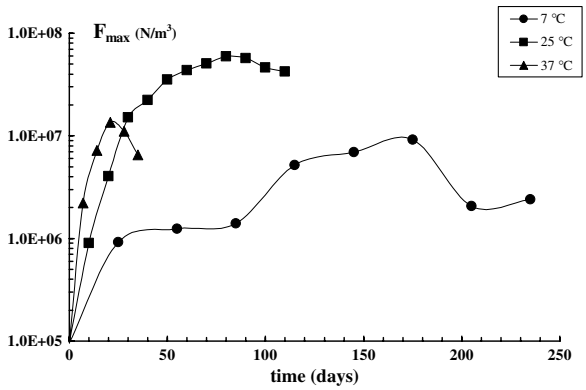


Fig. 2. Comparison between the hardness values, F_{max}, obtained for the GG/Ga hydrogel samples kept at 7, 25 and 37 °C, reported as a function of ageing time. $c_p = 1.5\%$ (w/V); $r = 4.0$; $N = 3$.

Table 2
Mechanical parameters obtained with the GG/Ga hydrogel kept at 7, 25 and 37 °C for different periods of time ($N = 3$)

T (°C)	Time (days)	[a] Hardness $\times 10^{-6}$ (N/m^3)	[a]Cohesiveness $\times 10^{-3}$ (J/m^3)	[a]Adhesiveness (J/m^3)	Young modulus, E (Pa)
7	25	0.92 ± 0.19	1.17 ± 0.03	60.40 ± 7.87	109.99 ± 1.59
7	175	9.17 ± 0.67	5.94 ± 0.50	31.53 ± 18.23	707.91 ± 32.88
7	235	2.40 ± 0.42	2.23 ± 0.22	79.92 ± 5.38	228.98 ± 13.69
25	10	0.90 ± 0.02	0.99 ± 0.03	66.91 ± 0.69	88.29 ± 1.35
25	80	59.53 ± 10.10	30.91 ± 7.88	25.10 ± 0.10	4105.26 ± 754.21
25	110	42.50 ± 13.12	16.61 ± 4.31	2.75 ± 0.04	3120.19 ± 513.55
37	7	2.22 ± 0.90	1.67 ± 0.06	158.20 ± 26.81	193.92 ± 9.37
37	21	13.51 ± 1.30	5.34 ± 1.14	34.15 ± 19.88	939.71 ± 60.73
37	35	6.53 ± 0.75	3.08 ± 0.22	26.58 ± 18.74	427.53 ± 43.55

[a] The given parameters are normalized per unit volume.

Table 3
Relative increase of c_p, and the corresponding increase in hardness and cohesiveness at different temperature and ageing time

T (°C)	Time (days)	Δc_p (%)	ΔHardness (%)	ΔCohesiveness (%)
7	175	$\sim 2\times10^2$	$\sim 9\times10^2$	$\sim 4\times10^2$
25	80	$\sim 6\times10^2$	$\sim 7\times10^3$	$\sim 3\times10^3$
37	21	$\sim 4\times10^2$	$\sim 5\times10^2$	$\sim 2\times10^2$

$\Delta X\,(\%) = ((X_m - X_0)/X_0)\times 100$, where X_0 represents the value of parameter X measured the first time and X_m represents the maximum value recorded in the time interval explored.

syneresis and an increment in hardness and cohesion comparable with those found at 37 °C. On the contrary, the samples kept at 37 °C undergo a degradation process in a very short time, due to the high temperature, and the percentage of F_{max} and cohesiveness increases are smaller than those observed at 7 °C. Peculiar are the results related to the samples kept at 25 °C: they show the highest syneresis but also the highest absolute value and the highest percentage relative increment of hardness and cohesiveness. It should be pointed out that the syneresis, though different for the three samples, is of the same order of magnitude; on the contrary the relative increments of cohesion and hardness, detected at 25 °C, are one order of magnitude higher than those obtained at the other two temperatures. In conclusion it can be summarized that at 7 °C the network strengthening occurs quite slowly because of the low temperature and the degradation is correspondingly reduced, while at 37 °C both kinetics are much faster leading to a degradation that overcomes much earlier the network settlement. At 25 °C the rate of the network formation is, as expected, higher than that occurred at 7 °C, but, at the same time, the degradation process is not as rapid as at 37 °C, thus the systems are able to reach hardness and cohesiveness values that are higher of one order of magnitude with respect to the other two temperatures.

3.3. Relaxation experiments

In order to acquire information about the mechanical properties of the hydrogels and, consequently, on the network characteristics, hydrogel relaxation behaviour can be matched resorting to the generalised Maxwell model [30]. This model assumes that the viscoelastic properties of the gel matrix can be represented by a mechanical device made up by a series combination of a Hookean spring of rigidity E_i and a Newtonian dashpot of viscosity η_i. For uniaxial stress relaxation, this model yields to:

$$\sigma(t) = \int_0^t \phi(t-t')\frac{\partial\varepsilon}{\partial t}\partial t' \tag{1}$$

where σ is the tension, ε the deformation, t the time and ϕ the relaxation modulus whose expression is:

$$\phi(t) = E_0 + \sum_{i=1}^{n} E_i \exp\left(-\frac{E_i}{\eta_i}t\right) \tag{2}$$

The relaxation test is preceded by a compression phase lasting t_1 and therefore Eq. (1) needs to be integrated with the following conditions:

$$\frac{\partial\varepsilon}{\partial t} = \frac{\varepsilon_0}{t_1}; \quad 0 < t < t_1 \text{ compression} \tag{3}$$

$$\frac{\partial\varepsilon}{\partial t} = 0; \quad t > t_1 \text{ relaxation} \tag{4}$$

where ε_0 is the final constant deformation applied. Accordingly, we get:

$$\sigma(t) = \frac{\varepsilon_0}{t_1}\left[E_0 t_1 + \sum_{i=1}^{n}\eta_i \exp\left(-\frac{E_i}{\eta_i}t\right)\times\left(\exp\left(\frac{E_i}{\eta_i}t_1 - 1\right)\right)\right] \tag{5}$$

In Fig. 3a, as an example, the relaxation spectra of GG/Ga hydrogels kept a 7 °C for different periods of time are shown, while in Fig. 3b the experimental values and the best fitting obtained applying the generalized Maxwell model for the sample kept at 7 °C for 175 days are given.

With increasing the ageing time the relaxation spectra show profiles qualitatively similar while the stresses opposed by the network steeply increase up to a maximum registered at day 175th. After that the network looses its strength and this is reflected also by the relaxation profiles whose curves assume gradually smaller values. In Fig. 4 the mechanical spectra, obtained reporting the coefficients E_i as a function of τ_i ($\tau_i = \eta_i/E_i$) calculated with the generalized Maxwell model, are shown in the case of the samples kept at 7 °C.

Generally the relaxation behaviour of GG/Ga could be sufficiently described in terms of three Maxwell elements plus a pure elastic element, E_0. Apart from the pure elastic element, the behaviour of the remaining viscoelastic part is typical of liquid-like materials, since the elements characterized by low relaxation times τ_i are associated with higher values of the spring constants E_i and

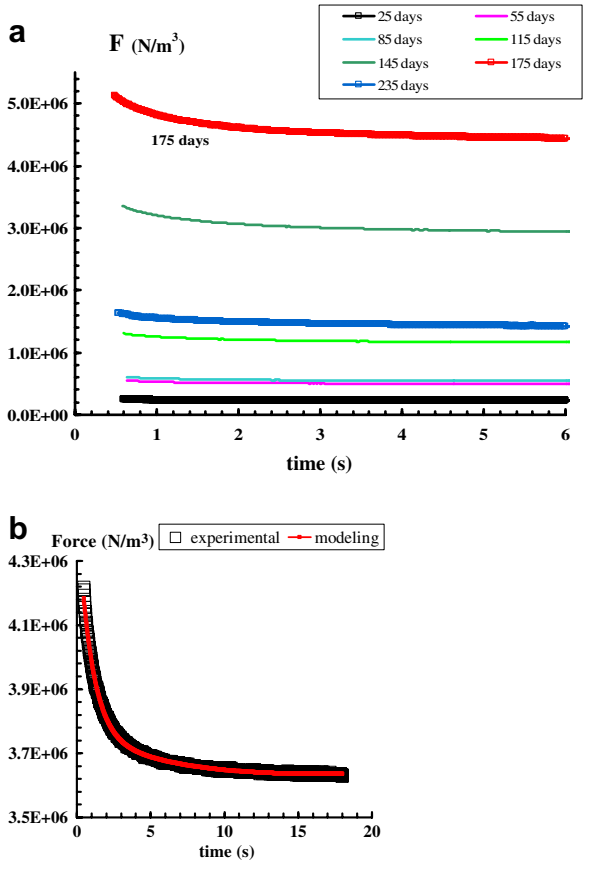


Fig. 3. Relaxation spectra of GG/Ga hydrogels kept at 7 °C for different ageing time (a). Experimental values and the best fitting (red line) obtained applying the generalized Maxwell model for the sample kept at 7 °C for 175 days (b). $c_p = 1.5\%$ (w/V); $r = 4.0$; $N = 3$.

consequently they prevail in determining the time-dependent response. In particular the spring constants E_i assume higher values with time until the systems reach their maximum hardness. Then, as the degradation process becomes predominant and therefore the network starts to loose its connectivity, also the mechanical spectrum is correspondingly influenced and the spring constants E_i begin to decrease. A similar remark can be made for the mechanical spectra (data not shown) related to the samples kept at 25 and 37 °C, obviously with different absolute values for the E_i elements. In Fig. 5 the time dependence of the pure elastic element for all tested systems is reported. On the basis of the linear viscoelasticity theory the E_0 values are directly related to the cross-linking density of the network. It is clear that a 7 °C the cross-linking density increases slowly with time while this process is much faster at 25 °C and even more at 37 °C. It is reasonable that, being the network built up by chemical linkages among the Ga and the GG chains, also physical interactions among the macromolecular chains should be taken into account. In fact the

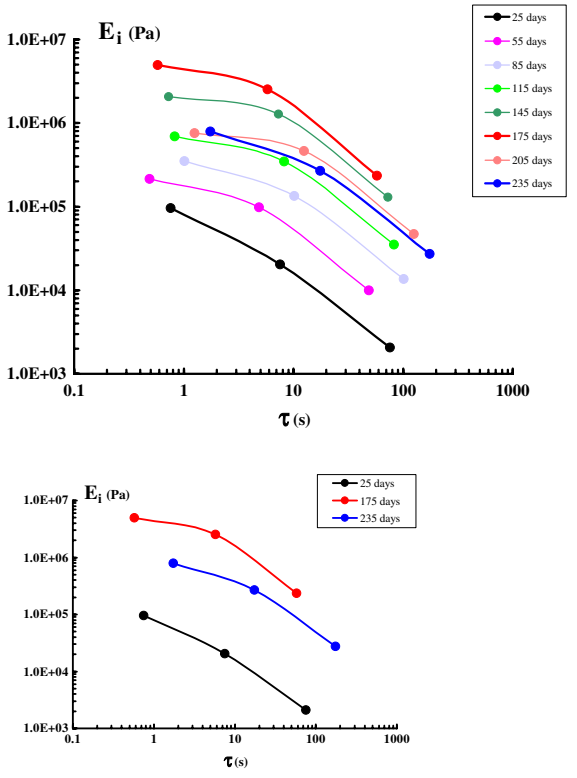


Fig. 4. Dependence of E_i on τ_i for GG/Ga samples kept at 7 °C at different ageing times. The solid lines are only a guide to the eye. In the insert the data obtained after 25 days (first measurement), after 175 days (maximum registered values) and after 235 days (last measurement) are reported. $c_p = 1.5\%$ (w/V); $r = 4.0$. $N = 3$.

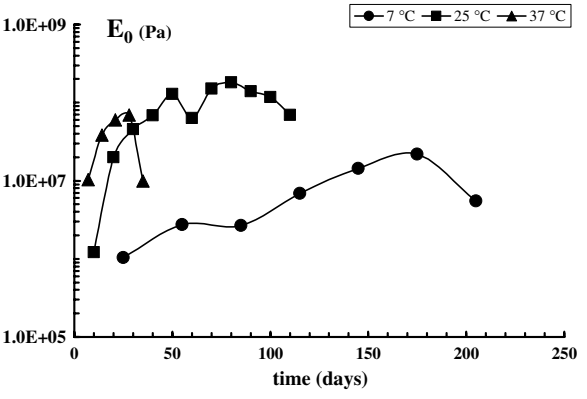


Fig. 5. Time dependence of E_0 for the hydrogels kept at 7, 25 and 37 °C.

average cross-link density can change over the time also because additional topological entanglements take place together with the syneresis process. Both these aspects influence the polymeric mesh sizes with the effect that E_0 increases with time. Obviously, when in the network structure prevails the degradation process, because of the long ageing time (as in the case of the samples kept at 7 °C), or because of the relative high temperature (as in the case of the samples kept at 37 °C), or because of the sum of the two effects (as in the case of the samples kept at 25 °C), also E_0 starts to decrease.

This aspect can be very important in the case, for example, of gels loaded with drugs and used for implantation in the human body: after ca. one month the gels, at 37 °C, loose their strength and degrade leading to biocompatible products and, at the same time, releasing all the drug still present in the matrix.

3.4. Rheological experiments

A chemical network is formed with the polymeric chains showing interconnections by covalent bonds with the cross-linker molecules. Theoretically the gel point is reached when the largest supramolecular cluster diverges to infinity, i.e. the second moment of the molecular weight distribution increases enormously. Though this critical point cannot be directly measured, nevertheless the rheological properties are very sensitive indicators and in the last decades have been widely applied, together with static and dynamic light scattering measurements, to elucidate the sol–gel transition. Winter and Chambon where the first ones reporting a power law behaviour for the shear modulus over a wide range of shear frequencies of a permanently gelling system [31] and they generalized a scaling law of the two moduli:

$$G'(\omega) \propto G''(\omega) \propto \omega^n \quad \text{with } 0 < n < 1.$$

In the case of the gelation of GG with the Ga molecules the reaction was followed at 37 °C, monitoring the behaviour of the shear storage and loss moduli over a wide range of frequencies (Fig. 6), immediately after the addition of the cross-linker, and until the gel was completely cured. As expected, at the beginning the mixture behaved as a macromolecular semi-dilute solution (Fig. 6a) and the loss modulus, G'', prevails over the storage modulus, G', showing the crossover frequency point of the moduli at about 1 Hz. Furthermore, both G' and G'', exhibit a significant dependence on the frequency and have rather low absolute values, as expected in the case of a concentrated solution. In fact, the variation of the moduli with frequency

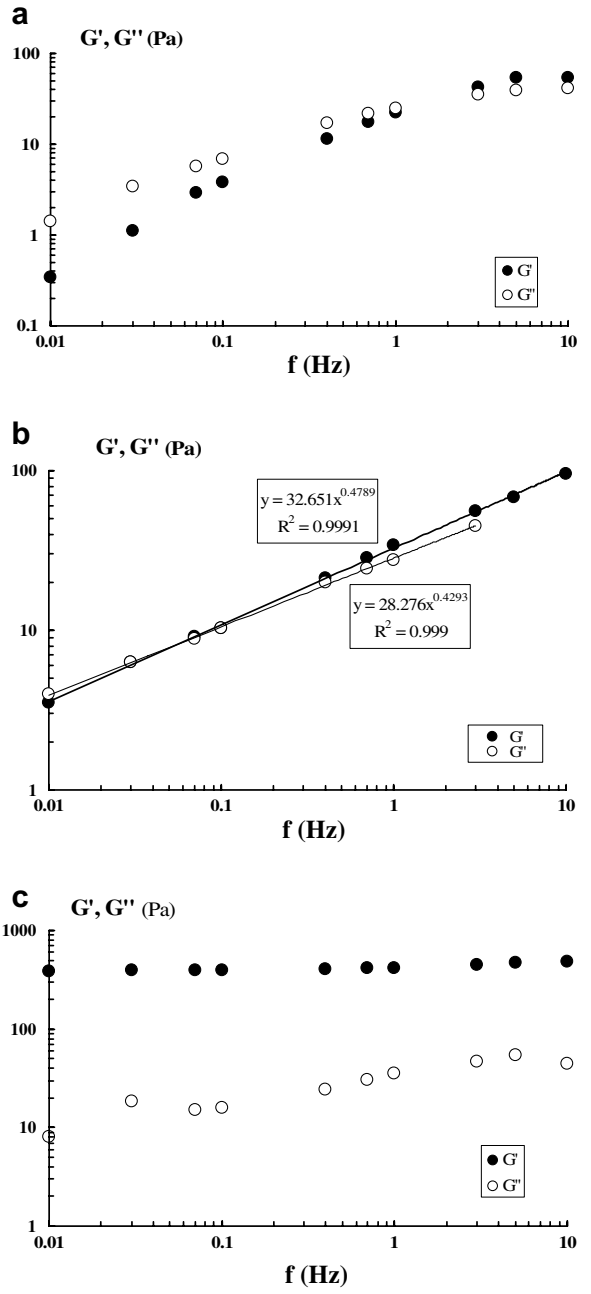


Fig. 6. Double-logarithmic plot of the storage $G'(\omega)$ and loss $G''(\omega)$ shear moduli as a function of angular frequency recorded at three different times after Ga addition to a GG solution kept at 37 °C. (a) ca. 4 min after the addition of Ga; (b) ca. 70 min after the addition of Ga; (c) ca. 4 h after the addition of Ga. $c_p = 1.5\%$ (w/V); $r = 4.0$. G': full symbols; G'': empty symbols.

follows a power law with an exponent of ~0.8 for G'' and ~1.0 for G'; these values are far below the theoretical ones (1 for G'' and 2 for G') since the investigated GG solutions have a $c_p = 1.5\%$, i.e. much above the c^*, overlapping concentration, that is estimated to be ~0.19% [32] and even much above the second critical concentration, c^{**}, estimated to be 0.28% [33]. Furthermore, it is known that GG molecules exhibit a dependence of the specific viscosity on concentration much higher than that usually observed for other polymers interacting purely by physical entanglements thus indicating the presence of more specific polymer–polymer interactions or hyperentanglements [34]. Subsequently, after ca. 1 h, a power law behaviour is detected, with the two moduli being almost parallel and coincident, in the explored frequency interval (Fig. 6b):

$$G'(\omega) \propto \omega^{0.48}, \quad G''(\omega) \propto \omega^{0.43}$$

Such power law is present over almost 3 decades in the frequency range of $\omega = 0.01$–10 rad s^{-1}. Obviously the real gel point is not matched perfectly and this is due to the fact that, during the experiment, the system continues to evolve and the extent of reaction to increase: as a consequence the two exponents cannot show the same value and therefore the given n value has to be taken as an estimation in the vicinity of the gel point.

According to different theories the predicted value of n changes significantly: for the percolation theory n should be equal to 0.73 [35], for the model without hydrodynamic interactions between the polymeric clusters (Rouse model) n should be equal to 0.66 [36], while for the mean-field theory n should be equal to 1 [37]. It is now well ascertained that there is no universal value for n. In fact, according to experimental data, the exponent value depends on several parameters, such as polymer concentration, molecular weight and the specific pre-gel history [38–46]. Furthermore, the relaxation exponent, n, assumes smaller values when the cross-linker concentration is higher than the stoichiometric ratio r [47]. Accordingly, our samples, prepared with an excess of cross-linker ($r = 4$), showed a critical exponent $n = 0.46 \pm 0.03$, smaller than that predictable when stoichiometric quantities are employed. A low n value implies that the material is mostly an elastic body with the limit of $G'' = 0$ at $n = 0$ and *vice versa* [47].

At the end of the experiment the two moduli run parallel to each other and are independent on the frequency (Fig. 6c). Furthermore, the storage modulus, at this time, reaches absolute values of ca. 400 Pa and is higher than the loss modulus of a factor almost 10^2: according to these characteristics the gel can be classified as a strong gel or a viscoelastic solid.

In Fig. 7 the evolution of tan δ of GG/Ga sample during the isothermal cross-linking reaction at 37 °C with the frequency is reported. tan δ decreases with reaction time, as expected from theory, and it is equal to 1 at the gel point:

$$G''(\omega)/G'(\omega) = \tan\delta \tag{6}$$

The phase angle (δ) between stress and strain is independent of frequency (ω) but proportional to the relaxation exponent:

$$\delta = n\pi/2 \tag{7}$$

The frequency independence of the loss tangent in the vicinity of the gel point has been widely examined for chemical and physical gels and has also been employed to determine the gel point [47–50].

The gel point, which is easily detected by its self-similarity (δ = constant over the terminal frequency range), occurred about 70 min after Ga addition. This value appears to be in good agreement, considering the experimental errors, with the mechanical spectra reported in Fig. 6b. This time is experimentally important even if it cannot be completely interpreted because the cross-linking reaction has already proceeded during the sample measurement.

The power law variation of the dynamic moduli at the gel point was related also to the self-similarity or fractal nature of the cluster that, at the percola-

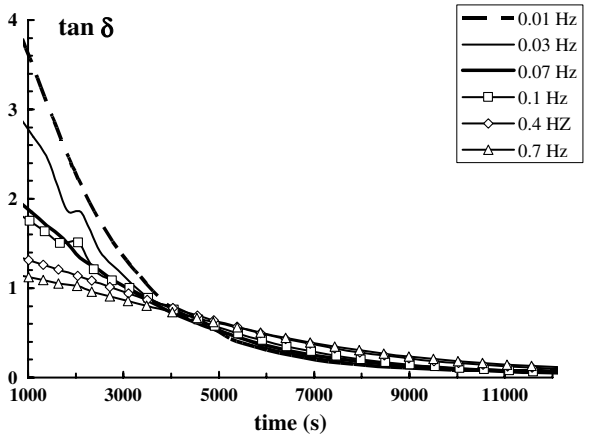


Fig. 7. Evolution, at 37 °C, of tan δ with the frequency for the GG/Ga sample after the addition of Ga. The maximum strain amplitude was 10%.

tion threshold, expands through the whole sample becoming infinite. The fractal dimension, d_f, measures how open or packed a structure is: lower fractal dimensions indicate a more open system while higher fractal dimensions indicate a more packed system. Theories relating d_f to the relaxation exponent, n, are based on whether the excluded volume of the polymer chains is screened or unscreened under conditions near the gel point [51,52]. The excluded volume of the macromolecular chains is progressively screened as the polymer concentration is increased, the size of the chains eventually approaching their unperturbed dimensions. Such screening is expected to occur near the gel point where the situation is similar to that found in a polymer melt, at least as far as the excluded volume effects are concerned. In the case of a polydisperse solution of polymers, when the excluded volume is fully screened, the relaxation exponent can be related to the fractal dimension at the gel point by the following equation [51]:

$$n = \frac{d(d+2-2d_f)}{2(d+2-d_f)} \tag{8}$$

where d is the space dimension, which in this case is 3. By applying Eq. (8), and inserting the average value of n estimated for GG/Ga, a fractal dimension of 2.0 is obtained. This is a relative high value, indicative of a rather "tight" network as it should be expected in presence of a high molecular weight polymer ($M_w = 2.7 \times 10^6$ g/mol) having small values of both critical concentrations c^* and c^{**} ($c^* = 0.19\%$; $c^{**} = 0.28\%$), and that was gelled at a high c_p value ($c_p = 1.5\%$) and with an excess of cross-linker.

4. Conclusions

The hydrogel prepared by chemical reaction between GG and Ga appears to be suitable as a matrix for modified drug delivery. According to the mesh size of the system, the network is capable to discriminate between two guest molecules of different steric hindrance. This effect is maintained, although at a different extent, when the hydrogel is used for the preparation of tablets. Furthermore, the study carried out as a function of temperature shows that, when the gels are kept at 37 °C, slightly after one month, they start to degrade. Implantation systems for relative long-term therapies, can benefit of this aspect, especially considering that the degradation products are known to be non toxic and biocompatible. The leaching of the drug could be supported by the shrinking process at the beginning and later on by the chemical rupture of the network structure.

The dynamo-mechanical studies, carried out at three temperatures, show the different stability in terms of hardness and cohesiveness. At 7 °C the gels show the lowest syneresis and also the slowest rate to reach the highest hardness value. At 25 °C a shorter time interval is needed to reach the maximum value of hardness, while at 37 °C all the processes occurring in the systems are significantly faster. According to the relaxation experiments these gels can be classified as solid viscoelastic materials as they show, at all investigated temperatures, an elastic residual modulus, E_0, that increases with time until the maximum values are reached and then decreases when the degradation phenomenon prevails. Mechanical spectra reveal a predominance of the viscous behaviour and, also in this case, the temperature increase leads to higher values of the moduli E_i.

The sol–gel transition of the system kept at 37 °C could be detected by means of oscillatory tests: the power law behaviour for the two moduli was evidenced in correspondence of a critical time of ca. 1 h. The evaluated critical exponent, $n = 0.46$, lies in the experimental range of values already found for several other systems where chemical linkages are formed in the presence of an excess of cross-linkers. This result confirms the statement that, though the gel point has a critical nature, the relaxation exponent has not a universal value being deeply dependent on stoichiometry, on chain length and on concentration of the polymeric system. Furthermore, as theoretically expected, at the gel point the phase angle between stress and strain, δ, is independent of frequency. The rather low value of δ found for our system, $\sim 33°$, is indicative of a network with a relative high elastic component, as already evidenced by the Maxwell generalized model applied to the relaxation experiments. Finally, the fractal dimension of GG cross-linked with Ga at 37 °C was estimated assuming a fully screened system; the obtained value of d_f equal to 2 is indicative of a very packed network.

As far as we know, this is the first time that both techniques, rheology and texture analysis, are being applied to gel systems and the parameters obtained at the critical point from the frequency analysis are correlated to those obtained with the dynamometer approach.

This aspect is very important also in relation to the practical application that such kind of hydrogels can have in the biomedical field as matrices for modified drug delivery.

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