Hydrogels from Scleroglucan and Ionic Crosslinkers: Characterization and Drug Delivery

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ABSTRACT: The polysaccharide scleroglucan (Sclg), exploited as a matrix suitable for modified drug delivery, was crosslinked in the presence of three ions: borate, aluminum, and iron. A rheological investigation indicated the main differences between the hydrogels in their viscoelastic linear response: the Sclg/borax system showed the highest strength when deformed by elongation, whereas the strength of the other systems broke down, in terms of viscosity, at much lower values of the imposed strain. Tablets prepared from the gels showed remarkable differences in their water uptake and dimensional swelling. On the other hand, the tablets, loaded with drugs of different steric hindrances, showed similar release behavior, regardless of the crosslinking agent. Scanning electron microscopy analysis was related to the delivery and rheological profiles. Texture analysis, carried out on tablets swollen for 5 h, showed different values of cohesion. Furthermore, when the generalized Maxwell model was applied to the relaxation data, the obtained mechanical spectra showed a more pronounced solidlike character of the Sclg/iron network in comparison with the prevailing viscous behavior of the other matrices. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 3610–3622, 2010

Key words: drug delivery systems; hydrogels; mechanical properties; rheology; swelling

INTRODUCTION

The use of hydrogels for biomedical and pharmaceutical applications has been widely exploited during the last years, these systems being very versatile materials with respect to their physicochemical properties.[1,2] Special attention has also been devoted to the study of gel preparation by physical and chemical approaches.

It is well known that borax is an efficient crosslinker for polymers bearing hydroxyl groups and that its presence can lead to the formation of gels. The kinds of linkages actually formed are still not completely defined, and up to now, at least two models have been described. The most popular model implies a chemical linkage between borax and two polymeric chains.[3–5] An alternative model, proposed in the past by Shibayama et al.[6] for poly(vinyl alcohol) and again recently suggested for the polysaccharide scleroglucan (Sclg),[7–10] predicts the formation of both chemical and physical linkages.

Within the field of pharmaceutics, borax was proposed for the preparation of a polysaccharide hydrogel suitable as a colon delivery system,[11] and more recently, it was used for the formulation of modified release matrices based on Sclg.[7]

Thus, it seemed interesting to perform further studies exploiting the ability of Sclg to interact with other ions. For this purpose, aluminum [Al(III)] and trivalent iron [Fe(III)] ions were selected.

We report here the results obtained with new hydrogels prepared with Sclg and Al(III) and with Sclg and Fe(III). All the new systems showed the property of being self-sustaining. Thus, it appears worthwhile to characterize the networks, which can be used for biomedical applications, as freshly prepared hydrogels or, after freeze drying, as tablets. The novel systems were studied with a rheological approach (frequency sweep, stress sweep, and flow curves). Furthermore, the gels were loaded with model drugs of different steric hindrances [theophylline (TPH), vitamin B12, and mioglobin (MGB)], freeze-dried, and then used to prepare tablets. The delivery from the oral dosage forms was monitored and compared with that obtained when only Sclg was used. The tablets were characterized in terms of the water uptake and relative increase in height. Peculiar behaviors were observed for the different

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systems. The matrices were also studied by means of a dynamometer: the cohesion and adhesion of the swollen tablets were evaluated. Particularly interesting was the behavior recorded when relaxation experiments were performed on the samples, and the data were analyzed with the generalized Maxwell model. Thus, the dynamic mechanical analysis, carried out on the swollen tablets, provided a description complementary to that obtained, by means of rheological testing, for the starting gel networks. A comparison of the two approaches revealed the crucial role that industrial processes, such as freeze drying and tableting, can exert on such polymeric systems, regardless of the crosslinker agent.

EXPERIMENTAL

Materials

Sclg was provided by CarboMer, Inc. (www.carbomer.com) (USA) with a degree of polymerization of 800. The borax was a Carlo Erba (Italy) product; TPH [van der Waals radius = 3.7 Å, molecular weight (MW) = 180], $AlCl_3 \cdot 6H_2O$, and $FeCl_3 \cdot 6H_2O$ were Acros Organic (Belgium) products; and vitamin B12 (van der Waals radius = 8.5 Å, MW = 1355), MGB (van der Waals radius = 21.0 Å, MW = 17,800), phenol, and sulfuric acid were purchased from Fluka (Germany).

All other products and reagents were analytical-grade. Distilled water was always used.

Purification of Sclg

Although the producer guarantees the purity of the product, it is normally a good practice to carry out dialysis to make a polymer free of low-molecular-weight contaminants (e.g., salts). For this purpose, a given amount of the polymer (5 g/L) was dissolved in distilled water and then kept under magnetic and mechanical stirring at room temperature for 24 h. The obtained solution was exhaustively dialyzed at 4°C against distilled water with dialysis membranes with a cutoff of 12,000–14,000, and then it was freeze-dried. The lyophilized product was stored in a desiccator until use.

Hydrogel preparation

The gels were obtained in a beaker by the addition of a calculated amount (i.e., moles of borax, aluminum chloride, or iron chloride = moles of repeating units of Sclg) of a borax, $AlCl_3 \cdot 6H_2O$, or $FeCl_3 \cdot 6H_2O$ solution (0.1*M*) to the polymer solutions previously prepared; the mixtures were magnetically stirred for 5 min and then left for 48 h at 7°C for gel setting. The final polymer concentration (c_p) was 0.7% (w/v). The concentration was selected as the lowest polymer content allowing the formation of a well appreciable self-sustaining gel of Sclg/borax.

When it was necessary, the Sclg/borax, Sclg/Al(III), and Sclg/Fe(III) gels were loaded with the model drugs (TPH, vitamin B12, and MGB). For this purpose, a given amount of the drug (20 mg) was dissolved in the polymer solution before the addition of borax.

Hydrogel preparation for rheological measurements

The hydrogels were prepared according to the following procedure: 70 mg of Sclg was dissolved under magnetic stirring in 9 mL of distilled water for at least 24 h. A borax, $AlCl_3 \cdot 6H_2O$, or $FeCl_3 \cdot 6H_2O$ solution (0.1*M*, 1 mL) was then added, and the solutions were stirred for 5 min. The samples were then transferred into beakers with a diameter greater than the plate–plate geometry of the rheometer (6.0 cm) for the settling of the gels at room temperature for an additional 48 h. To prepare a sample easy to manipulate and not too thick to invalidate the rheological measurements, the volume of the polymer solution was appropriately chosen so that a hydrogel thickness in the range of 1.0–3.0 mm would be obtained.

Tablet preparation

For the preparation of the tablets, about 160 mg of Sclg and 20 mg of the model drug were magnetically stirred in water for 24 h. Then, the calculated amount of a 0.1*M* borax solution (or $AlCl_3 \cdot 6H_2O$ or $FeCl_3 \cdot 6H_2O$) was added, and the system was kept under magnetic stirring for 5 min. The obtained gel (c_p = 0.7% w/v) was kept for 48 h at 7°C and then freeze-dried.

Tablets were finally prepared from the freeze-dried sample with an IR die (hydraulic press, PerkinElmer, Monza, Italy) with a force of 5.0 kN for 30 s. The weight of the tablets was 230 ± 10 mg; the diameter and the thickness, measured with a caliper (sensitivity = 0.05 mm), were 13.00 ± 0.05 and 1.40 ± 0.10 mm, respectively.

For comparison, tablets with only Sclg and the model drugs were also prepared.

For the water uptake experiments, the tablets were prepared as described before but without the drug-loading step.

Methods

Water uptake and dimensional increase studies

Tablet swelling was carried out via the soaking of the tablets in distilled water at 37°C. At fixed time

intervals, the tablets were withdrawn, the excess of water was removed with soft filter paper for 5 s, and then the corresponding dimensional variations along the longitudinal axis were determined with a ruler with a sensitivity of 1 mm.

All analyses were performed on three replicate samples. The mean values are reported in the figures, and in each case, all experimental values were below 10% of the mean.

Release experiments with the tablets

Release experiments with the model dosage forms were carried out in distilled water (pH = 5.4) according to the sixth edition of the European Pharmacopoeia with a rotating basket apparatus at 37.0 ± 0.1°C and 100 rpm.

Aliquots of the dissolution medium were taken at fixed time intervals, and the amounts of released TPH, vitamin B12, or MGB were spectrophotometrically determined at the appropriate wavelengths (TPH, $\lambda = 272$ nm; vitamin B12, $\lambda = 361$ nm; MGB, $\lambda = 409$ nm) with quartz cells with a pathlength of 1.0 or 0.1 cm. The release experiments were carried out in triplicate. Mean values are reported in the figures, and in each case, all experimental values were below 10% of the mean.

The results are reported as the relative release percentage, $M_t/M_\infty \times 100$, where M_t is the amount of the drug released at time t and M_∞ indicates the release of the total amount of the drug loaded in the formulation.

The possible erosion of the matrices, in terms of polymer dissolution in the medium during the release experiments, was quantitatively determined with a colorimetric method[12] with phenol in the presence of sulfuric acid.

Scanning electron microscopy (SEM)

The SEM images were obtained with an FEI Quanta 400 FEG apparatus (Eindhoven, the Netherlands). Lyophilized hydrogels were mounted on appropriate stubs and examined *in vacuo* (50 Pa), with no need of the gold-coating technique, at an accelerating voltage of 15 kV. All images were acquired digitally with xT Microscope Control software and a 400× magnification.

Texture analyzer

A software-controlled dynamometer (TA-XT2*i* texture analyzer, Stable Micro Systems, Godalming, Surrey, UK) with a 5-kg load cell, a force measurement 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.[13,14] The tablet resistance to compression of the probe was measured after 5 h of imbibition in water at 37°C. The trigger force was set equal to 0.002 N, the pretest speed was set at 2.0 mm/s, the test speed was set at 1.0 mm/s, the posttest speed was set at 2.0 mm/s, and the compression depth was variable, a fixed deformation of 10% being imposed with an acquisition rate of 200 points/s. The probe was an aluminum cylinder with a diameter of 35 mm (P35). For the relaxation experiments, the pretest speed was set at 2.0 mm/s, the test speed was set at 4.0 mm/s, and the posttest speed was set at 1.0 mm/s. A 10% deformation was imposed on the samples, and then the relaxation of the system was recorded until the baseline was reached. Before the compression and relaxation tests were performed, the linear viscoelasticity range was monitored for all samples for both kinds of experiments; a linear response up to a deformation of 12% was detected, and the imposed value of 10% deformation was then chosen to carry out the experiments.

The study was carried out at room temperature (~ 25°C). Before each measurement, the swollen tablets were allowed to reach the appropriate temperature in about 20 min. All analyses were performed on three replicate samples. Mean values are reported in the figures, and in each case, all experimental values were within 10% of the mean.

Rheological measurements

The rheological characterization of the samples was performed with a controlled-stress rheometer (RheoStress RS300 and Thermo DC50 water bath, Haake, Karlsruhe, Germany). Two geometries were used to reduce the extent of the wall-slippage phenomenon: a cone–plate device (C60/1 Ti with a cone diameter of 60 mm and a cone angle of 1° and an MP60 steel 8/8″ plate with a diameter of 60 mm, Haake) for the Sclg sample and a grained plate–plate device (PP35/S with a diameter = 35 mm, Haake) for the hydrogel samples.[15] To perform the measurements, the hydrogel, obtained with a thickness of 1.0–3.0 mm, was removed with the aid of a small spatula from the beaker in which it had settled, and it was laid with care on the lower plate of the rheometer. The upper plate was then lowered until it reached the hydrogel surface. Gap-setting optimizations were undertaken according to a procedure described elsewhere.[16] Samples were loaded at a fixed temperature of 25°C and coated around their periphery with a light silicone oil to minimize the loss of water. When only Sclg was tested, an appropriate amount of the polymer solution was spread onto the plate geometry to obtain a sample of the appropriate height. Rheological properties were studied in oscillatory experiments; mechanical spectra were recorded in the frequency range of 0.001–10 Hz. The linear viscoelastic

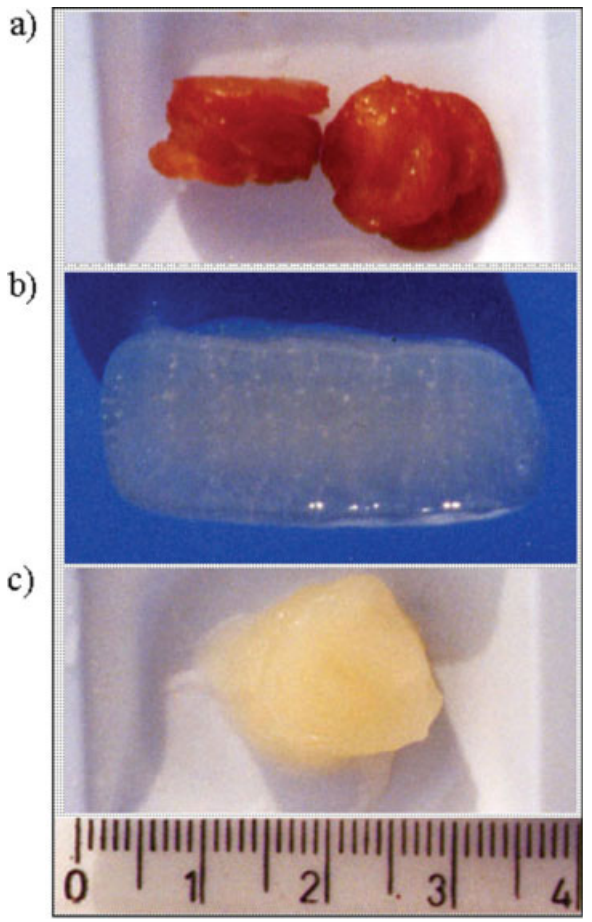

Figure 1 Pictures of tablets prepared with (a) Sclg/Fe, (b) Sclg/borax, and (c) Sclg/Al swollen in distilled water for 24 h at 37°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

region was assessed at 1 Hz by stress sweep experiments, and in the subsequent measurements, a deformation constant (γ) of 0.01 was used. Flow curves of the samples were obtained in the range of 0.1–30 Pa.

RESULTS AND DISCUSSION

Water uptake and dimensional increase studies of the tablets

It is well known that a gel system swells isotropically in all directions.[17] When the Sclg/borax gel was freeze-dried and compressed to prepare tablets, anomalous anisotropic swelling could be observed[9] when the tablets were soaked in distilled water at 37°C [see Fig. 1(b)]: the compressed matrix elongated essentially in its axial direction, whereas radial swelling was almost negligible. No anomalous swelling was ever detected for tablets prepared with only Sclg.[7] When the experiment was carried out with the other two systems, Sclg/Al(III) and Sclg/Fe(III), different behavior was found [see Fig. 1(a,c)]. In the presence of Al(III), the swelling was almost isotropic, whereas in the presence of Fe(III), the tablets showed very limited swelling, mainly along one direction, without remarkable variations of the diameter (as in the case of Sclg/borax). The corresponding water uptake data are reported in Figure 2(a,b).

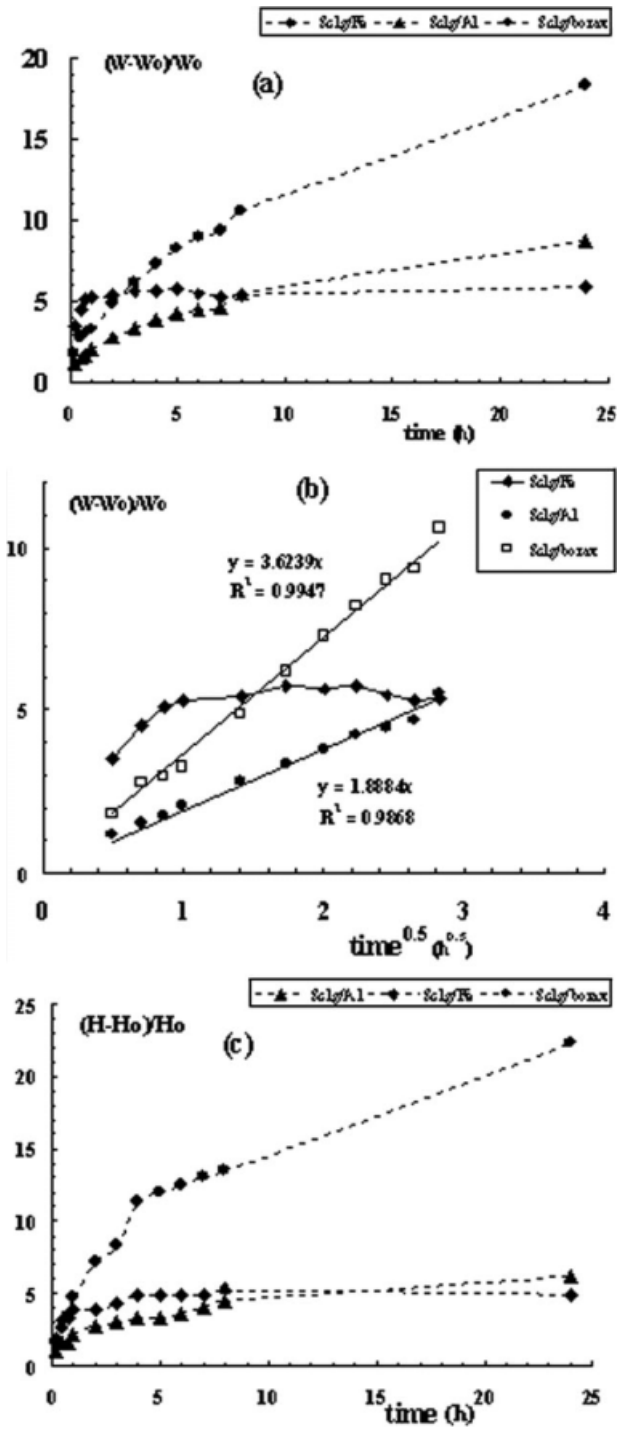

Figure 2 Water uptake of tablets prepared with Sclg/borax, Sclg/Al, and Sclg/Fe as a function of (a) time and (b) the square root of time. The relative increase of height, of the above mentioned tablets, as a function of time (c). W = weight of the swelled tablet; Wo = weight of the dry tablet.

In the case of borax, the maximum water uptake was detected; this uptake can be described in terms of a Fickian process [Fig. 2(b)], as indicated by the linear trend of the data reported against the square root of time. In the case of Sclg/Al(III), the water uptake again followed a Fickian process [Fig. 2(b)], but the overall increase in the weight was less than one-half of that observed with the Sclg/borax sample [Fig. 2(a)]. In the case of Sclg/Fe(III), the water uptake increased only during the first hour and then remained almost constant; thus, the diffusion of water into the network can be ascribed to a Fickian process only up to 1 h. At the same time, when the elongation of the tablets in their axial direction was examined as a function of time, the maximum effect was again detected for the Sclg/borax tablets [see Figs. 1(b) and 2(c)], whereas the Sclg/Al(III) and Sclg/Fe(III) matrices showed an elongation roughly 3 to 4 times lower than that of the Sclg/borax system. Although some models have been proposed for the Sclg/borax network with and without guest molecules,[7,9,10] no investigation has been carried out so far on these new systems. At this stage, an interaction between the positive ion [Al(III) or Fe(III)] and the hydroxyl groups of the glucopyranose units can be proposed. The physical linkages among the polysaccharide chains, mediated by the trivalent ions, lead to a supramolecular structure. The macroscopic properties of the Sclg solution are deeply influenced by the presence of the crosslinkers but to different extents depending on the strength of the formed Sclg–crosslinker linkage (as evidenced by the dynamic mechanical measurements, the rheological experiments, and the SEM analysis, as discussed later).

In this sense, it can be asserted that borate ions act as the most effective crosslinker agent with respect to the elongation in comparison with the other two ions, which form hydrogel networks by means of different types of interactions with the macromolecular chains.

Although other anisotropic swelling has been reported in the literature,[18,19] nevertheless, the longitudinal elongations reported here are always significantly higher (see Table I).

Our hypothesis is that the three crosslinking agents (borax, aluminum chloride, and iron chloride) are capable of linking, to different extents, the triple helices of Sclg, stabilizing intertriplex interactions and thus leading to the formation of ordered domains (as well-known junction zones present in other polysaccharide physical gels such as agarose, alginate, and carrageenan) connected to other domains that are less structured.[20] The high pressure applied during the tablet formation increases the extent of such regular arrangements, which are essentially retained during the subsequent swelling, when the crosslinker is present. The observed swelling (i.e., the water uptake and anisotropic elongation), significantly different for the three crosslinkers, represents only the macroscopic and final effects of molecular mechanisms acting in network formation.

TABLE I
Relative Increases in the Height [$(h - h_0)/h_0$] of Tablets of Sclg/Borax, Sclg/Al, and Sclg/Fe After 8 h in Water at 37°C Versus Data Calculated from the Literature for Tablets Prepared with Other Polymeric Matrices

Tablet matrix	$(h - h_0)/h_0$	
	8 h	24 h
Sclg/borax	13.5 ± 0.6	22.4 ± 0.8
Sclg/Al	4.5 ± 0.2	4.9 ± 0.2
Sclg/Fe	5.3 ± 0.2	6.2 ± 0.4
Hydroxypropyl methylcellulose (Methocel K100 M)	1.3[a]	–
Xanthan (RheoGel)	0.9 (1 h, plateau value)[b]	–

[a] Calculated from Papadimitriou et al.[18]
[b] Calculated from Talukdar and Kinget.[19]
h = height of the swelled tablet; h_0 = height of the dry tablet.

Drug release from the tablets

It is already known from previous studies carried out in our laboratories that tablets prepared with the Sclg/borax hydrogel are capable of modulating the release of guest molecules according to their different steric hindrances.[7,9,10] To determine if other crosslinkers of Sclg affect in a similar manner the release of the same tested molecules, the delivery was evaluated from dosage forms prepared with Sclg/Al(III) and Sclg/Fe(III). Figure 3 shows the experimental release profiles from the different tablets containing TPH [Fig. 3(a)], vitamin B12 [Fig. 3(b)], and MGB [Fig. 3(c)] in distilled water at 37°C. As can be observed in Figure 3(a), the release of TPH was quite similar for all three crosslinked systems, although a slightly higher release was detected in the case of Sclg/borax. The quite unexpected result was that the delivery from all the crosslinked structures was faster than that obtained from the tablets prepared without crosslinkers. This means that the introduction of a crosslinker leads to the formation of meshes within the networks that facilitate the diffusion out of the matrices of the smallest tested molecule. In the case of the release from tablets prepared with the uncrosslinked Sclg, the swollen system is actually formed by entanglements of the polymeric chains that deeply hinder the motion of the loaded guest drugs, regardless of molecular dimensions.

A quite different situation was detected in the case of vitamin B12. As shown in Figure 3(b), the

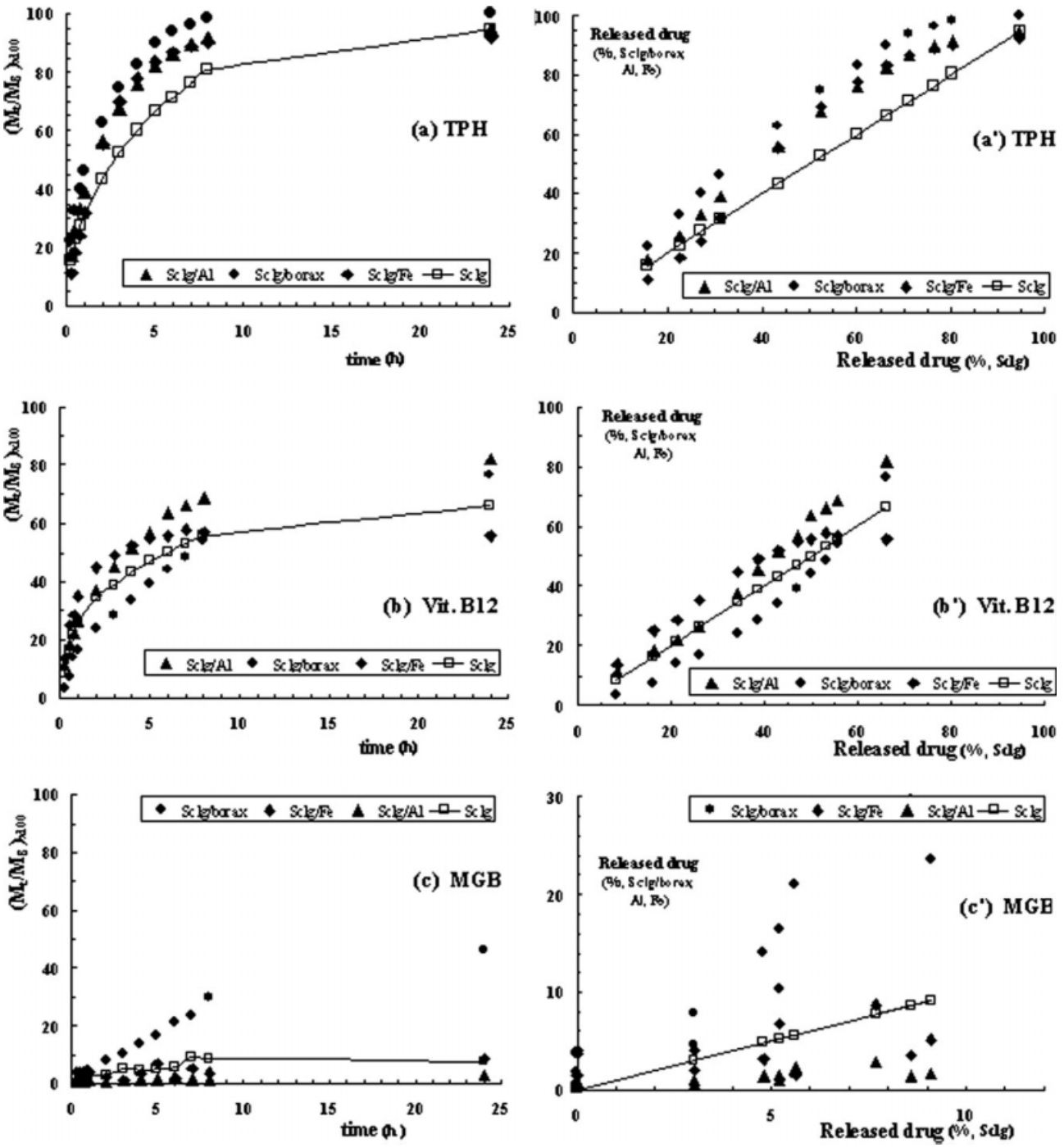

Figure 3 Comparison of the release profiles of (a) TPH, (b) vitamin B12, and (c) MGB from Sclg, Sclg/borax, Sclg/Al, and Sclg/Fe tablets in water at 37°C. The same data are also reported as the percentage of the drug released from Sclg/borax, Sclg/Al, and Sclg/Fe tablets as a function of the corresponding delivery from tablets prepared with only Sclg: (a′) TPH, (b′) vitamin B12, and (c′) MGB.

presence of the various crosslinkers led to different effects on the release rate. In the case of Sclg/borax, a slight decrease in delivery in comparison with the Sclg tablets was observed, probably because of the presence in the hydrogel of channels with dimensions comparable to those of vitamin B12.[9] When tablets of Sclg/Al(III) and Sclg/Fe(III) were tested, the release profiles were slightly higher than those obtained with the matrix without the crosslinker. Up to now, no model has been available for the linkage between Al(III) and Sclg or between Fe(III) and Sclg; nevertheless, from the experimental data, it is evident that some kind of network is formed in the presence of the tested crosslinkers that leads to more or less anisotropic elongation of the tablets during swelling. The microscopic structure of the Sclg

TABLE II
Delivery Percentages of TPH, Vitamin B12, and MGB from Tablets of Sclg, Sclg/Borax, Sclg/Al, and Sclg/Fe After 8 and 24 h in Water at 37°C

	TPH	Vitamin B12	MGB
Sclg			
$t_{50\%}$ (h)	3.0 ± 0.1	7.1 ± 0.1	≫24
$M_t/M_\infty \times 100$ (8 h)	80.0 ± 1.0	56.3 ± 0.5	9.2 ± 0.9
$M_t/M_\infty \times 100$ (24 h)	95.4 ± 0.6	74.3 ± 0.5	8.0 ± 0.7
Sclg/borax			
$t_{50\%}$ (h)	0.9 ± 0.1	7.0 ± 0.1	≫24
$M_t/M_\infty \times 100$ (8 h)	99.0 ± 1.0	54.2 ± 1.4	25.1 ± 0.8
$M_t/M_\infty \times 100$ (24 h)	99.8 ± 0.2	76.1 ± 0.7	46.3 ± 0.5
Sclg/Al			
$t_{50\%}$ (h)	1.9 ± 0.1	4.0 ± 0.1	≫24
$M_t/M_\infty \times 100$ (8 h)	92.0 ± 1.1	69.3 ± 1.2	1.0 ± 0.1
$M_t/M_\infty \times 100$ (24 h)	100.0 ± 0.1	82.1 ± 1.0	3.0 ± 0.2
Sclg/Fe			
$t_{50\%}$ (h)	2.0 ± 0.1	2.1 ± 0.1	≫24
$M_t/M_\infty \times 100$ (8 h)	90.0 ± 1.3	65.1 ± 0.8	4.0 ± 0.4
$M_t/M_\infty \times 100$ (24 h)	92.1 ± 1.0	65.0 ± 0.6	9.1 ± 0.4

$t_{50\%}$ = time at which 50% of the loaded molecules were delivered from the networks.

network is certainly much different if borax, Al(III), or Fe(III) is used; consequently, the loading process of guest molecules with different steric hindrances has a different disturbing effect on the network formation. When small molecules are loaded (e.g., TPH), then the diffusion is always easier in the presence of a crosslinker. When bigger molecules (e.g., vitamin B12) are loaded within the network, the disturbing effect is different and is dependent on the relative dimensions of the guest drug and the mesh size formed in the presence of the specific crosslinker.

The same considerations are valid in the case of MGB release [Fig. 3(c)]. In the presence of guest drugs of high steric hindrance, when borax is used as a crosslinker, the network formation is significantly modified by the allocation of MGB molecules.[9] This is reflected by a release that is faster than that obtained from tablets prepared with plain Sclg [Fig. 3(c)]. When Al(III) or Fe(III) is used as a crosslinker agent, the networks should have a different structure, as revealed also by the different water uptake and the different anisotropic swelling [see Fig. 1(a,c)]. Such different structures lead to release profiles almost superimposable with those obtained from the Sclg tablets.

These statements are further supported by Figure 3(a′,b′,c′), in which the different release percentages of the crosslinked samples are reported, at the same time, as a function of the delivery from the Sclg matrix without the crosslinker.

The experimental data indicate that the structures of the different gels and, consequently, the release rates are affected, to different extents, by the presence and kind of the crosslinker and by the dimensions of the guest molecule. In the case of TPH, all the release rates were higher than those for the tablets prepared with the plain Sclg [Fig. 3(a′)]; this means that for guest molecules with low steric hindrance (TPH; van der Waals radius = 0.7 nm), the presence of the crosslinker leads to faster release (i.e., the structure of the matrix becomes more open). More complex is the situation when the loaded molecule is vitamin B12 (van der Waals diameter = 1.7 nm): in the case of Sclg/borax, for which the formation of channels with a size near the diameter of vitamin B12 was proposed,[9,10] the diffusion of the molecule was slowed, whereas in the other two cases, in which no formation of channels could be supposed, the delivery was again slightly faster than that from the tablets of plain Sclg. In the case of the biggest guest molecule (MGB; van der Waals diameter = 4.2 nm), the overall release was very limited from all the tested samples. It is interesting to note that for Sclg/borax, in which wider channels were supposed to form in the presence of MGB,[9] the delivery was somehow faster than that from the plain Sclg. On the other hand, the release from the Sclg/Al(III) and Sclg/Fe(III) tablets was even more limited than that from the plain Sclg.

Thus, all the delivery data indirectly confirm that only borax is capable of forming channels, whereas the other two crosslinkers, Al(III) and Fe(III), act with a completely different mechanism.

As a further comparison of the three crosslinked hydrogels and the tablets prepared with only Sclg, in Table II, the release percentages of the model molecules at 8 and 24 h, together with the times at which 50% of the loaded molecules were delivered from the networks, are reported.

To verify if and when erosion of the matrices of Sclg with the crosslinkers can be considered negligible, the amount of the polymer dissolved in the medium during the release experiments of the model drugs was detected as a function of time by the colorimetric method. The results, very similar for all three investigated systems, showed very limited erosion also in the presence of the loaded molecules, as reported in Table III. These data confirm that the

TABLE III
Dissolution Percentages of Sclg from Tablets of Sclg, Sclg/Borax, Sclg/Al, and Sclg/Fe After 8 and 24 h in Water at 37°C in the Presence of the Loaded Molecules

	$M_t/M_\infty \times 100$	
Sample	8 h	24 h
Sclg	2.5 ± 0.1	4.6 ± 0.4
Sclg/borax	3.3 ± 0.2	4.7 ± 0.2
Sclg/Al	3.1 ± 0.1	3.7 ± 0.3
Sclg/Fe	7.7 ± 0.2	11.1 ± 0.2

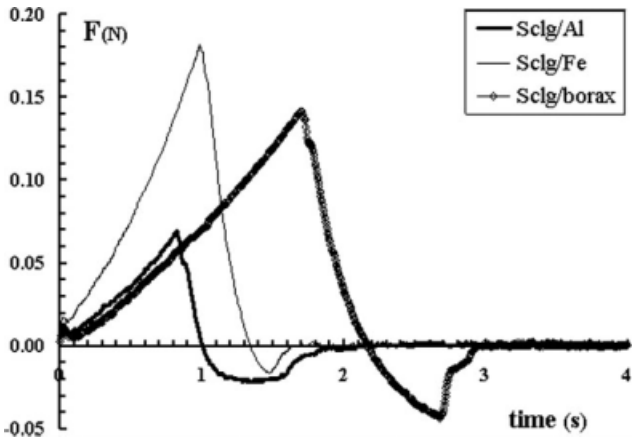

Figure 4 Compression profiles obtained from Sclg/borax, Sclg/Al, and Sclg/Fe tablets after swelling in distilled water for 5 h at 37°C. The areas under the curves to the maximum value represent the work of cohesion, whereas the negative areas are equal to the work of adhesion of the samples to the probe. The hardness of the samples is given by the maximum positive value of the applied force, *F*.

release profiles are related only to the peculiar structure of the polysaccharide matrix, both with and without the crosslinker, and they cannot be related to the tablet erosion.

The overall behavior of the tested matrices indicates that the three systems appear to be suitable for the preparation of modified-release oral dosage forms. In particular, the possibility of modulating the delivery rate as a function of the steric hindrance of the drug molecule can be appealing for innovative formulations of tablets. Furthermore, the new matrices can be proposed for the delivery of high-molecular-weight molecules, such as proteins, provided that a preliminary gastroresistant coating is applied to the tablet surface.

Texture analysis

Compression measurements of the swollen tablets

From the compression analysis, important parameters of the networks were acquired: the hardness (i.e., the maximum positive force required to attain a given deformation), the work of cohesion (i.e., the part of the positive area under the force–time curve up to the maximum value), and the work of adhesion (i.e., the negative area under the force–time curve). The experimental profiles, taken at room temperature ($\sim$ 25°C) for tablets previously swollen in water for 5 h at 37°C, are shown in Figure 4, whereas the obtained parameters are reported in Table IV.

The work of cohesion, which represents the work needed to overcome the internal bonds of a material, has its highest value for the swollen Sclg/borax tablets. The Sclg/Fe(III) system shows a value of cohesiveness reduced by about 30%, whereas for the Sclg/Al(III) system, a much lower value is recorded. Obviously, this difference is strictly related to the kind of linkage between the different crosslinkers and the polysaccharide chains. The strongest tablet network, in terms of cohesion, is Sclg/borax, whereas the weakest one is Sclg/Al(III), which shows cohesiveness 4 times lower than that of Sclg/Fe(III). On the other hand, when the hardness is considered, Sclg/Fe(III) becomes the strongest system (i.e., it is the system that opposes the highest force against deformation); it is followed by Sclg/borax and, with a much lower value, by Sclg/Al(III). This result is related both to the strength of the internal linkages and to the degree of swelling. In terms of cohesion, Sclg/borax is the strongest, whereas the hardness is lower than that of Sclg/Fe(III), which anyhow swells to a much lower extent.

The work of adhesion, which represents the work needed to pull the probe away from the sample, is highest in the case of the swollen Sclg/Al(III) tablets and lowest in the case of Sclg/Fe(III). Indeed, when a sample is very hard and shows high cohesiveness, it also has a low degree of adhesion. Furthermore, the specific adhesiveness values can differ according to the different materials of the probe (in our case aluminum), which is responsible for the adhesion process.

Relaxation measurements of the swollen tablets

To acquire information about the mechanical properties of hydrogels and consequently about network characteristics, the relaxation behavior of hydrogels can be matched with the generalized Maxwell model.[21] Briefly, this model assumes that the viscoelastic properties of a gel matrix can be represented by a mechanical device made up of 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 becomes

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

TABLE IV
Parameters Obtained from Compression Experiments Carried Out at Room Temperature with a TA-XT2i Texture Analyzer on Tablets Prepared with Sclg/Borax, Sclg/Al, and Sclg/Fe Swollen for 5 h in Distilled Water at 27°C

Sample	Hardness (mN)	Cohesiveness (J/m^3)	Adhesiveness (J/m^3)
Sclg/borax	141 ± 8	305 ± 15	52 ± 5
Sclg/Al	69 ± 5	49 ± 5	109 ± 8
Sclg/Fe	182 ± 12	205 ± 10	8 ± 1

The given parameters were normalized per unit of volume.

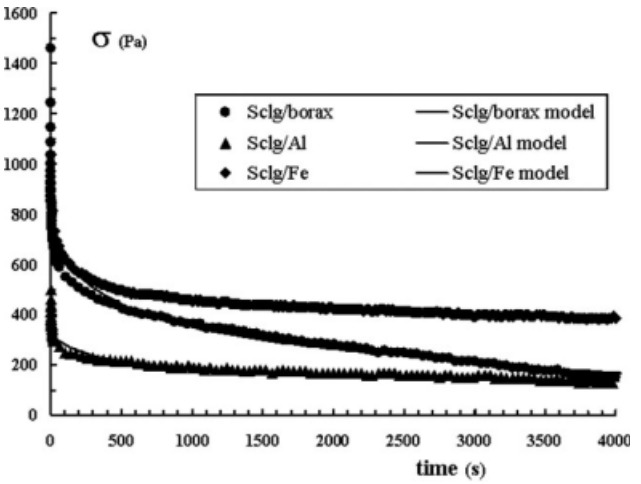

Figure 5 Relaxation spectra of the Sclg/borax, Sclg/Al, and Sclg/Fe samples. The solid lines are the best fits obtained by the application of the generalized Maxwell model.

where σ is the tension, ε is the deformation, t is the time, t' is the dummy variable for time, and ϕ is the relaxation modulus. The expression of ϕ is

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

where E_0 is the elastic modulus for a first spring element of the generalized Maxwell model (Pa) and n is the number of elements in the generalized Maxwell model.

As the relaxation test is unavoidably preceded by a compression phase lasting for time t_1, eq. (1) needs to be integrated with the following conditions:

$$\frac{\partial \varepsilon}{\partial t} = \frac{\varepsilon_0}{t_1}, 0 < t < t_1 \text{ for compression} \quad (3)$$

$$\frac{\partial \varepsilon}{\partial t} = 0, t > t_1 \text{ for relaxation} \quad (4)$$

where ε_0 is the final applied constant deformation.

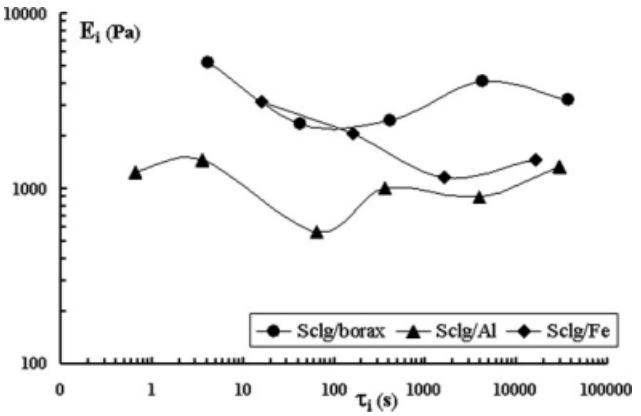

Figure 6 Dependence of E_i on τ_i for Sclg/borax, Sclg/Al, and Sclg/Fe (c_p = 0.7 w/v). The solid lines are only guides for the eyes.

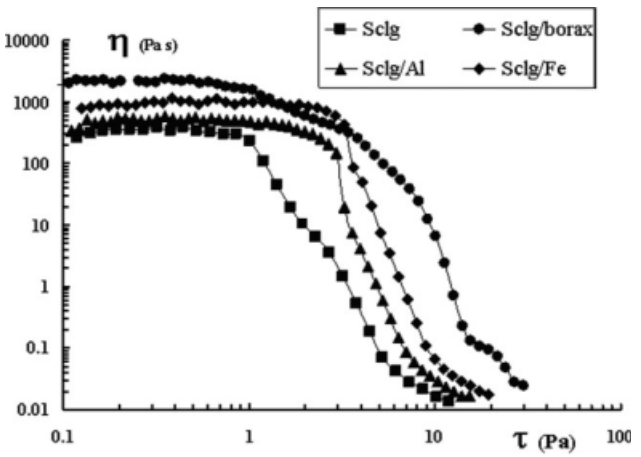

Figure 7 Flow curves at 25°C for Sclg, Sclg/borax, Sclg/Al, and Sclg/Fe (c_p = 0.7 w/v).

Accordingly, we get

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

Tablets of Sclg/borax, Sclg/Fe(III), and Sclg/Al(III) were first swollen for 5 h at 37°C in distilled water, and the matrices were then tested for the relaxation experiment. The experiment was carried out after 5 h to obtain reproducible and comparable results because, for longer time intervals, the Sclg/Al(III) matrix showed some minor mechanical stability problems. The obtained spectra were then analyzed according to the generalized Maxwell model. As an example, Figure 5 shows good agreement between the relaxation experimental data (symbols) and the best fitting by eq. (5) (solid lines) for the three samples, with ε_0 set at 0.10.

Interestingly, three to five Maxwell elements, plus a pure elastic element characterized by E_0, were sufficient to represent the relaxation behavior of the matrices. The presence, in all systems, of the first pure elastic element can be related also to a core of the tablets not completely swollen after 5 h. Anyhow, the E_0 values found for the three systems are meaningful: Sclg/Al(III) has E_0 = 1371 Pa, whereas Sclg/borax has E_0 = 2028 Pa and Sclg/Fe(III) has E_0 = 2594 Pa. Again, the different intrinsic strengths of the networks also emerge from the evaluation of this parameter.

Figure 6, which shows the mechanical spectra of the three systems [the spring constant (E_i) vs the relaxation time ($\tau_i = \eta_i/E_i$)] obtained with the generalized Maxwell model, evidences how, apart from the first pure elastic element, the behavior of the remaining viscoelastic part is typical of gel-like materials because the elements characterized by low

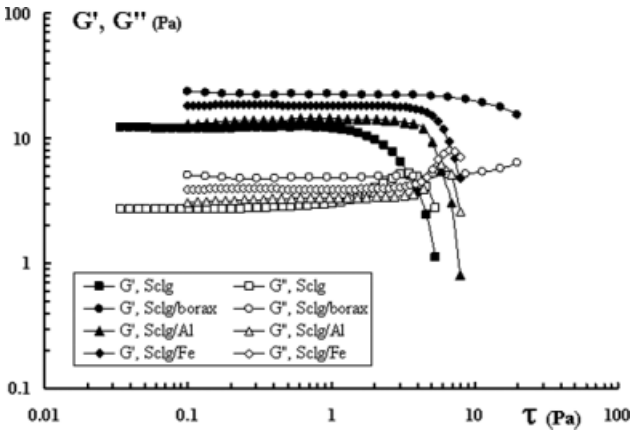

Figure 8 Stress sweeps for Sclg and for Sclg/borax, Sclg/Al, and Sclg/Fe at 1 Hz and 25°C ($c_p = 0.7$ w/v).

τ_i values and the elements characterized by high τ_i values are associated with similar values of E_i. Accordingly, E_i influences in a similar manner the time-dependent response of the three matrices. Furthermore, the Sclg/borax E_i values are always higher than those of Sclg/Al(III), thus showing a higher strength of the network prepared in the presence of borax. The E_i values for Sclg/Fe(III) are between those of the other two samples, and they are lacking the lowest τ_i values, as expected for a hydrogel with a rather tight network and as revealed by the low water uptake shown by this matrix.

Rheological analysis of the hydrogels

Figure 7 reports the flow curves for the Sclg aqueous solutions without and with the tested crosslinkers. All the systems showed a high degree of pseudoplasticity, and it was not possible to detect the Newtonian plateau at a high shear rate. The effects of the different crosslinkers are also evident at a low shear rate: Sclg shows the lowest viscosity, and the Sclg/borax hydrogel shows the highest one. The viscosities of Sclg/Fe(III) and Sclg/Al(III) are between them. These results indicate again the importance of the different crosslinkers to the network structure: borax exerts the maximum effect, with an increase in the viscosity in comparison with Sclg of a factor of almost 10, whereas Al(III) and Fe(III) have weaker effects. This is clearly related to the microscopic interactions among the polysaccharide chains and the crosslinker agents. All the curves in Figure 7 indicate that the flow properties of the polysaccharide, with and without the crosslinker, are typical of a macromolecular system with shear-thinning behavior and a Newtonian region at low shear stress values. The presence of the crosslinkers does not qualitatively modify the dependence of viscosity, η on stress, τ, whereas it determines a noticeable increase in the Newtonian plateau and a shift toward higher values of the shear stress at which the system starts to flow (τ_f), corresponding to the breakage of the gel structure. For Sclg, $\tau_f \approx 1$ Pa, and this critical value increases about 3 times for Sclg/Al(III) and even more for Sclg/Fe(III) and Sclg/borax.

Similar and even more evident information can be acquired from the stress–sweep experiments shown in Figure 8. The range of linear viscoelasticity is wider for all the systems prepared in the presence of the crosslinkers: in fact, the critical stress (τ_c) holds until approximately 1 Pa for Sclg, whereas it increases up to approximately 4 Pa for Sclg/Al(III), up to approximately 5 Pa for Sclg/Fe(III), and up to approximately 10 Pa for Sclg/borax. If we remember the following relation between τ_c and deformation (γ_c)[15]

$$\gamma_c = \frac{\tau_c}{\sqrt{(G')^2 + (G'')^2}} \tag{6}$$

we can see that γ_c is approximately 0.08 for the Sclg sample, whereas it increases up to approximately 0.31 for Sclg/Al(III), up to approximately 0.32 for Sclg/Fe(III), and up to approximately 0.57 for Sclg/borax.

Based on the results obtained in the stress sweep experiments (with and without crosslinkers), a frequency sweep analysis at a constant deformation ($\gamma = 0.01$) was carried out in the range of linear viscoelasticity. Also, the mechanical spectra (see Fig. 9) reveal the different assemblies of the Sclg chains when different crosslinkers were used. In all cases, the spectra indicate the formation of weak gels as the storage modulus (G') was always greater than the loss modulus (G'') in the overall frequency window explored, and both viscoelastic quantities, with rather low absolute values, still showed a dependence on the imposed frequency of oscillation.[15] As already indicated by the flow and stress sweep

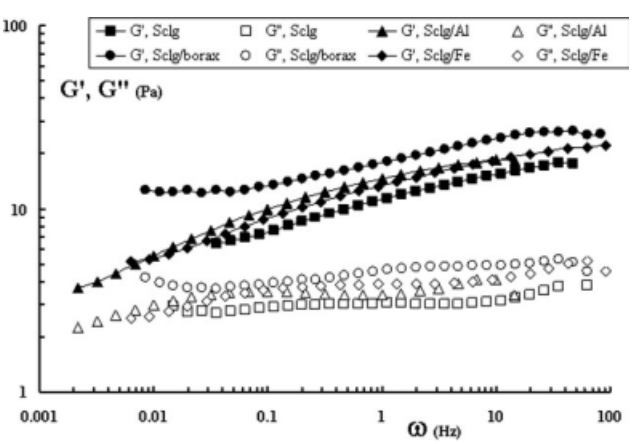

Figure 9 Frequency (ω) sweeps for Sclg, Sclg/borax, Sclg/Al, and Sclg/Fe at 25°C and $\gamma = 0.01$ ($c_p = 0.7$ w/v).

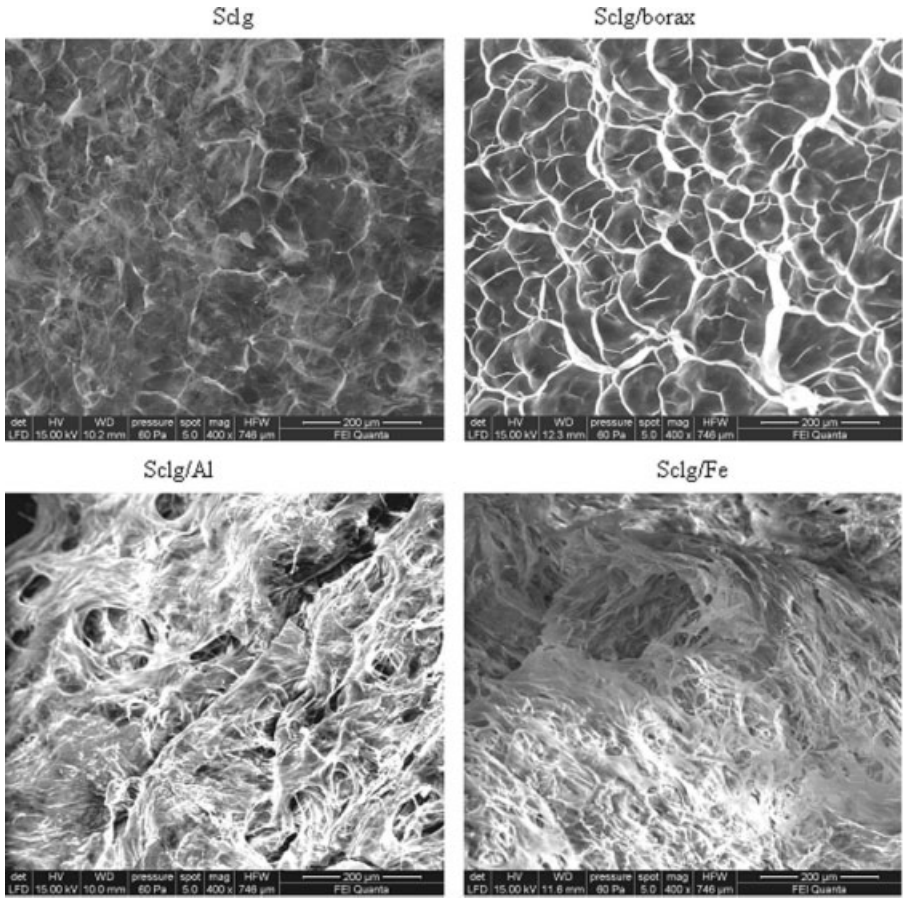

Figure 10 SEM images of freeze-dried samples of Sclg, Sclg/borax, Sclg/Al, and Sclg/Fe (magnification = 400×, c_p = 0.7 w/v).

curves, the highest effect, in terms of the strength of the gel, was found for the Sclg/borax system. In this case, the spectra are significantly different, even at very low frequencies, thus evidencing the formation of a rather stable network. In the case of Sclg/Al(III), no significant increases in G' and G'' were detected in comparison with Sclg alone, and this indicates that the moduli were only slightly affected by the presence of Al(III). A similar effect was already found in the flow and stress sweep curves, in which no appreciable difference was detected, at low shear stresses between Sclg and Sclg/Al(III) (see Fig. 7). A similar consideration is valid for Sclg/Fe(III), for which the moduli were always only slightly higher in comparison with those of Sclg alone and superimposable to those of Sclg/Al(III). Thus, the rheological analysis indicates the formation of a hydrogel whose increased strength is deeply influenced by the nature of the crosslinker in comparison the network built up with only entangled Sclg chains.

SEM characterization

To determine the morphological characteristics of the hydrogels, SEM images were recorded on lyophilized samples of Sclg and Sclg/crosslinker systems. The micrographs, shown in Figure 10, show how the introduction of the crosslinker changed the internal morphological features. In comparison with Sclg, the Sclg/borax lyophilized hydrogel showed a highly porous and interconnected interior structure with a spongelike aspect. It could be inferred that the hydrogel had a high water retention capacity (as was actually found with the water uptake experiments; see Fig. 2), and both small and large molecules could diffuse freely through it.

The presence of Al(III) or Fe(III) changed the overall internal structure, which appeared to be somehow less loose than that shown by the Sclg/borax sample. In fact, the release of a large molecule, such as MGB, from the Sclg/Fe(III) and Sclg/Al(III) tablets was completely forbidden. On the other hand, for small molecules such as TPH, faster release was always detected in comparison with the Sclg matrix. Similar behavior was observed for molecules such as vitamin B12: the swollen matrices allowed faster release (in comparison with that of Sclg), with the exception of Sclg/borax, for which the mesh size was supposed to be comparable to the dimensions of the guest molecules.[9] The morphologies of the

Sclg/Al(III) and Sclg/Fe(III) hydrogels were less regular than that evidenced in the presence of borax. Furthermore, in the case of Sclg/Al(III), a texture with wide holes was evidenced. Indeed, as confirmed by rheological measurements, the hydrogel with Al(III) showed a reduced capability to keep its shape in comparison with Sclg/Fe(III) and Sclg/borax, but it was always higher than that of the Sclg sample (see Fig. 7).

CONCLUSIONS

The characterization of polysaccharide hydrogel systems, such as those obtained with Sclg and various crosslinkers, is important for shedding light on their structures and consequently for improving our knowledge of their performance for biomedical applications and more specifically for innovative drug formulations.

In particular, in the presence of the three crosslinkers borax, Al(III), and Fe(III), Sclg is able to form hydrogels that show quite different properties in terms of water uptake when tested as tablets. In fact, although in the case of borax very peculiar anisotropic swelling is detected along one direction, in the presence of Al(III) and Fe(III), such an event is drastically reduced, and this indicates that the crosslinking reaction may occur in a different manner that leads to remarkable differences at the macroscopic level. As suggested also elsewhere,[9] the imbibition process of the Sclg/borax tablets somehow stabilizes preexisting ordered assemblies, which lead to the observed peculiar anisotropic swelling. Furthermore, for the Sclg/borax and Sclg/Al(III) networks, the water uptake follows a pure diffusive process, whereas this does not occur for the Sclg/Fe(III) tablets.

The tested networks, when loaded with model drugs of different steric hindrances and prepared in the form of tablets, show qualitatively similar delivery profiles. In comparison with the tablets prepared with plain Sclg, the presence of the crosslinker always induced an increase in the relative delivery of the smallest guest molecules (TPH), the effect being most relevant in the case of Sclg/borax. This effect can be explained by the formation of regular meshes that lead to easier diffusion out of the swollen tablet in comparison with the matrix without a crosslinker. The release profiles from tablets loaded with molecules of higher steric hindrance (e.g., vitamin B12 and MGB) allow us to discriminate among the three crosslinkers, indicating again a different type of network, which is related to the relative interactions among the chains and the crosslinking agents. The reported results for the behavior of crosslinked Sclg tablets appear to be promising: the systems can be proposed as monolithic swellable matrices for modified-release formulations, as they are capable of modulating the delivery of molecules as a function of the molecular dimensions. Furthermore, in comparison with the delivery from Sclg tablets, a fine tuning of the release of guest molecules is possible by the crosslinker agent being changed.

Also, the texture analysis gives us useful information on the mechanical properties of the swollen tablets, discriminating among the crosslinkers: the most cohesive network was the Sclg/borax system, whereas the maximum hardness was found for Sclg/Fe(III). The Sclg/Al(III) network showed the lowest values of hardness and cohesion together with the highest adhesion. According to the relaxation experiments, all the tested swollen tablets could be classified as solid viscoelastic materials: they showed residual stress E_0, and the mechanical spectra revealed a predominance of viscous behavior. Again, the different crosslinkers had different values of modulus E_i.

The rheological investigation, carried out on the prepared gels before the freeze-drying and compression steps, showed the changes occurring in the Sclg sample when a crosslinker was added. Both moduli, G' and G'', increased, and this indicated a strength that was once more dependent on the crosslinking agent. All the samples, including Sclg alone, from a rheological point of view were gels, G' always being higher than G'' in the whole frequency range explored. Nevertheless, these gels were classified as weak gels because the absolute values of G' were quite low and because the moduli still showed a dependence on the frequency that disappears in strong gels. Also, the rheological results showed that the various crosslinkers had a different effect: borax led to the maximum variation of G' and G'' and of the viscosity in comparison with Sclg alone. Also, the viscoelasticity range and the flow curves were influenced, to different extents, by the presence of the crosslinkers. Interestingly, in the flow curves and in the stress sweep experiments, the Sclg/crosslinker hydrogels followed the same order already found in the compression test applied to the swollen matrices. From all the collected data, it can be concluded that the rheological parameters are affected to different extents by the crosslinkers according to the following order:

$$\text{Borax} > \text{Fe(III)} > \text{Al(III)}$$

If we take into account the mechanical data, the rheological data, and the delivery profiles as well as the SEM analysis, it can be concluded that, for the studied systems and for the tested model drugs, the main factors affecting the release process are (1) the crosslinkers, which act as spacers among the polymeric chains, leading to the formation of meshes

whose size depends on the kind of reagent and on the kind of formed linkage, and (2) the steric hindrance of the guest molecules with respect to the network mesh size. Less important seem to be the rheological differences detected in the viscoelasticity range when the crosslinking agent was added to the Sclg sample and the dynamic mechanical parameters recorded on the swollen tablets.

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