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Research paper

Physical gels of a carboxymethyl derivative of scleroglucan: Synthesis and characterization

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Abstract

A carboxymethyl derivative of scleroglucan (Scl-CM) was synthesized and characterized through FT-IR, ^{1}H NMR and potentiometer titration. Rheological studies allowed evidencing the effect produced by the introduction of the carboxymethyl moiety on the native polymer. The mechanical spectrum of the scleroglucan solution showed a weak gel behaviour, while the derivative one looked like a system near the gel point, that evolved to a gel state depending on the concentration. This difference could be related to conformational changes due to the introduction of the negative charges on the chains. Different concentrations of Ca^{2+}, added to the aqueous solutions of Scl-CM, were able to deeply modify the resulting system, showing a sharp transition toward a gel like behaviour. Acyclovir was loaded into the hydrogels obtained with different amounts of polymer and salt. The release rate of the drug from these systems was strictly related to both concentrations of salt and polymer. The obtained results suggest a possible employment of these new hydrogels for topical formulations or in situ implantation.

Keywords: Carboxymethyl scleroglucan; Physical hydrogels; Rheology; Acyclovir; Controlled release

1. Introduction

Scleroglucan (Scl) is a water-soluble extracellular polysaccharide produced by fungi of the genus *Sclerotium*. It consists of a backbone of (1 → 3)-β-linked glucose residues substituted with single (1 → 6)-β-D-glucopyranosyl residues on every third backbone unit [1]. X-ray fibre diffraction data indicate that scleroglucan adopts a triple helix structure that is destroyed in solutions at pH higher than 12 or in dimethylsulfoxide (DMSO) [2,3]. Scleroglucan and some of its derivatives at different oxidation degrees have been employed as starting materials for the synthesis of chemical and physical hydrogels suitable as matrices for the controlled release of drugs [4–9]. We have recently reported the synthesis of chemical gels of scleroglucan obtained by reaction of the native polymer with 1,ω-dicarboxylic acids with a different number of carbon atoms in the chain [10]. Other chemical gels have been prepared with different derivatives of scleroglucan, obtained by the controlled oxidation of the polymer. These reactions yield the opening of the glucose in side chain, giving rise, respectively, to scleraldehyde and carboxylated scleroglucan (sclerox), employed for the synthesis of chemical gels [11–13]. Furthermore it is well known that many charged polysaccharides can form physical gels in the presence of uni- and/or multivalent ions. In the literature physical gels of scleroglucan have been prepared starting from sclerox in the presence of cations [14]. Moreover, scleroglucan is able to form physical gels with the addition of borax; the structural characteristics and the employment of these hydrogels for the controlled delivery of drugs of different steric hindrance have extensively been investigated [15,16]. Now

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we report the preparation and the characterization of new physical hydrogels of scleroglucan obtained from its carboxymethyl derivative (Scl-CM) in the presence of different amounts of Ca^{2+}. This method does not need the previous oxidation of the polymer, avoids the opening of the glucose ring in the side chain and allows obtaining networks with different properties compared with the reported ones. After the characterization of the derivative, the rheological behaviour of the systems prepared with different amounts of polymer and salt was investigated. The characteristics of the hydrogels obtained with Ca^{2+} suggested them as possible controlled delivery systems of drugs. With this aim acyclovir was loaded into the hydrogels; the release rate of the drug from these systems seems to be strictly related to the concentration of the salt and to the molar ratio carboxylated repetitive units of the polymer/Ca^{2+}.

2. Materials and methods

2.1. Materials

All used reagents were of analytical grade. Scleroglucan was provided by Carbomer; it had $M_w = 1.4 \times 10^6$ as evaluated by viscosimetric measurements in 0.01 M NaOH. Dimethylsulfoxide (DMSO), $CaCl_2{\cdot}2H_2O$ and chloroacetic acid were purchased from Fluka (Switzerland). D_2O, DMSO-d_6 and DOWEX 50WX4-50 ion-exchange resin were purchased from Aldrich (England). Acyclovir was kindly provided as a gift by Recordati (Italy).

2.2. Synthesis of carboxymethyl scleroglucan (Scl-CM)

Scleroglucan (1.0 g) was dissolved in water (20 ml) at 90 °C and to the solution, transferred in ice bath, NaOH (14.3 g) was added. After complete dissolution, chloroacetic acid (5.0 g) in water (25 ml) was added dropwise to the solution at 60 °C. The solution was maintained under stirring at 60 °C for 24 h. After neutralization with glacial acetic acid, the solution was submitted to exhaustive dialysis. In order to obtain all the carboxylic residues in the undissociated form, the dialyzed solution was eluted through DOWEX 50WX4-50 ion-exchange resin column previously treated with 2.0 M HCl. After freeze-drying the new polymer was characterized by FT-IR and ^{1}H NMR spectra. FT-IR spectra were recorded with a Perkin-Elmer Paragon 1000 spectrophotometer (USA) in the range 4000–400 cm^{-1} using KBr pellets (number of scans 100, resolution of 1 cm^{-1}). ^{1}H NMR spectra were obtained with a Bruker AC-400 instrument (Germany). First of all, the samples were dissolved in D_2O, maintained under stirring for 2 h and then freeze-dried. This procedure, already reported in the literature for other (1 → 3)-β-glucans [17], was repeated twice. The obtained polymer was dissolved in DMSO-d_6 and submitted to ^{1}H NMR analysis at the temperature of 75 °C. In order to determine the number of carboxylic groups introduced into scleroglucan, samples of the derivative (100 mg) were dissolved in water and submitted to potentiometer titration with 1×10^{-2} M NaOH. The amount of acid in mmol (equal to the mmol of employed base) was inserted into the equation:

$$\text{amount of polymer (mg)} = X\ \text{PM}_{\text{repetitive unit}} + (\text{mmol of acid})\ \text{PM}_{\text{acid group}}$$

and allowed the calculation of X, the mmol of repetitive units of the polymer. The ratio between the mmol of acid and the mmol of the repetitive units of the polymer multiplied by 100 gave the degree of derivatization (DD, number of carboxylic groups for 100 repetitive units) that was 65 ± 5.

2.3. Hydrogels preparation

To a solution of Scl-CM (0.050 and 0.100 g in 5 ml of water, Cp = 1 and 2% w/v) maintained at 60 °C under stirring, $CaCl_2{\cdot}2H_2O$ (0.075, 0.200 and 0.400 g) was added in order to obtain salt concentrations Cs = 0.10, 0.25 and 0.50 M, respectively. After complete dissolution, the solutions were left to cool to room temperature. The sample at Cp = 1% (2%) w/v and Cs = 0.01 M were prepared dissolving 0.050 g (0.100 g) of Scl-CM in water (2.5 ml) and adding under stirring 2.5 ml of 0.02 M solution of the salt at 60 °C.

The hydrogels containing acyclovir were prepared adding the polymer (0.050 and 0.100 g, Cp = 1% and 2% w/v) to a solution of the drug 0.25% w/v (0.013 g in 5 ml of water) maintained at 60 °C. After complete dissolution, $CaCl_2{\cdot}2H_2O$ (0.075, 0.200 and 0.400 g) was added in order to obtain Cs = 0.10, 0.25 and 0.50 M, respectively.

2.4. Rheological measurements

Rheological experiments were performed with a Haake RheoStress 300 Rotational Rheometer (Germany) equipped with a Haake DC10 thermostat. Oscillatory experiments were performed at 25.0 ± 0.2 °C in the range 0.01–10 Hz on aqueous solutions (1%, 2% and 3% w/v) of Scl and Scl-CM (1% and 2% w/v). Mechanical spectra were also recorded for hydrogels obtained adding a solution of $CaCl_2$ to the 1% and 2% w/v Scl-CM solutions to obtain a final salt concentration 0.01, 0.10, 0.25 and 0.50 M. Enough quantity of each sample was carefully poured to completely cover the 6-cm cone-plate geometry (angle of 1°). For each sample the linear viscoelastic range was evaluated: a 1% maximum deformation was used.

2.5. Scanning electron microscopy (SEM)

Freeze-dried samples of scleroglucan, carboxymethyl scleroglucan and of its physical gels with Ca^{2+} were mounted onto an aluminium stud and sputter-coated with gold employing an Emitech-K Sputter Coater in order to make it conducting. The samples were submitted to SEM analysis employing an acceleration voltage of 15.0 kV. A

scanning electron microscope Philips XL 30 (Netherland) was employed for the photographs.

2.6. Release studies

Release experiments were carried out on the samples (2.5 g) of freshly prepared hydrogels (Cp = 1% and 2% w/v and Cs = 0.10, 0.25 and 0.50 M) containing acyclovir (0.25% w/w), with the rotating basket technique at 37.0 ± 0.1 °C and 100 rpm according to U.S.P. XXIV. The experiments were carried out with a SOTAX AT7 Smart (Switzerland) in phosphate buffer (PB, pH 7.4) and acetic buffer (pH 5.0) as release media (0.5 L). The final concentration of the drug (12.5 mg/L) was much smaller than 10% of its solubility in water (1.40 g/L) [18], so that sink conditions could be assumed. The gel was carefully inserted into the basket and the drug release was followed by means of HPLC analysis, monitoring the amount of acyclovir at 255 nm. HPLC apparatus consisted of a Perkin-Elmer Series 200 LC pump, equipped with a 235 Diode Array (USA). HPLC analyses were carried out using a Merck Hibar LiChrocart (250-4, 5 μm) RP-18 column, with a flow rate of 1 ml/min. CH_3CN/H_3PO_4 10^{-2} M mixture (7:3) was used as eluant for the analyses. All the experiments were carried out in triplicate and the results are reported as mean values ± SD.

3. Results and discussion

3.1. Synthesis and characterization of carboxymethyl scleroglucan (Scl-CM)

The derivatization of scleroglucan was carried out according to a procedure already described in the literature [19]. The scheme of the synthesis is reported in Fig. 1.

After purification and freeze-drying, Sgl-CM was characterized by FT-IR spectroscopy. For an easy comparison the spectra of the native scleroglucan and of its derivative are reported in Fig. 2.

It is evident that the introduction of the carboxymethyl group produces a modification of the spectrum of the native polymer. In particular a new band is present in the sample of Scl-CM at 1617 cm^{-1} that can be related to the asymmetric stretching vibration of the carboxylate group. Two other strong bands are also present at 620 and 479 cm^{-1} probably due to the rocking in-plane and out-of-plane deformation vibrations of the carboxylic ion.

^1H NMR spectra of samples of Scl and Scl-CM in DMSO-d_6, obtained at 75 °C, are reported in Fig. 3.

As reported in the literature [17], the spectra were performed at high temperature in order to achieve a better spectral resolution. The polymers were dissolved in D_2O, maintained under stirring for 2 h and freeze-dried. This procedure, repeated twice, was necessary to eliminate the huge signal of the water, yet evident in Scl spectrum (around 3 ppm). Furthermore in this way, OH peaks were eliminated allowing the H-1 protons to appear clearly (at 4.55, 4.26 and 4.09 ppm). In Scl-CM spectrum, the signal at 4.09 ppm is more complex probably due to the presence of the substituent OCH_2CO. Unfortunately the integrals of the signals of this zone of the spectrum were not so accurate to allow a precise determination of the number of acid groups introduced for every repetitive unit of the polymer. For this reason the evaluation of the number of acid groups was performed by potentiometer titration (see Section 2.2): the derivatization degree calculated with this method was 65 ± 5.

3.2. Rheological behaviour of solutions of Scl and Scl-CM

The reaction conditions used for the derivatization of Scl and the introduction of charged moieties on the polymer chains are expected to affect its conformation, originating a system showing a different rheological behaviour. In fact, Scl in water has a triple helix conformation that can be denatured by the strong alkaline reaction conditions used in the synthesis [1]. The carboxylated moieties present on Scl-CM are expected to prevent the triple helix regeneration – usually achieved for the Scl by lowering the pH of the solution – leading to a random coil macromolecular system, in which the single chain should be more rigid due to the charges carried.

In Fig. 4 mechanical spectra recorded in the range 0.01–10 Hz for Scl and Scl-CM 1% aqueous solutions are reported. Scl shows a G' and G'' profile typical of a weak gel [20], with the moduli roughly parallel to the frequency axis and the G'/G'' ratio lesser than 10.

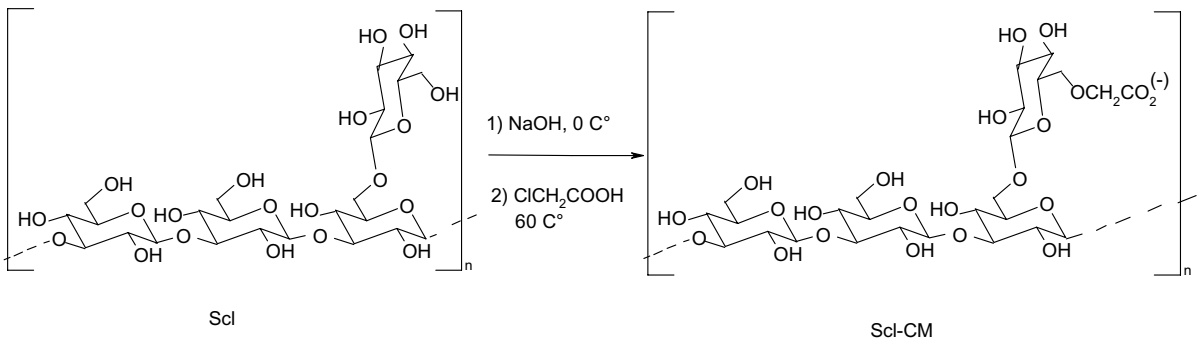

Fig. 1. Scheme of the synthesis of carboxymethyl scleroglucan.

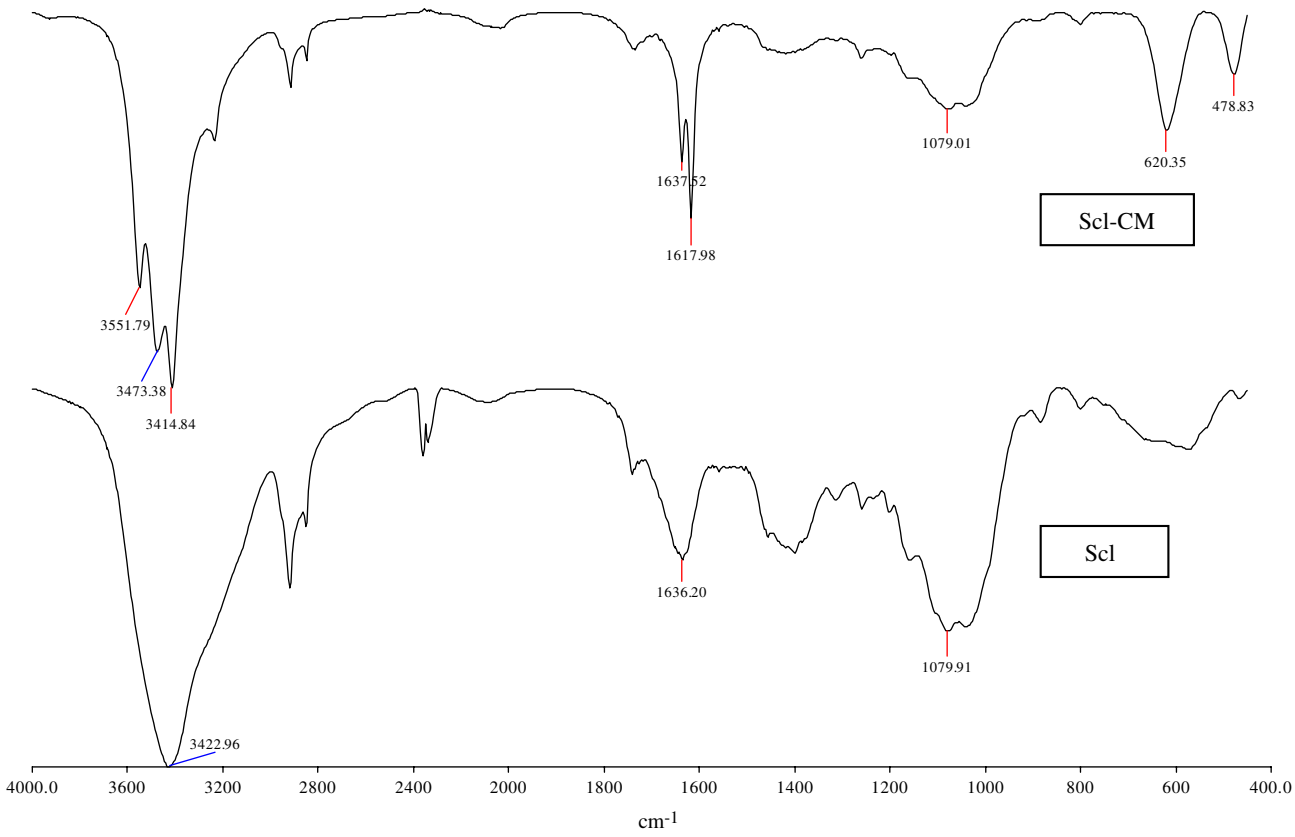

Fig. 2. FT-IR spectra of carboxymethyl scleroglucan (Scl-CM) and scleroglucan (Scl).

As expected, the mechanical spectrum of Scl-CM is quite different: the slopes of the G' and G'' profiles are about the same and different from zero, with G'' higher than G', indicating that the aqueous system is near the gel point [21].

Scl and Scl-CM show a different evolution of the mechanical spectra as a function of the polymer concentration; this effect is superimposed on the quite obvious increase of the absolute values of the moduli. As the polymer concentration is raising (2% w/v), an increase of the moduli values for the Scl system is observed, whereas for the Scl-CM an important moduli profiles modification is evident (Fig. 5a).

In fact G' became higher than G'' until a cross-over point located near 1 Hz, indicating an evolution from a near gel point to a real gel system In Fig. 5b the aqueous solution of Scl-CM at concentration of 3% w/v finally evolved to a weak gel system.

The obtained results suggest that the polymer derivatization should prevent the chain–chain aggregation because of electrostatic repulsions. The increase of the polymer concentration is able to restore the gel state for Scl-CM, structuring a network that should have a different organization with respect to the Scl one.

3.3. Preparation of physical gels of Scl-CM with Ca^{2+}

Preliminary experiments were performed in order to verify the amounts of polymer and salt that were able to form physical hydrogels. Polymer concentrations smaller than 1% w/v do not yield the gel formation even in the presence of a concentration of salt >0.5 M. On the other hand, if the salt concentration is too small (<0.01 M) the hydrogel formation does not take place. For a better understanding of the composition of the hydrogels under study, the concentrations of Scl-CM and $CaCl_2$ for each sample are reported in Table 1.

It is important to underline that when the molar ratio between the salt and the carboxylic groups is <1, the gel formation does not occur, whereas when it is very high (>25) the sample does not look homogeneous.

The morphology of the hydrogels was analysed by SEM. Fig. 6 reports SEM micrograph of the freeze-dried hydrogel; a porous surface is evident with salt inclusions (see the spots into the circle) whose nature was confirmed by mass spectroscopy.

3.4. Rheological measurements of physical gels of Scl-CM with Ca^{2+}

The formation and the mechanical properties of the physical gels of Scl-CM were monitored by means of rheological measurements. The mechanical spectra of 1% and 2% Scl-CM aqueous solutions were recorded after the addition of Ca^{2+} ions. In Fig. 7 the curves relative to the 1% polymer solutions are reported.

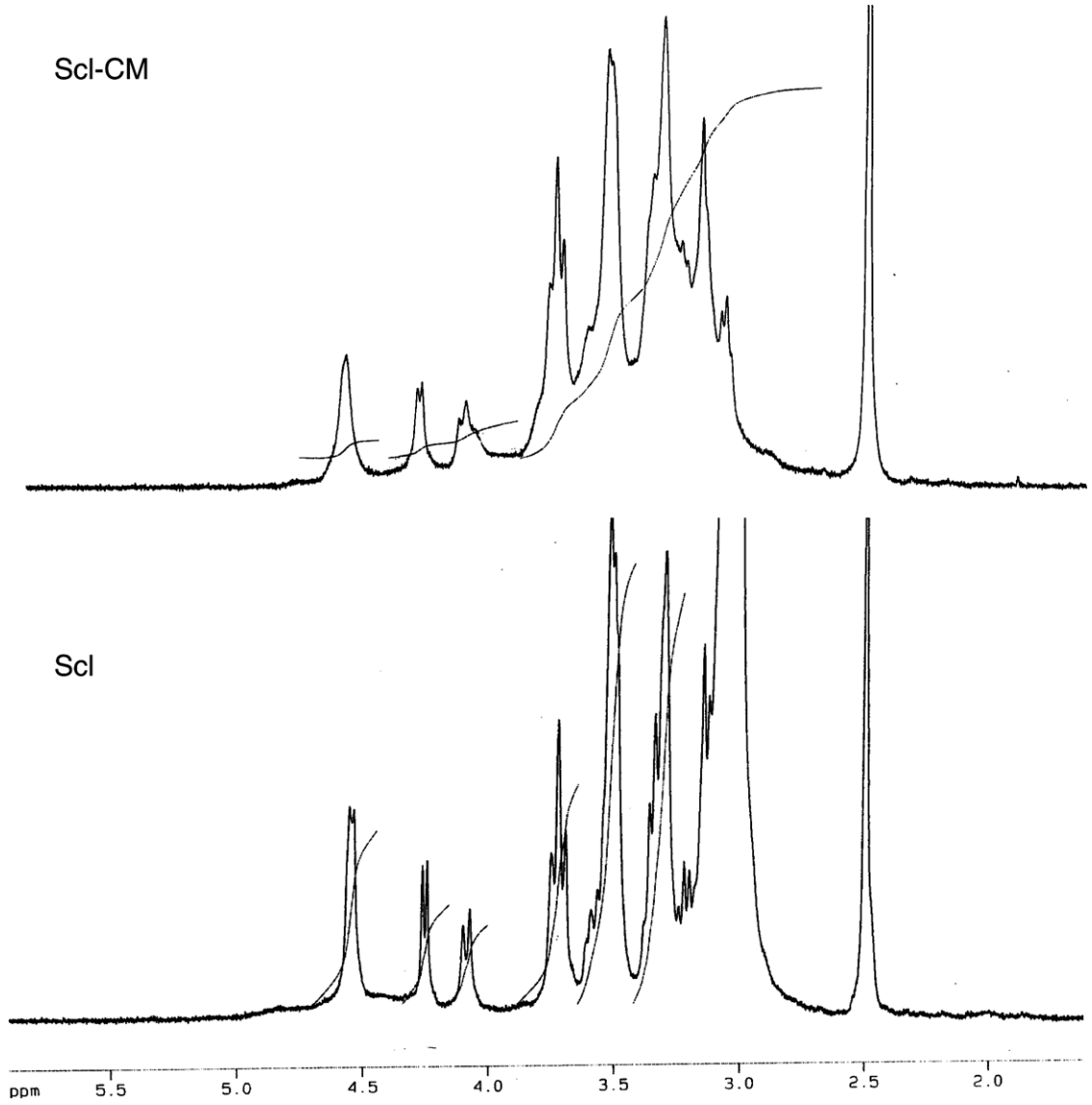

Fig. 3. ^{1}H NMR spectra of Scl-CM and Scl in DMSO-d_6 carried out at 75 °C.

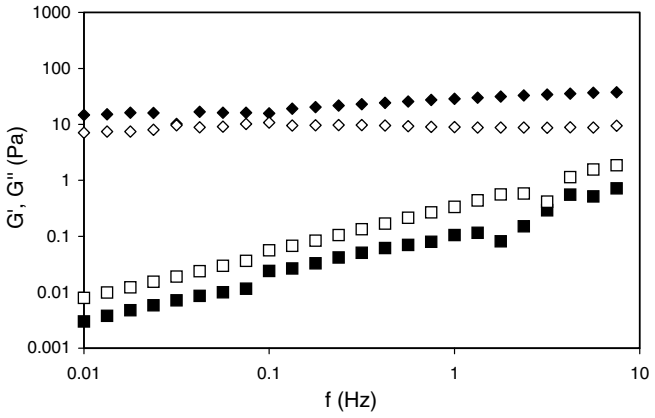

Fig. 4. Mechanical spectra of 1% w/v aqueous solution of Scl (♦, G'; ◇, G'') and Scl-CM (■, G'; □, G'') at 25 ± 0.2 °C.

The salt addition produces an evolution of the Scl-CM system from a near gel point state, as previously seen in Fig. 4, to a gel state, showing an increase of the moduli and a quite parallel profile for the curves relative to the higher concentration of Ca^{2+}. In Fig. 8, the data are reported for the 2% polymer solutions. In this case the gel state is reached at [Ca^{2+}] 0.1 M. The G' value – a measure of the strength of the gel – has the same order of magnitude of Scl 3% in water solution, and roughly irrespective of the salt concentration.

3.5. Physical gels of Scl-CM and Ca^{2+} loaded with acyclovir: release studies

Hydrogels loaded with the antiviral drug acyclovir were submitted to release studies in PB solution (pH 7.4). Acyclovir was chosen as model drug because it is very often formulated for topical application and can potentially take advantage of a hydrogel formulation. Moreover it is reported that scleroglucan has antiviral properties that

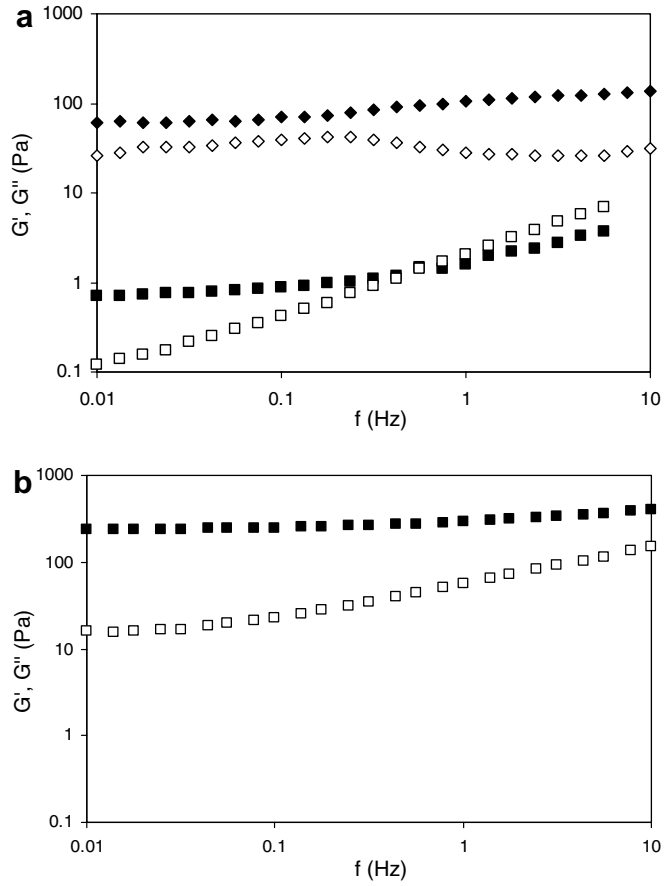

Fig. 5. Mechanical spectra of (a) 2% w/v aqueous solution of Scl (♦, G'; ◇, G'') and Scl-CM (■, G'; □, G'') and (b) 3% w/v aqueous solution of Scl-CM (■, G', □, G'') at 25 ± 0.2 °C.

Table 1
Concentrations of polymer and $CaCl_2$ employed for the preparation of the hydrogel samples

Polymer concentration (w/v)	$CaCl_2$ (0.01 M)	$CaCl_2$ (0.10 M)	$CaCl_2$ (0.25 M)	$CaCl_2$ (0.50 M)
1%	C_p1-$C_s0.01$ [b]	C_p1-$C_s0.10$	C_p1-$C_s0.25$	[a]
2%		C_p2-$C_s0.10$	C_p2-$C_s0.25$	C_p2-$C_s0.50$

[a] Inhomogeneous sample.
[b] The hydrogel does not form.

could enhance the activity of the preparations [22]. Dissolution studies were carried out on the freshly prepared hydrogels starting from solutions at 1% and 2% of the derivatized polymer and different amounts of salt. The release profiles of the drug are reported in Figs. 9 and 10.

The release rate seems to be strictly related to the concentration of the salt. In fact, for equal concentrations of the polymer, the amount of drug released in the first 6 h depends on the concentration of $CaCl_2$ and as the concentration of the salt increases the amount of released drug decreases. For a better understanding, the amounts of the drug released in 6 and 24 h from the different samples are reported in Table 2.

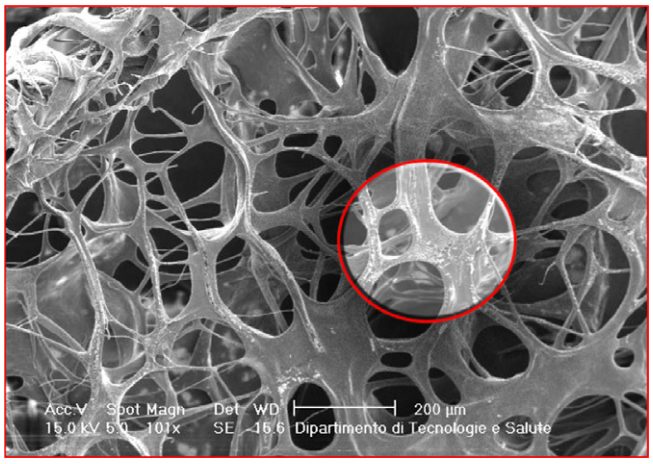

Fig. 6. SEM photograph of the outside surface of a sample of freeze-dried gel of Scl-CM and $CaCl_2$.

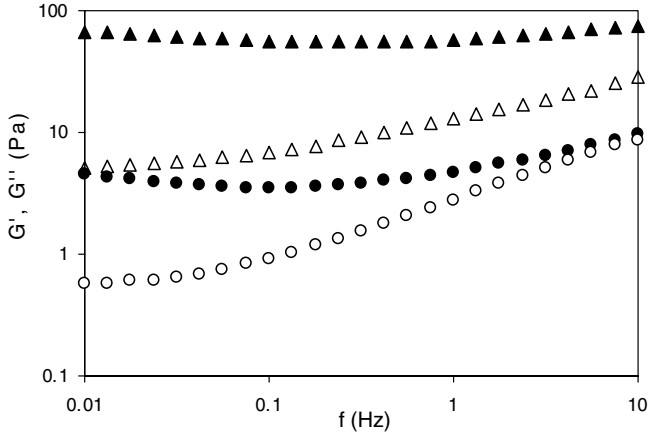

Fig. 7. Mechanical spectra of the hydrogels C_p1-$C_s0.10$ (●, G'; ○, G'') and C_p1-$C_s0.25$ (▲, G'; Δ, G'') at 25 ± 0.2 °C.

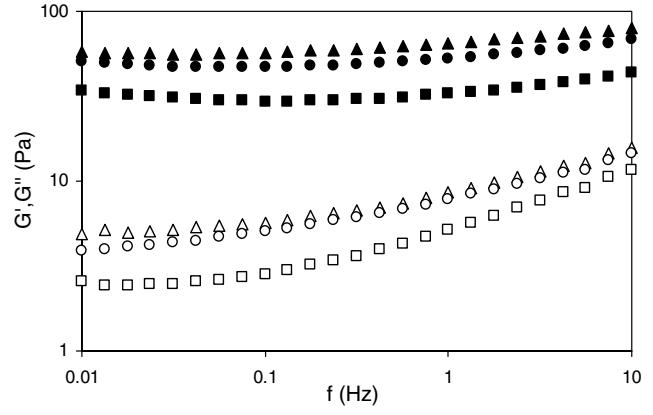

Fig. 8. Mechanical spectra of the hydrogels C_p2-$C_s0.10$ (■, G'; □, G''), C_p2-$C_s0.25$ (●, G'; ○, G'') and C_p2-$C_s0.50$ (▲, G'; Δ, G'') at 25 ± 0.2 °C.

On the contrary, comparing the amount of drug released by the samples C_p1-$C_s0.10$ and C_p2-$C_s0.10$, prepared with the same concentration of salt and different polymer amounts, the concentration of the latter seems to have an

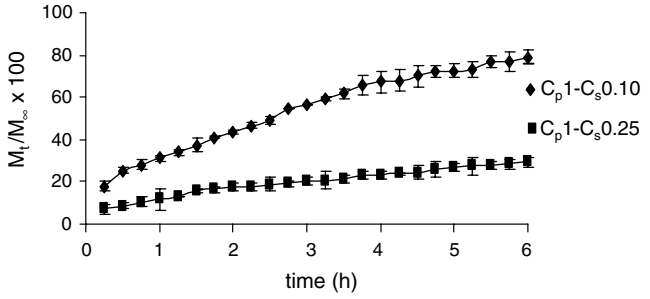

Fig. 9. Release profiles $[(M_t/M_\infty) \times 100]$ of acyclovir from hydrogels of Scl-CM (1% w/v) obtained with different concentrations of salt maintained in PB solution (pH 7.4) at 37.0 ± 0.1 °C for 6 h.

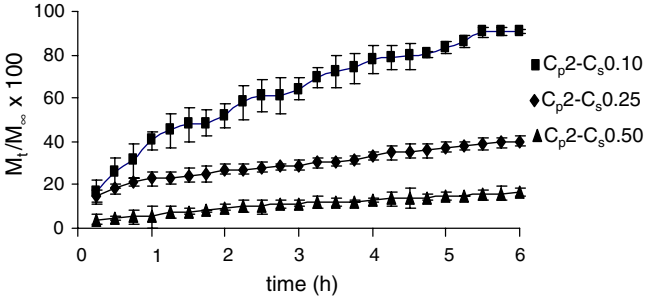

Fig. 10. Release profiles $[(M_t/M_\infty) \times 100]$ of acyclovir from hydrogels of Scl-CM (2% w/v) obtained with different concentrations of salt maintained in PB solution (pH 7.4) at 37.0 ± 0.1 °C for 6 h.

Table 2
Drug released from the different samples of gels in 6 and 24 h

Sample	Released drug in 6 h (%)	Released drug in 24 h (%)
C_p1-$C_s0.10$	79.0 ± 3.1	96.9 ± 1.9
C_p1-$C_s0.25$	29.3 ± 2.3	74.0 ± 1.1
C_p2-$C_s0.10$	90.6 ± 1.2	98.5 ± 1.2
C_p2-$C_s0.25$	40.2 ± 2.5	63.6 ± 0.5
C_p2-$C_s0.50$	16.2 ± 1.9	21.5 ± 1.2

opposite influence on the drug release. In fact the dissolution of acyclovir after the first 6 h is much higher (90.6% against 79.0%) in the sample having the higher polymer concentration. It is likely that the amount of salt in the sample at higher polymer concentration is not enough to have the maximum interactions among carboxylic/alcoholic groups and Ca ions. As a confirmation of this hypothesis, the samples $C_{sp}1$-$C_s0.25$ and C_p2-$C_s0.50$, having the same molar ratio mole of carboxylated repetitive unit/Ca^{2+}, show an amount of released drug that decreases as the polymer concentration increases. From the analysis of the obtained data, it is evident that the amount of released drug can be easily modulated by changing the salt amount in samples having the same polymer concentration, and/or in general by changing the molar ratio between carboxylated repetitive units of the polymer and Ca^{2+}. The obtained results agree with the rheological behaviour of the hydrogels, showing that the release rate of acyclovir is directly related to the characteristics of the system. In

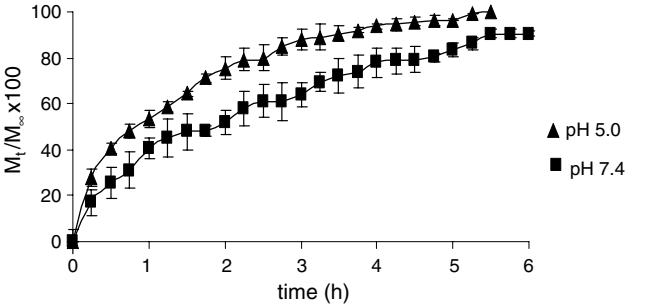

Fig. 11. Release profiles $[(M_t/M_\infty) \times 100]$ of acyclovir from hydrogels C_p2-$C_s0.10$ in pH 7.4 and 5.0 solutions at 37.0 ± 0.1 °C.

general as the strength of the gel increases, the rate of the drug release decreases according to a trend often reported in the literature [23].

Finally release studies from samples C_p2-$C_s0.10$ loaded with acyclovir were carried out in medium at pH 5.0 simulating the vaginal and skin ones. For an easier comparison the release profiles obtained in the two media are reported in Fig. 11.

It is evident that the release rate in acidic medium is higher than in the neutral one. The solubility of the drug is equal in the two media [18], but the pH of the solution could influence the strength of the gel. It is likely that some of the carboxylic groups are in the undissociated form in the lightly acidic medium [24]; the decrease of the electrostatic interactions between dissociated carboxylic groups and calcium ions could yield a less compact matrix that releases the drug faster. In any case it is evident that the release is quite modulated in the time of permanence in the application site (about 6 h), therefore the system can be proposed for the preparation of topical formulations as well as for in situ implantation.

4. Conclusions

A carboxymethyl derivative of scleroglucan (Scl-CM) was synthesized and characterized. Its rheological behaviour was studied and compared with that of the starting polymer. New physical gels were prepared and characterized adding opportune amounts of $CaCl_2$ to solutions at different concentration of Scl-CM. The rheological studies carried out on the gels allowed evidencing that the gel strength depends on the polymer and $CaCl_2$ concentrations. In order to verify a possible employment of these hydrogels as drug delivery systems, acyclovir was loaded into the networks. Dissolution studies carried out on the gels showed that the release rate of the drug is a function of the salt concentration as well as of the molar ratio polymer carboxylated repetitive units/Ca^{2+}. If compared with other analogous systems reported in the literature our hydrogel shows significant advantages. The synthesis of the derivative is very easy and performed in one step. The formation of the gel takes place by cooling the solution polymer-salt obtained at 60 °C. Moreover the system is

very flexible because it allows the modification of the release rate of the drug by only changing the salt amount during the gel preparation.

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References

[1] T. Coviello, A. Palleschi, M. Grassi, P. Matricardi, G. Bocchinfuso, F. Alhaique, Scleroglucan: a versatile polysaccharide for modified drug delivery, Molecules 10 (2005) 6–33.
[2] S. Kitamura, T. Hirano, K. Takeo, H. Fukada, K. Takahashi, B.H. Falk, B.T. Stokke, Conformational transitions of schizophyllan in aqueous alkaline solution, Biopolymers 39 (1995) 407–416.
[3] B. Guo, A. Elgsaeter, B.T. Stokke, Scleroglucan gel volume changes in dimethylsulphoxide/water and alkaline solutions are partly caused by polymer chain conformational transitions, Carbohydr. Polym. 39 (1999) 249–255.
[4] E. Touitou, F. Alhaique, F.M. Riccieri, G. Riccioni, E. Santucci, Scleroglucan as sustained release oral preparations. Part I. In vitro experiments, Drug Des. Deliv. (1989) 141–148.
[5] F. Alhaique, M. Carafa, F.M. Riccieri, E. Santucci, E. Touitou, Studies on the release behaviour of a polysaccharide matrix, Pharmazie 48 (1993) 432–435.
[6] S. Rizk, C. Duru, D. Gaudy, M. Jacob, F. Ferrari, M. Bertoni, C. Caramella, Physicochemical characterization and tabletting properties of scleroglucan, Int. J. Pharm. 112 (1994) 125–131.
[7] V. Crescenzi, M. Dentini, F. Silvi, M. Paci, G. Paradossi, L.D. Bellini, Z. Righetto, Studies of physical and chemical gels based on microbial polysaccharides, Bioactive Compatible Polym. 10 (1995) 235–248.
[8] T. Coviello, M. Dentini, G. Rambone, P. Desideri, M. Carafa, E. Murtas, F.M. Riccieri, F. Alhaique, A novel co-crosslinked polysaccharide: studies for a controlled delivery matrix, J. Control. Release 55 (1998) 57–66.
[9] N.J. Francois, A.M. Rojas, M.E. Daraio, Rheological and drug-release behaviour of a scleroglucan gel matrix at different drug loading, Polym. Int. 54 (2005) 1613–1619.
[10] M.A. Casadei, G. Pitarresi, F. Benvenuti, M. Giannuzzo, Chemical gels of scleroglucan obtained by cross-linking with 1,ω-dicarboxylic acids: synthesis and characterization, J. Drug Deliv. Sci. Technol. 15 (2005) 145–150.
[11] B.E. Christensen, E. Aasprong, B.T. Stokke, Gelation of periodate oxidised scleroglucan (scheraldehyde), Carbohydr. Polym. 46 (2001) 241–248.
[12] T. Coviello, M. Grassi, G. Rambone, F. Alhaique, A crosslinked system from scleroglucan derivative: preparation and characterization, Biomaterials 22 (2001) 1899–1909.
[13] T. Coviello, M. Grassi, G. Rambone, E. Santucci, M. Carafa, E. Murtas, F.M. Riccieri, F. Alhaique, Novel hydrogel system from scleroglucan: synthesis and characterization, J. Control. Release 60 (1999) 367–378.
[14] T. Coviello, F. Alhaique, C. Parisi, P. Matricardi, G. Bocchinfuso, M. Grassi, A new polysaccharidic gel matrix for drug delivery: preparation and mechanical properties, J. Control. Release 102 (2005) 643–656.
[15] T. Coviello, M. Grassi, R. Lapasin, A. Marino, F. Alhaique, Scleroglucan/borax: characterization of a novel hydrogel system suitable for drug delivery, Biomaterials 24 (2003) 2789–2798.
[16] T. Coviello, M. Grassi, A. Palleschi, G. Bocchinfuso, G. Coluzzi, F. Banisheib, F. Alhaique, A new scleroglucan/borax hydrogel: swelling and drug release studies, Int. J. Pharm. 289 (2005) 97–107.
[17] H.E. Ensley, B. Tobias, H.A. Pretus, R.B. McNamee, E.L. Jones, I.W. Browder, D.L. Williams, NMR spectral analysis of a water-insoluble (1 → 3)-β-glucan isolated from *Saccharomyces cerevisiae*, Carbohydr. Res. 258 (1994) 307–311.
[18] C.A.S. Bergström, U. Norinder, K. PLuthman, P. Artursson, Experimental and computational screening models for prediction of aqueous drug solubility, Pharm. Res. 19 (2002) 182–189.
[19] A.E.J. de Nooy, V. Rori, G. Masci, M. Dentini, V. Crescenzi, Synthesis and preliminary characterization of charged derivatives and hydrogels from scleroglucan, Carbohydr. Res. 324 (2000) 116–126.
[20] A.H. Clark, S.B. Ross-Murphy, Structural properties of biopolymer gels, Adv. Polym. Sci. 83 (1987) 57–192.
[21] P. Matricardi, M. Dentini, V. Crescenzi, S.B. Ross-Murphy, Gelation of chemically cross-linked polygalacturonic acid derivatives, Carbohydr. Polym. 27 (1995) 215–220.
[22] I. Giavasis, L.M. Harvey, B. McNeil, Scleroglucan in: Biopolymers, Wiley-VCH Verlag GmbH, Weinheim, Germany, 2002.
[23] M.M. Talukdar, I. Vinckier, P. Moldenaers, R. Kinget, Rheological characterization of xanthan gum and hydroxypropylmethyl cellulose with respect to controlled-release drug delivery, J. Pharm. Sci. 85 (2002) 537–540.
[24] M. Giannuzzo, M. Feeney, P. Paolicelli, M.A. Casadei, Synthesis and characterization of pH-sensible hydrogels of dextran, J. Drug Deliv. Sci. Technol. 16 (2006) 49–54.