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Scleroglucan/borax/drug hydrogels: Structure characterisation by means of rheological and diffusion experiments

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ABSTRACT

This paper is aimed to the macro- and microscopic characterisation of a scleroglucan–borax gel system eventually hosting model drugs. A rheological study was carried out, also as a function of temperature, on samples at different polymer concentrations in the presence and without guest model drug. Applying the generalised Maxwell model the mechanical spectra, showing the gel-like characteristics dependent on polymer concentration, were obtained. Flory's theory and the equivalent network approach were applied to the rheological data in order to describe the molecular arrangement of the scleroglucan chains in the presence of borate ions with an estimation of the network mesh size, ξ. Diffusion experiments of three model drugs, with different van der Waals radius, allowed the determination of the diffusion coefficients. An evaluation of the network mesh size was also obtained by means of a modified free volume theory and a good agreement between the two sets of ξ values was found.

1. Introduction

Scleroglucan (SCLG), a water soluble polysaccharide produced by fungi of the genus *Sclerotium*, consists of a main chain of (1–3)-linked β-D-glucopyranosyl units bearing, every third unit, a single β-D-glucopyranosyl unit linked (1–6). It is known that SCLG assumes a triple-stranded helical conformation in aqueous solution and a single coiled disordered conformation in methylsulphoxide or at high pH values (NaOH >0.2 M) (Norisuye, Yanaki, & Fujita, 1980; Sato, Norisuye, & Fujita, 1981; Sato, Norisuye, & Fujita, 1983; Yanaki, Kojima, & Norisuye, 1981; Yanaki, Norisuye, & Fujita, 1980). Due to its peculiar properties, SCLG was extensively used for various commercial applications (secondary oil recovery, ceramic glazes, food, paints, cosmetics, etc.) (Giavasis, Harvey, & McNeil, 2002); it was also investigated for modified/sustained release formulations (Coviello, Coluzzi, et al., 2003; Coviello, Grassi, Lapasin, Marino, & Alhaique, 2003; Coviello et al., 2005; Palleschi, Coviello, Bocchinfuso, & Alhaique, 2006; Rizk et al., 1994.) and ophthalmic preparations (Romanelli, Alhaique, Riccieri, Santucci, & Valeri, 1993); furthermore, also oxidized scleroglucan was proposed for a controlled delivery from oral dosage forms (Alhaique, Riccieri, Santucci, Crescenzi, & Gamini, 1985; Coviello, Alhaique, et al., 2005). In aqueous solutions, up to a concentration of 0.25% (w/V), SCLG shows a pseudoplastic behaviour that becomes plastic for higher concentrations (Grassi, Lapasin, & Pricl, 1996) evidencing a transition from the sol state to a weak gel.

More recently, by addition of borax to a SCLG solution, we obtained a new stronger gel that, in the form of tablets, can be used for a modified delivery of guest molecules: furthermore, the obtained matrices show a remarkable asymmetric swelling (Coviello, Grassi, Lapasin, Marino, & Alhaique, 2003; Coviello, Grassi, et al., 2005; Coviello et al., 2003). Borax has been previously studied as a cross-linker for polymers containing hydroxyl groups, and it has been already used, in the field of pharmaceutics, with guar gum for a colon delivery formulation (Rubinstein & Gliko-Kabir, 1995). It is well known that to improve the release properties of polymeric systems, it is crucial to describe and to understand the macro- and microscopic properties of the matrices. For this purpose, a rheological study was carried out on samples of scleroglucan–borax hydrogel in order to analyse the effect that the polymer concentration (keeping constant the ratio between the cross-linker moles and the moles of repeating units of polymer), the temperature, the pH and the presence of guest molecules (of different steric hindrance) can exert on the macromolecular network. Thus, stress sweep experiments were carried out to determine the linear viscoelastic range. Frequency sweep experiments were also performed and, applying the Flory's theory, an evaluation of the cross-linking density is given. From this value, according to the equivalent network model (Schurz, 1991), an estimation of the average mesh size,

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ξ, of the hydrogel networks is given together with the dependence of this parameter on the temperature and on the presence of host molecules (e.g. Theophylline, Vitamin B_{12} and Myoglobin).

The comparison of this information, with that from the experimental determination of the diffusion coefficients of different model drugs, leads to an excellent agreement between the two values of ξ and it allows to depict a possible microscopic structure of the network. As far as we know, this is the first time that two completely different approaches, the rheological study and the diffusion experiments, lead to an almost coincident estimation of such microscopic parameter, very important for the description of gel networks and therefore very useful for the understanding of the delivery properties of this kind of matrix.

2. Materials and methods

2.1. Materials

Scleroglucan (SCLG) (Actigum CS 11) was provided by Mero-Rousselot-Satia (France) (molecular weight = 1.4×10^6 from viscometric measurements). Theophylline (TPH, molecular weight 198) and borax were Carlo Erba products (Italy), while Vitamin B_{12} (B_{12}, molecular weight 1355) and Myoglobin (MGB, molecular weight 17,800) were purchased from Fluka (Germany). All other products and reagents were of analytical grade. Distilled water was always used.

2.2. Hydrogel preparation

SCLG purification consisted in dissolving 5 g of SCLG in distilled water and allowing system homogenisation *via* magnetic and mechanical stirring at room temperature for 24 h. The solution was exhaustively dialyzed at 7 °C against distilled water and then freeze-dried.

Drug loaded hydrogels were obtained by adding a known amount of drug to distilled water, followed by addition of a calculated amount of purified SCLG. Systems homogenisation was ensured by 24 h magnetic stirring. SCLG cross-linking was carried out by addition of 0.1 M borax solution to the homogeneous drug–polymer system in order to get a unitary value of the ratio *r* between borax moles and moles of the repeating SCLG units. Mixture was magnetically stirred for 5 min and then left for 2 days at 7 °C for gel setting. Final polymer concentrations (C_p) were 0.5, 0.6, 0.7, 1.0, 1.5 and 2.3 g/100 cm^3 (in the following also referred to as 0.5%, 0.6%, 0.7%, 1.0%, 1.5% and 2.3%) while drug concentration was always 5 mg/cm^3. The investigated polymer concentration range was selected because 0.7% represents the lowest polymer content allowing the formation of a well appreciable self-sustaining gel, while 2.3% is approximately equal to the polymer concentration occurring in completely swollen tablet obtained by compression of dried SCLG–drug–borax systems (Coviello, Grassi, et al., 2003). Drug free hydrogels were obtained according to the same procedure except for drug absence in the initial SCLG solution.

In the presence of borax, the system is self buffered at pH 9.0; in same cases the gel was prepared in NaOH aqueous solutions at different concentrations (pH 10, 12, 13 and 14) to test the network stability at high pH.

2.3. Release experiments from gels

The gel, freshly prepared in a beaker (C_p = 0.7%), was obtained, as described above, in the presence of the guest molecule, with the cylindrical shape of the glass vessel (height = 1.0 cm, diameter = 2.2 cm). During the release experiment, carried out in distilled water (200 cm^3, pH 5.5) at 37 °C, the gel was kept at a certain height from the bottom of the container by a thin web, while the medium was gently magnetically stirred. Samples (3 cm^3) were withdrawn from the solution at appropriate time intervals and replaced by the same amount of fresh solvent (concentration data were corrected for dilution). TPH, B_{12} or MGB were spectrophotometrically detected at 272, 361 and 409 nm, respectively, (Perkin-Elmer, lambda 3a, UV–Visible spectrometer) using quartz cells with path-lengths of 1.0 or 0.1 cm. All experiments were carried out in triplicate and the values reported in the present paper represent mean values and lay within 10% of the mean.

The possible erosion of the gel, in terms of polymer dissolution in the medium during the release experiments, was quantitatively determined by a colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using phenol in the presence of sulphuric acid. Obtained results indicate that such erosion, in the first 8 h, is almost negligible ($\leq$4%).

In order to evaluate model drug diffusion coefficient inside the gel network, release data were fitted by a mathematical model relaying on the observation that in the first 8 h our gel did not undergo significant erosion or further swelling when placed in the release environment. Accordingly, model drug release could be studied by Fick's second law. Due to gel symmetry, the intrinsically three-dimensional diffusive problem could be reduced to a simpler two-dimensional one:

$$\frac{\partial C}{\partial t} = \frac{D}{R}\frac{\partial}{\partial R}\left(R\frac{\partial C}{\partial R}\right) + D\frac{\partial^2 C}{\partial Z^2} \tag{1}$$

where D is the drug diffusion coefficient in the gel, t is time, C is the drug concentration (mass/volume) in the cylinder, R and Z are the radial and axial axes, respectively. This equation must satisfy the following initial and boundary conditions:

Initial conditions:

$$C(Z,R) = C_0, \qquad -Z_c \leqslant Z \leqslant Z_c, \quad 0 \leqslant R \leqslant R_c \tag{2}$$

$$C_r = 0 \tag{3}$$

Boundary conditions:

$$C(Z,R_c,t) = C(\pm Z_c,R,t) = k_p C_r(t) \tag{4}$$

$$V_r C_r(t) = \pi R_c^2 2Z_c C_0 - \int_0^{Z_c}\int_0^{R_c} C(Z,R,t)\,2\pi R\,dR\,dZ \tag{5}$$

where $2Z_c$ and R_c are, respectively, cylinder height and radius, C_0 is the initial drug concentration in the cylinder, C_r and V_r are the drug concentration and the volume of the release medium while k_p is the drug partition coefficient between the cylindrical gel and the environmental release fluid. Eqs. (2) and (3) state, respectively, that the gel is uniformly loaded with a drug at C_0 concentration, while the release environment is initially drug free. Eq. (4) expresses the partitioning condition at the cylinder/release fluid interface, while Eq. (5) is a drug mass balance for the gel/release fluid system allowing to state the relation between C_r and $C(Z, R, t)$. In order to account for the diffusion resistance exerted by the thin web surrounding the cylindrical gel (it slightly reduces release surface and favours the formation of a thick aqueous boundary layer at the gel/release environment interface), an interfacial diffusion coefficient (D_i), lower than the bulk one (D), is introduced. Thus, the proposed model is characterised by two fitting (unknowns) parameters as k_p was set equal to 1. Model numerical solution was performed according to the control volume method (Patankar, 1990). This approach is based on the calculation domain subdivision into a number of non-overlapping control volumes (rings, in our 2D frame) where Eq. (1) is integrated assuming uniform model drug concentration in each control volume. The interfacial diffusion coefficient D_i is assigned to the external surface of the control volumes facing the release

environment. By doing so, we render its effect independent of control volume thicknesses in the radial or longitudinal directions. In order to ensure numerical solution reliability, the domain was subdivided into 100 control volumes in the radial and axial direction (for a total of 10^4 control volumes) and integration time step was set equal to 22.5 s.

It is worth noticing that the knowledge of the drug diffusion coefficient in the polymeric network (D) and in the swelling agent (water in our case, D_0) allows an approximate estimation of the polymeric network average mesh size ξ (intended as the average distance between two consecutive cross-links). Indeed, Peppas and co-workers (Peppas, 1984; Peppas, Bures, Leobandung, & Ichikawa, 2000; Peppas & Korsmeyer, 1998, chap. 6) combining the free volume theory (Vrentas, Duda, Ju, & Liu, 1982) with the assumption that the probability that a solute of radius r has to pass through an opening of diameter ξ is linearly dependent on the ratio $2r/\xi$, determine the following relation between the ratio D/D_0, $2r/\xi$ and polymer volume fraction φ:

$$\frac{D}{D_0} = \left(1 - \frac{2r}{\xi}\right) \exp\left(-Y \frac{\varphi}{1-\varphi}\right) \tag{6}$$

where Y comes from the free volume theory and represents the ratio of the critical volume required for a successful translational movement of the solute molecule and the average free volume per molecule of the swelling agent. Although Y depends on many factors, the authors suggest that, lacking deeper information, it can be approximately assumed as equal to one. Accordingly, Eq. (6) allows ξ estimation once D/D_0, φ and r are known (Hubble & Zhang, 2008, chap. 16). Although polymer volume fraction increases with time due to drug release, in the light of its small variation imputable to drug disappearing in the gel, we assume that φ, and thus D (see Eq. (6)), are constant along the whole release experiment.

2.4. Rheological characterisation

Hydrogels rheological characterisation was performed by means of a controlled stress rheometer Haake Rheo-Stress RS150, using, as measuring device, a shagreened plate and plate apparatus (PP35 TI: diameter = 35 mm; gap between plates = 1 mm). This kind of device was needed in order to avoid possible sample slippage phenomena at the wall (Lapasin & Pricl, 1995). Rheological properties were studied under small and large deformations by applying different procedures: stress (f = 1 Hz, $\omega = 2\pi f$ = 6.28 rad/s) and frequency sweep (in the linear viscoelastic region; constant deformation γ = 0.05). Frequency sweep tests were interpreted by means of the generalised Maxwell model representing material rheological behaviour by means of a sequence of Maxwell elements in parallel, each one constituted by a spring and a dashpot in series. This model states that material elastic and viscous character are represented by the elastic (G') and viscous (G'') moduli that depend on pulsation ω according to the following expressions (Lapasin & Pricl, 1995):

$$G' = G_\infty + \sum_{i=1}^{n-1} G_i \frac{(\lambda_i \omega)^2}{1 + (\lambda_i \omega)^2}; \qquad G_i = \eta_i / \lambda_i \tag{7}$$

$$G'' = \sum_{i=1}^{n} G_i \frac{\omega \lambda_i}{1 + (\lambda_i \omega)^2} \tag{8}$$

where n is the number of Maxwell elements considered, G_i, η_i and λ_i represent, respectively, the spring constant, the dashpot viscosity and the relaxation time of the ith Maxwell element, while G_∞ is the spring constant of the last Maxwell element which is supposed to be a pure elastic element without its dashpot (G_∞ is typical of purely elastic network, i.e. of a chemically cross-linked network).

Among other interesting information about macroscopic behaviour (i.e. mechanical and relaxation spectra), rheological characterisation allows an estimation of the polymeric network nanostructure via the determination of the cross-link density ρ_x, defined as the moles of junctions between different polymeric chains per hydrogel unit volume. Indeed, Flory's theory (Flory, 1953) ensures the following relations between ρ_x and hydrogel shear modulus G:

$$\rho_x = (G/RT)\varphi^{2/3} \tag{9}$$

$$\rho_x = G/RT \tag{10}$$

where R is the universal gas constant, T is the temperature, φ is the polymer volume fraction in the swollen condition and G can be computed as the sum of the elastic contribution (G_i) pertaining to each element of the generalised Maxwell model describing hydrogel mechanical spectrum (Pasut et al., 2008). It has to be underlined that, while Eq. (9) holds for a gel system that was cross-linked in dry conditions (i.e. in the absence of the swelling solvent) and then swollen up to a polymer volume fraction equal to φ, Eq. (10) holds for the opposite case where network cross-linking is performed in wet conditions, i.e. polymeric chains undergo cross-linking in solution without undergoing further swelling (i.e. the situation of the present work). ρ_x knowledge, jointly with the equivalent network theory (Schurz, 1991), allow the estimation of the polymeric network average mesh size, ξ. Indeed, equivalent network theory, starting from the evidence that, in the majority of the situations, a detailed description of a real polymeric network is very hard (Hild, 1998) if not impossible, suggests to replace it by and idealised one made up by a collection of spheres whose diameter coincides with the average network mesh size ξ. Remembering the definition of cross-link density (moles of cross-links per hydrogel unit volume), it turns out that sphere volume is exactly equal to $1/(N_A \rho_x)$ (this is the volume competing to each cross-link):

$$\frac{4}{3}\pi\left(\frac{\xi}{2}\right)^3 = \frac{1}{\rho_x N_A} \tag{11}$$

where N_A is the Avogadro number.

Solving Eq. (11) for ξ leads to:

$$\xi = \sqrt[3]{6/\pi \rho_x N_A} \tag{12}$$

Eq. (12) allows ξ estimation on the basis of ρ_x knowledge which in turn depends on hydrogel shear modulus (G), determinable by rheological measurements.

3. Results and discussion

Rheological stress sweep tests performed at T = 25 °C, referring to hydrogels characterised by different polymer concentrations and not containing any model drug, indicate that the extension of the linear viscoelastic regime strongly depends on polymer concentration, as witnessed by Fig. 1. Indeed, Table 1, reporting the values of the critical percentage deformation (γ_c) and stress (τ_c) (linear viscoelasticity no longer holds when deformation and stress overcome γ_c and τ_c, respectively), shows a clear, non-linear increase of γ_c and τ_c with polymer concentration C_p. According to this evidence, frequency sweep tests were carried out at a constant deformation of 5%, a value lying within the linear viscoelastic field.

Fig. 2 sums up the effect of polymer concentration on hydrogel macroscopic rheological behaviour reporting the trend of the complex modulus $G^* = \sqrt{(G')^2 + (G'')^2}$ versus pulsation $\omega = 2\pi f$ (G' and G'' are the storage and loss moduli, respectively, while f is the solicitation frequency). It turns out that G^* is slightly dependent on ω and that the effect of C_p is not linear. Indeed, if the 0.5% and 0.6%

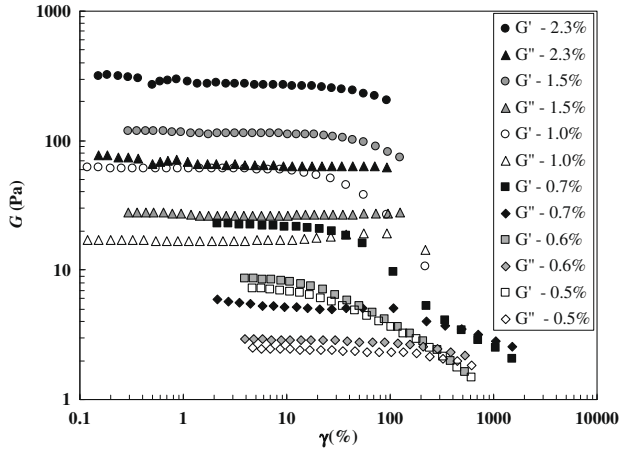

Fig. 1. Stress sweep tests (1 Hz and 25 °C) for different polymer concentrations C_p. γ is the deformation, while G' and G'' indicate, respectively, the storage (or elastic) and loss (or viscous) moduli.

Table 1
Dependence of the critical percentage deformation (γ_c) and critical stress (τ_c) on polymer concentration (C_p) for SCLG–borax samples.

T = 25 °C		
C_p (g/100 cm^3)	γ_c (%)	τ_c (Pa)
0.5	9	0.7
0.6	10	0.9
0.7	11	2.5
1.0	15	8.7
1.5	20	24.5
2.3	40	117.5

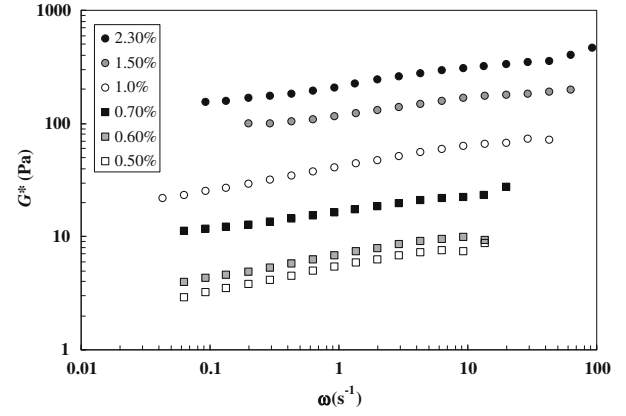

Fig. 2. Complex modulus $G^* = \sqrt{(G')^2 + (G'')^2}$ trend versus solicitation pulsation $\omega = 2\pi f$ (f is solicitation frequency) for different polymer concentrations (C_p) of the SCLG–borax system (T = 25 °C). G' and G'' indicate, respectively, the elastic (or storage) and viscous (or loss) moduli.

systems are very similar, the 0.7% system implies a significant increase of G^*. 1.0% system lies in between 0.7% and 1.5% system, while the 1.5% system is closer to the 2.3% system than to the 1.0% sample. 1.5% system is characterised by a modulus G^* that is about one order of magnitude higher than that competing to the 0.7% sample in the whole considered frequency range. Interestingly, the 2.3% system is characterised by a complex modulus G^* that is only two times of that competing to the 1.5% sample. A deeper insight of system rheological properties can be deduced by fitting the generalised Maxwell model (Eqs. (7) and (8)) to the experimental frequency sweep data according to a consolidated approach (Lapasin & Pricl, 1995). In particular, fitting was performed assuming that relaxation times λ_i (= η_i/G_i) are not independent each other but they are scaled by a factor 10 ($\lambda_i = 10 * \lambda_{i-1}$). Accordingly, only G_∞, η_i and λ_1 represent real model fitting parameters. In addition, the optimum number of Maxwell elements to be considered was that minimising the product ($\chi^2 * N_p$), where χ^2 is the sum of the squared errors while N_p indicates the number of fitting parameters considered. The resulting relaxation spectra make clear that for the higher polymer concentrations (2.3% and 1.5%) the typical gel behaviour occurred as G_i are substantially λ_i independent (see Fig. 3 and Table 2). Apart from higher moduli G_i, the C_p = 2.3% gel differs from the C_p = 1.5% sample mainly for a higher pure elastic contribution (see insert in Fig. 3). The relaxation spectrum of the C_p = 1.0% system would suggest a weak gel behaviour as G_i decrease with λ_i. Nevertheless, as its pure elastic contribute (see insert in Fig. 3) is higher than that competing to the 1.5% system, we can still affirm that elastic properties prevail on viscous ones. The relaxation spectrum of the C_p = 0.7% system still resembles that of a gel although a weak G_i dependence on λ_i can be observed and G_i are significantly lower that those competing to the C_p = 2.3%, 1.5% and 1.0% gels. Interestingly, also in this case the pure elastic contribution is considerable as it is just lower than that competing to the C_p 1.5% gel. The C_p = 0.6% and 0.5% systems, instead, show a mechanical spectrum characterised by a decreasing of G_i with increasing λ_i, a behaviour typical of liquid-like materials where the rheological characteristics substantially depend on fast relaxing Maxwell elements. In addition, G_∞ considerably decreases to get a vanishing value for the C_p = 0.5% system.

In order to reduce the number of fitting parameters in the generalised Maxwell model and to immediately see the gel behaviour of our systems, we can assume that G_i depend on λ_i according to $G_i = a * \lambda_i^b$ where a and b are fitting parameters. Indeed, when b approaches zero, G_i result independent of λ_i in a Log–Log plot diagram and this represents the typical gel condition. Table 3, reporting the results of data fitting for the different tested polymer concentrations, shows that while b is very small for C_p = 2.3%, 1.5% and 0.7%, for the other three systems it is neatly higher (in absolute terms). The anomalous behaviour of the 1.0% system in terms of parameter b value, is compensated by its high G_∞ value. Interestingly, for each system, the sum of the G_i, determined according to the two adopted strategies (first strategy, G_∞, η_i and λ_1 as fitting parameters; second strategy, a, b, G_∞ and λ_1), leads to similar results (see Table 3). This is very important as far as the determi-

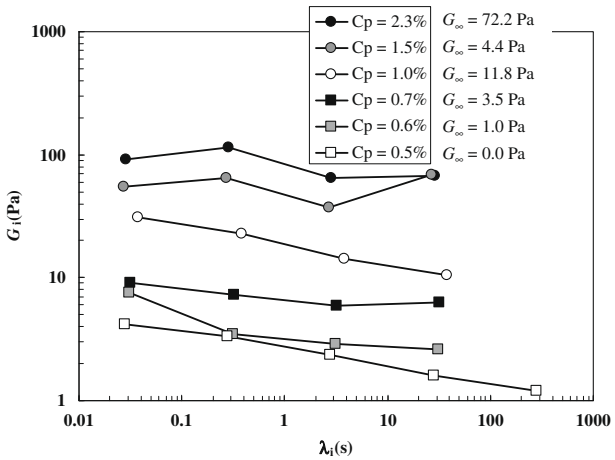

Fig. 3. Relaxation spectra dependence on polymer concentration, C_p, for the SCLG–borax system. G_i and λ_i indicate, respectively, spring modulus and relaxation time of the ith generalised Maxwell model element. G_∞ is the spring constant of the last Maxwell element which is supposed to be a pure elastic element without its dashpot.

Table 2
Results of frequency sweep data fitting according to the generalised Maxwell model assuming G_∞, λ_1 and G_i as fitting parameters (first fitting strategy). C_p represents the polymer concentration.

C_p (g/100 cm^3)	G_∞ (Pa)	λ_1 (s)	G_1 (Pa)	G_2 (Pa)	G_3 (Pa)	G_4 (Pa)	G_5 (Pa)
0.5	0.0	0.0279	4.2	3.3	2.3	1.6	1.2
0.6	1.0	0.0311	7.5	3.5	2.9	2.6	0.0
0.7	3.5	0.0319	9.1	7.3	5.9	6.3	0.0
1.0	11.8	0.0377	31.1	22.9	14.3	10.0	0.0
1.5	4.4	0.0270	55.5	64.0	37.4	69.4	0.0
2.3	72.2	0.0286	91.6	114.2	64.2	67.3	0.0

Table 3
Results of frequency sweep data fitting according to the generalised Maxwell model assuming G_∞, λ_1, a and b as fitting parameters ($G_i = a * \lambda_i^b$) (second fitting strategy). C_p represents the polymer concentration, N_m is the number of the generalised Maxwell model elements considered while $\sum G_{i1}$ and $\sum G_{i2}$ are, respectively, the sum of G_i determined according the first (see Table 2) and the second fitting strategy (this table).

C_p (g/100 cm^3)	0.5	0.6	0.7	1.0	1.5	2.3
G_∞ (Pa)	0.9	1.6	5.2	11.9	42.6	93.6
λ_1 (s)	0.044	0.019	0.029	0.038	0.007	0.014
a (Pa s^{-b})	2.7	3.4	6.8	18.6	53.0	82.2
b (–)	−0.156	−0.115	−0.067	−0.166	−0.003	−0.072
N_m (–)	4	4	4	4	4	4
$\sum G_{i1}$ (Pa)	13.0	18.0	33.0	90.6	231.0	409.0
$\sum G_{i2}$ (Pa)	13.3	17.0	33.2	90.8	255.0	447.7

nation of the cross-link density is concerned, as shown in the following.

On the basis of these considerations, we can say that if clear gel behaviour appears for $C_p \geq 0.7$, the strong gel behaviour takes place only for $C_p \geq 1.5\%$. Thus, the $C_p = 1.0\%$ system would lie in the transition zone separating the weak and strong gel behaviour. For $C_p < 0.7\%$, the resulting systems were no longer self sustaining. Accordingly, in the following, attention will be focussed on the $C_p = 0.7\%$ sample.

Fig. 4 reports the effect of pH on G^* for the SCLG–borax sample with $C_p = 0.7\%$ ($T = 25$ °C). It can be noticed that up to pH 12 no significant variations occur. On the other side, pH 13 leads to a small reduction of G^* while pH 14 witnesses polymeric network collapse, as already found in the past for the SCLG system alone (Bluhm, Deslandes, Marchessault, Perez, & Rinaudo 1982; Bo, Milas, & Rinaudo 1987; Kashiwagi, Norisuye, & Fujita, 1981; Tabata, Ito, Kojima, Kawabata, & Misaki, 1981). The destabilization of the triple helix is due to the breakage, by the NaOH, of the hydrogen bonds that are the main responsible for one of the highest molecular structure rigidity found in nature.

In the light of pharmaceutical applications, it is interesting to discuss the possible effects of different size model drugs inside hydrogel network in a relatively high concentration (5 mg/cm^3).

For this purpose, Fig. 5 presents the comparison between our pivot system ($C_p = 0.7\%$, $T = 25$ °C, no drug; white circles) and other three systems differing only for the presence of TPH, B_{12} or MGB, respectively, in the hydrogel. If, in general, the effect of all model drugs is very limited as G^* undergoes variation within two times the reference G^* value competing to the pivot system (white circles), TPH comports a very small increase of G^*, while B_{12} and MGB determine a small G^* decrease. This behaviour could be most probably attributed to the different van der Waals radius of the model drugs ($r_{TPH} = 3.7$ Å; $r_{B12} = 8.5$ Å; $r_{MGB} = 21$ Å) (Coviello, Coluzzi, et al., 2003).

As temperature can play a very important role in determining hydrogel properties (both rheological and diffusive), Fig. 6 shows the effect of temperature on G^* for the $C_p = 0.7\%$ system containing MGB (analogous consideration can be drawn for TPH and B_{12} loaded hydrogels). It can be seen that G^* progressively diminishes with temperature even if only for $T = 37$ °C this decrease is more evident.

As stated above, rheological characterisation can also provide information on gel nanostructure. Indeed, Eq. (10) allows the estimation of polymeric network cross-link density ρ_x. In turn, the equivalent network theory (Schurz, 1991) allows, via Eq. (12), the determination of gel average mesh size ξ.

Table 4, showing ξ values for some of the most interesting systems studied in this paper, evidences that ξ decreases with C_p and about five times C_p increase (0.5–2.3%) implies a reduction of about three times the mesh size. Remembering that for cross-linked polymeric systems it is not unusual having ξ values around 2–5 nm (Kuijpers et al., 1999), it is evident that our hydrogels are characterised by wide meshes. In addition, it can be seen that model drugs presence does not significantly affect the mesh size for

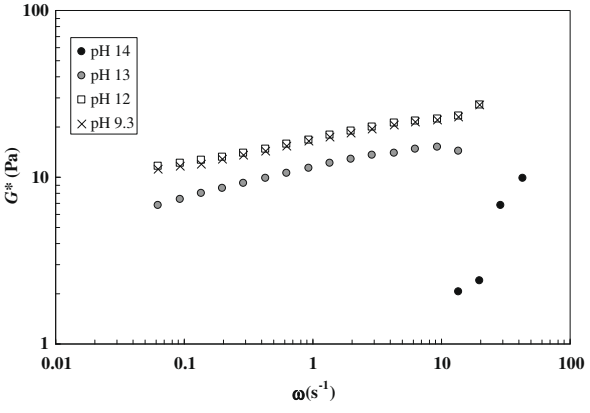

Fig. 4. Complex modulus $G^* = \sqrt{(G')^2 + (G'')^2}$ trend versus solicitation pulsation $\omega = 2\pi f$ (f is solicitation frequency) for different pH of the aqueous medium used to prepare the SCLG–borax hydrogel ($C_p = 0.7\%$, $T = 25$ °C).

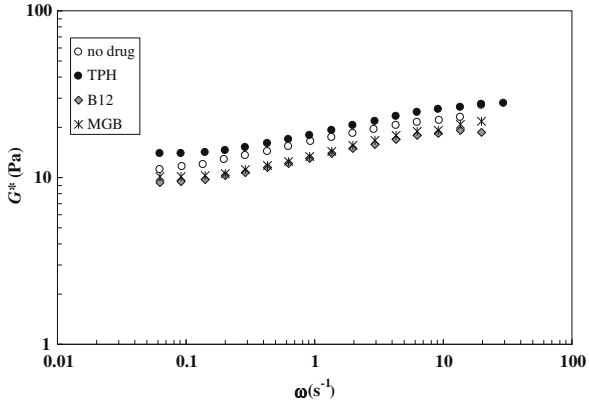

Fig. 5. Comparison between complex modulus $G^* = \sqrt{(G')^2 + (G'')^2}$ trend versus solicitation pulsation $\omega = 2\pi f$ (f is solicitation frequency) for the SCLG–borax system ($C_p = 0.7\%$, $T = 25$ °C) with and without loaded model drugs.

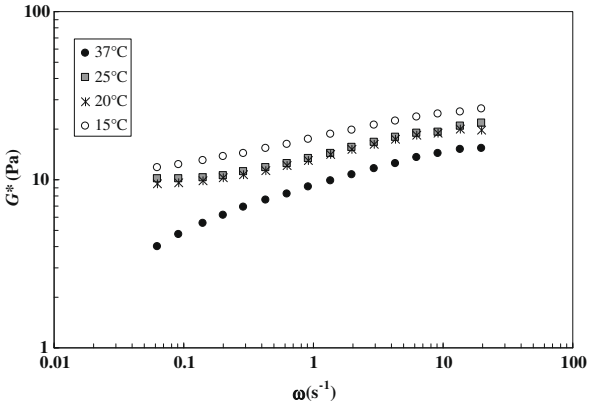

Fig. 6. Effect of temperature on complex modulus trend $G^* = \sqrt{(G')^2 + (G'')^2}$ versus solicitation pulsation $\omega = 2\pi f$ (f is solicitation frequency) for the SCLG–borax system containing Myoglobin (MGB) (C_p = 0.7%, T = 25 °C).

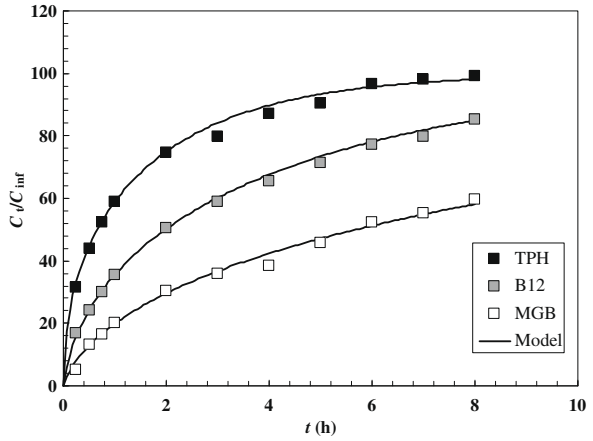

Fig. 7. Comparison between model best fitting (Eqs. (1)–(5), solid lines) and experimental data (distilled water, pH 5.5, 37 °C) referring to theophylline (TPH), vitamin B12 (B_{12}) and myoglobin (MGB) release from a cylindrical SCLG–borax hydrogel (C_p = 0.7%).

C_p = 0.7% and 25 °C. On the contrary, for T = 37 °C, a slight influence of model drugs seems to occur as ξ increases with increasing model drug van der Waals radius (r_{TPH} = 3.7 Å; r_{B12} = 8.5 Å; r_{MGB} = 21 Å) (Coviello, Coluzzi, et al., 2003). It is now interesting to compare these ξ estimations with those coming from release experiments. Accordingly, release mathematical model (Eqs. (1)–(5)) was fitted to experimental data referring to TPH, B_{12} and MGB delivery from C_p = 0.7 gels (37 °C).

Fig. 7 shows the very good agreement between model best fitting (solid lines) and experimental data (symbols). This visual evidence is also supported by F statistic values reported in Table 5.

Interestingly, in the case of TPH, the bulk diffusion coefficient (D) and the interfacial one (D_i) coincide (statistically speaking this means that only one model fitting parameter is needed for data fitting). Thus, the presence of the surrounding net does not represent a further significant resistance for TPH release from cylindrical gel. On the contrary, in the B_{12} and MGB cases, D and D_i are neatly different, being D_i approximately one order of magnitude lower than D (statistically speaking, this means that two fitting parameters are needed for data fitting). Accordingly, in these two cases, the surrounding net does exert an additional diffusive resistance for the release of the two model drugs. Due to the smaller THP radius in comparison to those of B_{12} and MGB, this finding seems reasonable. On the basis of the above found D_i values and the knowledge of model drug diffusion coefficient in water at 37 °C (D_0, see Table 5), Eq. (6) allows the determination of the average polymeric network mesh ξ (see Table 5). The comparison of these results (Table 5) with those shown in Table 4 indicates that rheological and diffusive estimation of ξ are almost coincident. Finally, it is worth mentioning that the results concerning the relation between mesh size and polymer volume fraction at 37 °C (TPH release tests) show a satisfactory agreement with what found by Barba and co-workers (Barba et al., 2009) who worked on TPH release from hydroxypropyl methylcellulose matrices. Indeed, while here we found that ξ = 66 nm when $\varphi = 4.5 \times 10^{-3}$, they found that ξ = 1.1 nm when φ = 0.1 this corresponding to D/D_0 = 0.36. According to Eq. (6), the ratio D/D_0 should be equal to 0.27 when φ = 0.1 and with Y = 1; therefore we can conclude that our findings are in substantial agreement with those of Barba and co-workers.

Table 5
Results of the release mathematical model (Eqs. (1)–(5)) best fitting to experimental data shown in Fig. 6. D is the drug diffusion coefficient in the gel, D_i is the interfacial drug diffusion coefficient, D_0 is the drug diffusion coefficient in water, F is the F-statistic parameter, C_p is the polymer concentration and φ is the polymer volume fraction. All diffusion coefficients are evaluated at 37 °C

	TPH	B_{12}	MGB
D (cm²/s)	$(8.07 \pm 0.85) \times 10^{-6}$	$(3.67 \pm 0.22) \times 10^{-6}$	$(1.21 \pm 0.21) \times 10^{-6}$
D_i (cm²/s)	$(8.07 \pm 0.85) \times 10^{-6}$	$(4.0 \pm 0.4) \times 10^{-7}$	$(1.6 \pm 0.3) \times 10^{-7}$
D_0 (cm²/s)[a]	8.20×10^{-6}	3.80×10^{-6}	1.30×10^{-6}
F(1,10,0.95)	<571	<2717	<376
ξ (nm)	65	57	65
C_p (g/cm³)	0.7		
φ	4.5×10^{-3}		

[a] Grassi, Grassi, Lapasin, and Colombo (2007).

4. Conclusions

Stress sweep tests demonstrated that the extension of the linear viscoelastic regime increases, in a not linear manner, with polymer concentration. Nevertheless, for all systems, a percentage deformation of 5% falls within the linear viscoelastic regime and that is the reason why frequency sweep tests were performed assuming this value for the deformation. These tests indicate that in the polymer

Table 4
Dependence of shear modulus (G), cross-link density (ρ_x) and mesh size (ξ) on polymer concentration (C_p), temperature (T) and model drug (Theophylline, TPH, van der Waals radius 3.7 Å; Vitamin B_{12}, B_{12}, van der Waals radius 8.5 Å; Myoglobin, MGB, van der Waals radius 21.0 Å). φ is the polymer volume fraction.

SCLG–borax					SCLG–borax–drug						
T = 25 °C					T = 25 °C				T = 37 °C		
C_p (g/100 cm³)	$\varphi \times 10^3$ (–)	G (Pa)	ρ_x (mol/ml)	ξ (nm)	C_p (g/100 cm³)	G (Pa)	ρ_x (mol/ml)	ξ (nm)	G (Pa)	ρ_x (mol/ml)	ξ (nm)
0.5	3.2	13	5.1×10^{-9}	85	0.7 (TPH)	34.0	1.4×10^{-8}	61	28.7	1.1×10^{-8}	66
0.6	3.8	18	7.2×10^{-9}	76	0.7 (B_{12})	26.6	1.1×10^{-8}	66	25.4	1.0×10^{-8}	67
0.7	4.5	33	1.3×10^{-8}	61	0.7 (MGB)	33.3	1.3×10^{-8}	62	20.0	8.0×10^{-9}	73
1.0	6.4	91	3.6×10^{-8}	44							
1.5	9.6	231	9.3×10^{-8}	32							
2.3	14.7	409	1.6×10^{-7}	27							

concentration range 0.5–0.6%, our systems are characterised by mechanical spectra showing, in the $\log(G_i)$ versus $\log(\lambda_i)$ diagram, a negative slope. This means that their behaviour is more influenced by fast relaxing components as it happens for liquid-like materials. On the other side, in the concentration range 0.7–2.3%, SCLG–borax systems show gel behaviour. Nevertheless only for $C_p \geqslant 1.5\%$ strong gels behaviour occur as supported by the significant increase of moduli (G_i). Interestingly, C_p increase exerts a non-linear effect on the system complex modulus G^*, embedding both elastic and viscous system characteristics. Indeed, while $C_p = 0.5\%$ and 0.6% systems do not significantly differ for what concerns G^*, a clear increase is observed for the 0.7% hydrogel. G^* increase is even much more pronounced for the 1.0% and 1.5% hydrogels while it is considerably reduced for the highest examined polymer concentration (2.3%). Notably, despite their quite high concentration, the presence of the model drugs does not exert a significant effect on 0.7% hydrogel G^*. This aspect is, of course, very important as delivery system designing can neglect one additional parameter represented by drug presence (assuming that no specific drug–polymer interaction occurs). In addition, temperature variations (15–37 °C) reflect in moderate reductions of G^* for the 0.7% hydrogel. Finally, if gel structure can hold for pH $\leqslant$13, for higher values it collapses.

The combination of frequency sweep tests, Flory theory on polymeric networks and equivalent network theory allows the estimation of the network mesh size ξ in relation to polymer concentration inside the hydrogel. It turns out that, as expected, ξ decreases with polymer concentration (even if not linearly) and that model drug presence does not significantly reflect in ξ variations at 25 °C. On the other side, a small ξ increment is observed at 37 °C for all model drugs. In all considered situations, it turns out that we realised polymeric networks characterised by wide meshes and, thus more suitable for controlling the release of big solutes, such as nucleic acid based drugs (Davia et al., 2009). Interestingly, for the system identified by polymer concentration equal to 0.7%, the estimation of network mesh size coming from the rheological characterisation is in agreement with that coming from the release experiments performed with different drugs. Finally, we would like to point out that a correct modelling of release profile requires assuming the presence of an interfacial diffusive resistance on gel surface determined by the presence of the thin net suspending the cylindrical gel in the release environment.

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