J Mater Sci: Mater Med (2009) 20:1081–1087
DOI 10.1007/s10856-008-3665-1

Carboxymethyl derivative of scleroglucan: a novel thermosensitive hydrogel forming polysaccharide for drug delivery applications

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Received: 19 August 2008 / Accepted: 9 December 2008 / Published online: 10 January 2009

Abstract A carboxymethyl derivative of scleroglucan (Scl-CM) with a derivatization degree of 65 ± 5% was synthesized. The rheological behaviour of this novel polymer was studied and compared with that of the starting polymer. We observed that the charged moieties carried on the chains could prevent the triple helix formation of Scl. Scl-CM aqueous solutions behave like true polymer solutions up to 1% w/v, whereas above this concentration a weak gel behaviour was observed. $CaCl_2$ addition to aqueous Scl-CM solutions led to a physical gel formation; the hydrogel strength was related to polymer and $CaCl_2$ concentrations. Temperature sweeps, registered at 1 Hz on hydrogels differing in $CaCl_2$ concentration, evidenced a gel → sol transition in the range of 30–40°C, depending on the molar ratio between carboxylic groups and Ca^{+2}. In order to verify a possible use of these hydrogels as drug delivery systems, acyclovir was loaded into the network. Rheological analysis evidenced that the loaded drug can affect the hydrogel elastic modulus. The release of acyclovir in phosphate buffer was evaluated at different temperatures in order to assess the suitability of this novel drug delivery system in topical applications.

1 Introduction

Scleroglucan (Scl) is a natural polysaccharide produced by fungi of the genus *Sclerotium.* It consists of a main chain of (1 → 3)-linked β-D-glucopyranosyl units substituted with single(1 → 6)-linked β-D-glucopyranosyl residues every third backbone unit [1]. Because of its peculiar rheological properties and its resistance to hydrolysis, temperature and electrolytes, scleroglucan has found applications in oil industry, as well as in cosmetic, food and pharmaceutical ones. The peculiar rheological properties of Scl can be related to the rigid rod structure that polymer chains have in aqueous solutions. In fact, in this condition Scl chains adopt a stable triple helix structure and the denaturation can occur in dimethyl sulfoxide (DMSO) or in water solutions at pH higher than 12.5 [2, 3].

The use of scleroglucan as antitumor, antiviral and antimicrobial compound, because its immune stimulatory effects, has also been exploited [4–7]. Furthermore Scl and some of its oxidation derivatives were used for the preparation of chemical and physical hydrogels, suitable as matrices for the controlled release of drugs [8–13] It was reported in the literature the preparation of a physical hydrogel of Scl obtained from the carboxylated scleroglucan (Sclerox) in the presence of cations [14]. This method involves the opening of the glucose ring in the side chain by means of $NaIO_4$ and the oxidation of the aldehydes formed by means of $HClO_2$ in the presence of acetic acid. We have already described the preparation of a new chemical carboxymethyl derivative of Scl (Scl-CM), and its physical hydrogel, obtained by interaction with Ca^{+2} ions [15]. In particular, the properties of this novel hydrogel as a matrix for the controlled release of acyclovir (ACV) were described. In the present article we report the rheological characterization of the aqueous solutions of Scl-CM and of the hydrogel obtained by the interaction between Scl-CM and Ca^{+2} ions. Scl-CM has the ring in the side chain intact and, in the presence of different amounts of Ca^{+2} ions it gives rise to gels of different strengths. In particular, the thermosensitive behaviour showed by the

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Scl-CM/Ca^{+2} hydrogels in a well defined range of polymer and salt concentrations is here described.

2 Experimental

2.1 Materials

All 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), $CaCl_2 \times 2H_2O$ 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

The synthesis of carboxymethyl scleroglucan has already been described [15]. Briefly, 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, a water solution of chloroacetic acid (5.0 g in 25 ml of water) was added dropwise to the polymer 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 dialyzed against distilled water until the conductivity reached 1.0 μS. 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. Then the resulting solution was freeze-dried. 100 mg of freeze dried samples were dissolved in water and a potentiometric titration with 1×10^{-2} M NaOH carried out in order to determine the degree of derivatization of the scleroglucan. The amount of acid in mmol (equal to the mmol of employed base) was inserted into the equation:

$$\text{Amount of polymer (mg)} = \text{X}PM_{\text{repetitive unit}} + (\text{mmol of acid})\, PM_{\text{acid group}}$$

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%.

The scleroglucan derivative was characterized by FT-IR and ^{1}H-NMR spectra as reported elsewhere [15]. FT-IR spectra were recorded with a Perkin Elmer Paragon 1000 spectrophotometer (USA) in the range 4,000–400 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). The samples were dissolved in D_2O, maintained under stirring for 2 h and then freeze-dried. This procedure, already reported in literature for other (1 → 3)-β-glucans, was repeated twice [16]. The obtained polymer was dissolved in DMSO-d_6 and ^{1}H-NMR spectra were collected at 75°C.

2.3 Preparation of physical gels of Scl-CM

Physical gels of Scl-CM was prepared adding to a polymer aqueous solution (0.050 and 0.100 g in 5 ml of water, $C_p = 1.0$ and 2.0% w/v), maintained at 60°C under stirring, $CaCl_2 \times 2H_2O$ (0.075, 0.200 and 0.400 g) in order to obtain salt concentrations $C_s = 0.10$, 0.25 and 0.50 M respectively. After complete dissolution, the solutions were left to cool to room temperature. The sample at $C_p = 1.0$ and 2.0% w/v and $C_s = 0.01$ M were prepared dissolving 0.050 g and 0.100 g respectively of Scl-CM in water (2.5 ml) and adding under stirring 2.5 ml of 0.02 M $CaCl_2 \times 2H_2O$ solution at 60°C.

The hydrogels containing acyclovir were prepared adding the polymer (0.05 and 0.10 g, $C_p = 1.0$ and 2.0% 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, $CaCl_2 \times 2H_2O$ (0.075 g) was added in order to obtain a salt concentration $C_s = 0.1$ M. The hydrogel setting was achieved refrigerating the solution to room temperature.

2.4 Rheological measurements

Rheological experiments were performed with a Haake RheoStress 300 Rotational Rheometer (Germany) equipped with a Haake DC10 thermostat. Flow curves of solutions of native Scl and Scl-CM 0.5, 1.0 and 2.0% w/v (0.025, 0.050, 0.100 g in 5 ml of water) were recorded at 25.0 ± 0.5°C with a shear stress in the range 0.01–10 Pa. In the same condition a flow curve of a Scl solution, treated as in the experimental conditions used for the synthesis but without adding chloroacetic acid (Scl "treated"), was carried out. Oscillatory experiments were performed at 25.0 ± 0.5°C in the range of 0.01–10 Hz on neutral aqueous solutions of Scl and Scl-CM at concentrations of 0.5, 1.0, 2.0 and 3.0% w/v. Mechanical spectra were also recorded for the physical hydrogels obtained adding a solution of $CaCl_2$ to the 1.0 and 2.0% w/v Scl-CM solutions to obtain a final salt concentration of 0.01 M, 0.10 M, 0.25 M and 0.50 M at 25.0 ± 0.5°C. Temperature sweeps, from 0 to 55°C, were performed on hydrogels at 1.0 and 2.0% w/v, prepared with a different amount of $CaCl_2$ (0.01, 0.10, 0.25, 0.50 M) and the G′ evolution at 1 Hz was recorded. Temperature sweeps were also performed on hydrogels C_p = 1.0 and 2.0% w/v

with 0.10 M of $CaCl_2$ containing acyclovir entrapped in the network. G′ evolution at 1 Hz was recorded: mechanical spectra were also recorded at 0 and 55°C.

For each analysis a sufficient quantity of sample was carefully poured to completely cover the 6 cm cone-plate geometry (Haake CP60/1, cone angle 1°). For each sample preliminary strain sweep tests were performed in order to evaluate the range of deformation in which the linear viscoelasticity occurs: a 1% maximum deformation was used.

2.5 Release studies

Release experiments were carried out on the samples (2.5 g) of freshly prepared hydrogels ($C_p = 1$ and $C_s = 0.10$ M) containing acyclovir (0.25% w/v), with the rotating basket technique (100 rpm) at 25.0, 37.0 and 45.0 ± 0.1°C. The experiments were carried out with a SOTAX AT7 Smart (Switzerland) in phosphate buffer (pH = 7.4) as release medium (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), 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 of 1 ml/min. CH_3CN/H_3PO_4 10^{-2} M mixture (7:3) was used as eluant for the analyses. The experiments were carried out in triplicate and the results are reported as mean value ± SD.

3 Results and discussion

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

The derivatization of Scl was carried out according to a procedure already reported [15]. In Fig. 1 is reported the scheme of the synthesis. One of the derivatives that can be obtained from the reaction is reported in the scheme.

The product obtained was purified, freeze-dried and characterized by FT-IR and ^{1}H-NMR spectroscopy [15]. To determine the number of acid groups introduced on the scleroglucan chain, a potentiometer titration was performed: the derivatization degree obtained was 65 ± 5%.

To study the influence of charged moieties present on the polymer chains, the rheological behaviour of Scl-CM was compared with that of the native Scl. In Fig. 2a and b the mechanical spectra, recorded at 25 ± 0.5°C, of Scl and Scl-CM at different polymeric concentrations ($C_p = 0.5$, 1.0, 2.0% w/v), were reported.

According to the literature [17], Scl sample above 0.23% w/v behaves like a weak gel, showing a parallelism between G′ and G″ with G′ > G″ and quite independent from frequency. The charged carboxylated moieties introduced on the polymer chains, in unscreened conditions—i.e. without salt added—are able to modify the behaviour with respect to that observed by Scl, as showed by the mechanical spectra of Fig. 2b. In fact, Scl-CM aqueous solutions show a solution behaviour at 0.5% w/v concentration, whereas a transition from a true solution to a weak hydrogel behaviour was observed in the range 0.5–3% w/v.

The flow curves of Scl at $C_p = 0.5$, 1.0 and 2.0% w/v, reported in Fig. 3a and those of Scl-CM reported in Fig. 3b, clearly indicate in solution a different molecular organization of the two systems due to the derivatization.

Comparing the Scl and Scl-CM (measured at shear stress $\sigma = 0.2$ Pa) at each concentration reported in Fig. 3, a decrease of the zero-stress applied flow viscosity of more than one order of magnitude is observed.

The overall behaviour is compatible with a model that describes an electrostatic repulsion among the polymer chains of Scl-CM caused by the charges of the carboxylic groups. This repulsion avoids the interchain association that is responsible for the weak gel behaviour of Scl. At higher polymer concentration, the hydrogel behaviour of Scl-CM is restored because of charge screening due to the presence of counter ions; in this case the storage modulus G′ at 1 Hz for Scl-CM 3.0% is comparable to the plain Scl storage modulus G′ at 1 Hz at 2.0%.

In order to investigate the role played by the reaction environment in the model above described, flow curves of Scl-CM, native Scl and "treated" Scl were compared (Fig. 4a). The strong alkaline reaction conditions used in the synthesis determine the loss of the triple helix structure of Scl [1]. For the Scl "treated" sample, the neutralization

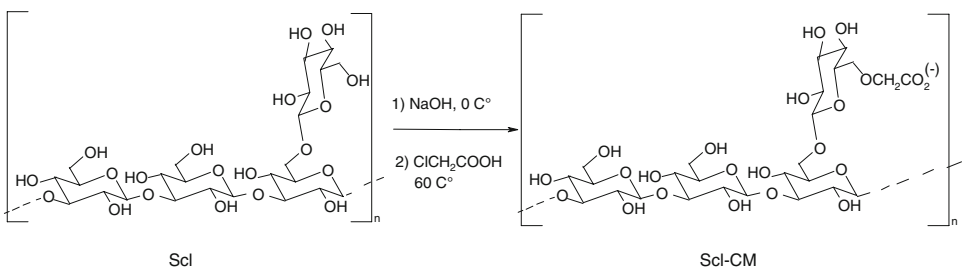

Fig. 1 Scheme of the carboxymethyl scleroglucan synthesis

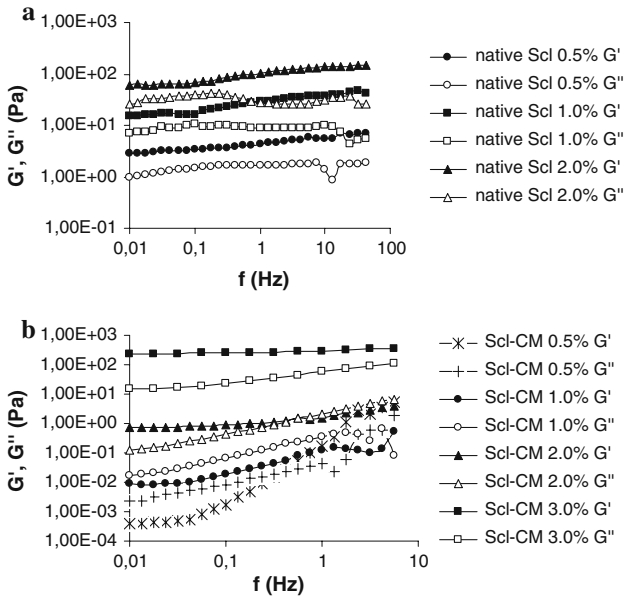

Fig. 2 Mechanical spectra of **a** Scl and **b** Scl-CM at different polymeric concentrations at 25.0 ± 0.5°C

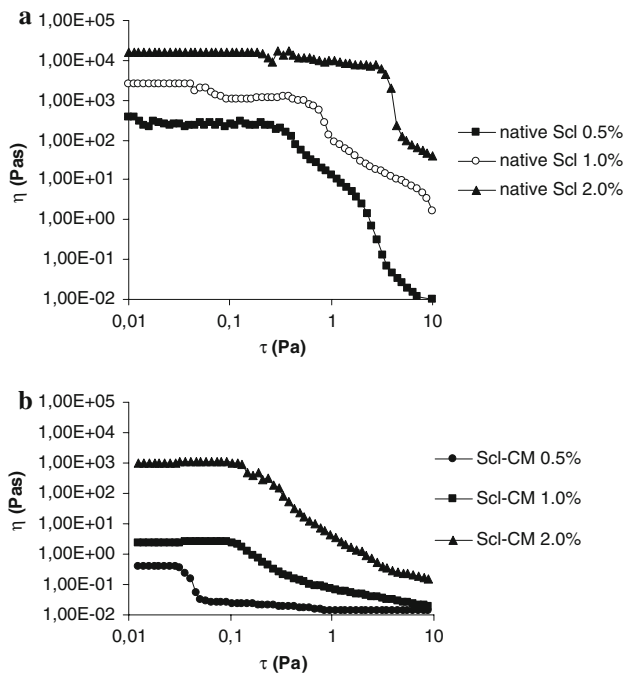

Fig. 3 Flow curves of **a** Scl and **b** Scl-CM at different polymeric concentrations (0.5, 1.0, 2.0% w/v) carried out at 25.0 ± 0.5°C

did not involve the reconstitution of the triple helix structure in the time range considered—at least 10 days, as the flow viscosity appeared to be unvaried. Analogously the Scl-CM is not able to reform the triple helix structure after neutralization, but it shows absolute values of η higher than that of "treated" Scl, probably because of increased

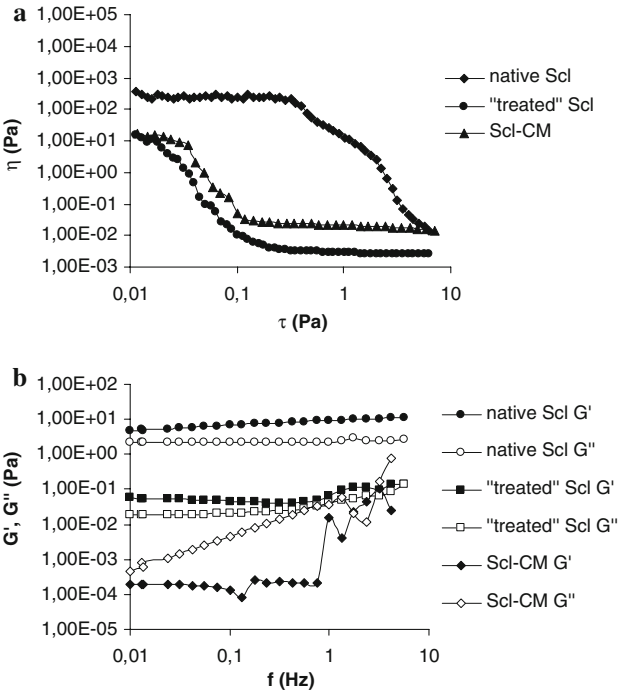

Fig. 4 Flow curves (**a**) and mechanical spectra (**b**) of different samples of 0.5% w/v aqueous solutions of Scl at 25.0 ± 0.5°C

rigidity of the polymer chain due to its polyelectrolyte nature. In Fig. 4b the mechanical spectra of the aqueous solutions of Scl, Scl "treated" and Scl-CM—$C_p = 0.5\%$ w/v—are shown. Scl "treated" shows a mechanical spectrum typical of a weak gel, similar to that of Scl, whereas in the same experimental conditions Scl-CM behaves like a solution, confirming that the electrostatic repulsions avoid the chain associations responsible of the weak hydrogel behaviour of Scl.

In Fig. 5 mechanical spectra of Scl, Scl "treated" and Scl-CM at 1.0% w/v concentration are reported. Also in this case a behaviour similar to that as above depicted was observed.

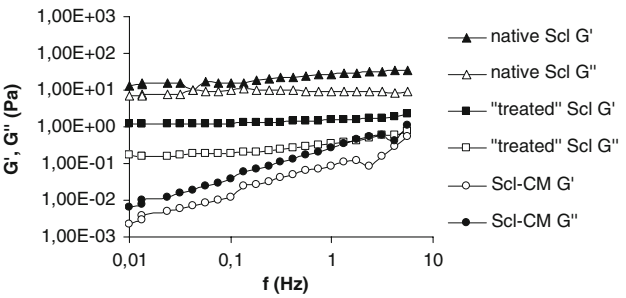

Fig. 5 Mechanical spectra of samples of 0.5% w/v aqueous solutions of Scl, Scl-CM and Scl "treated" at 25.0 ± 0.5°C

Table 1 Summary of the hydrogels samples of Scl-CM and $CaCl_2$ prepared

	$CaCl_2$ 0.01 M	$CaCl_2$ 0.10 M	$CaCl_2$ 0.25 M	$CaCl_2$ 0.50 M
C_p 1% w/v	$C_p1 - C_s0.01$ $r = 1.9 \times 10^{-1}$	$C_p1 - C_s0.10$ $r = 1.9 \times 10^{-2}$	$C_p1 - C_s0.25$ $r = 7.5 \times 10^{-3}$	Inhomogeneous sample
C_p 2% w/v	The hydrogel does not form	$C_p2 - C_s0.10$ $r = 3.78 \times 10^{-2}$	$C_p2 - C_s0.25$ $r = 1.51 \times 10^{-2}$	$C_p2 - C_s0.50$ $r = 7.56 \times 10^{-3}$

The molar ratio r (r = mol of Ca^{+2} vs. mol of polymer) is reported

3.2 Physical hydrogels of Scl-CM with Ca^{2+}

It was observed that aqueous solutions of Scl-CM were able to form hydrogels when $CaCl_2$ is added. From preliminary experiments it was observed that hydrogels were formed only in a well defined polymer concentration range [14]. Also the molar ratio "r" between the moles of salt added and the carboxylic groups carried on by Scl-CM was a critical parameter: when r is <1 the gel formation does not occur, whereas when it is very high, $r > 25$, the sample does not look homogeneous (Table 1).

The mechanical spectra of 1.0 and 2.0% w/v Scl-CM aqueous solutions, in the presence of different concentrations of Ca^{+2} (0.01 and 0.10 M for 1.0% and 0.10, 0.25, 0.50 M for 2.0%), clearly show that the addition of the salt determines an evolution of the system from a near gel state to a gel state (Fig. 6). Indeed, the elastic modulus G′ is higher than the dissipative modulus G″ and both of them are quite independent from the frequency applied.

With the aim of developing a drug delivery system suitable for topical applications, the thermosensitive properties of the Ca^{+2} hydrogels prepared were tested. The pharmaceutical products used in topical applications (e.g. vaginal ones) should have, from the mechanical point of view, thermosensitive properties in order to facilitate patient compliance, showing appropriate rheological properties.

In Fig. 7a G′, recorded at 1 Hz in temperature sweeps experiments in the range $0 \div 50$°C, of the samples of Scl-CM at $C_p = 1.0\%$ with different amounts of salt ($C_s = 0.01$, 0.1, 0.25 M) were reported.

As expected G′ decreases as the temperature increases for all the samples. The samples with C_s0.01 and 0.10 M show a gel behaviour at low temperatures and a solution behaviour at higher temperatures. The crossover temperature, where the inversion of the G′ and G″ is observed, is shifted towards higher values as the salt concentration is increased: 33 and 45°C for the samples with C_s0.01 and 0.10 M respectively. The mechanical spectra, recorded at 55°C, of the same samples confirm the solution behaviour (data not reported). The system containing 0.25 M Ca^{+2} always shows a $G' > G''$, clearly indicating that in the temperature range explored no thermosensitive effect is observed. Increasing the polymer concentration

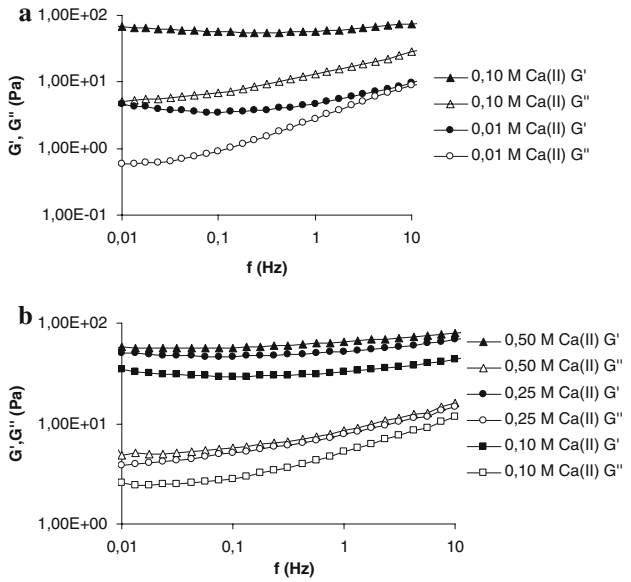

Fig. 6 Mechanical spectra of **a** 1.0% w/v and **b** 2.0% w/v of Scl-CM aqueous solutions in the presence of different amounts of Ca^{+2} ions

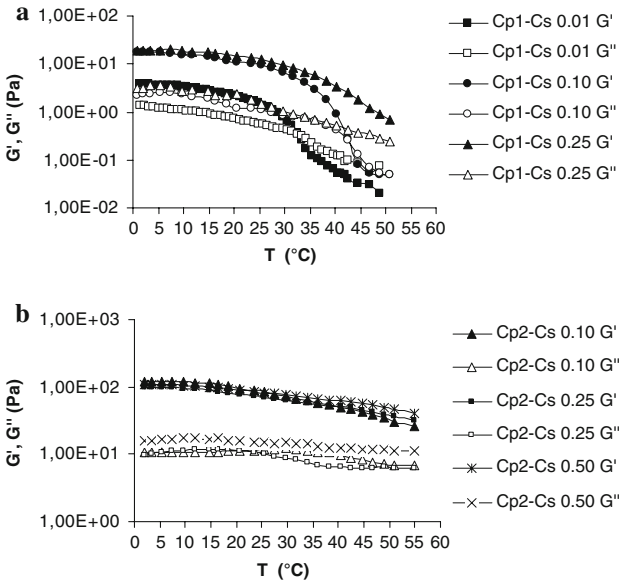

Fig. 7 Temperature sweeps of **a** 1.0% w/v and **b** 2.0% w/v of Scl-CM aqueous solutions in the presence of different amounts of Ca^{+2} ions

(C_p = 2.0% w/v) a different behaviour is observed: the salt concentration does not influence the G′ and G″ profiles (Fig. 7b) and no thermosensitive behaviour is observed.

3.3 Hydrogel of Scl-CM/Ca^{+2} containing acyclovir

Acyclovir, an antiviral drug, was chosen as model drug to test the release properties of the hydrogels prepared, because of its use in topical formulations. The thermosensitive behaviour that some gels, obtained with an opportune molar ratio between carboxylic groups and Ca^{+2}, should make easier the release of the loaded drug. Moreover, it is reported that scleroglucan has antiviral properties that could enhance the activity of the preparations [5–7]. Temperature sweep experiments were performed on the Scl-CM physical hydrogels loaded with acyclovir (C_p = 1.0 and 2.0% w/v, C_s = 0.10 M, ACV = 0.25% w/v) in order to verify if the presence of the drug could vary the rheological behaviour of the matrix. In Fig. 8 the temperature sweeps of 1.0 and 2.0% w/v Scl-CM gels with and without ACV were reported.

There are no significant changes in the trend of the curves. In the presence of the drug the gel obtained from 1.0% w/v Scl-CM solution shows slightly lower values of G′ and G″ that evidence a negative effect of ACV on the strength of the gel. For the samples at 2.0% w/v the curves are nearly coincident.

In a previous work the releases of this drug at 37°C from the Scl-CM/Ca^{+2} hydrogels were already reported. In the present work, in order correlate the mechanical and the drug release properties, hydrogels loaded with acyclovir were submitted to release studies in solution at pH = 7.4 at three different temperatures. The experiments were carried out on the freshly prepared hydrogels obtained from solutions at 1.0% w/w of the derivatized polymer and 0.10 M $CaCl_2$ at 25.0, 37.0 and 45.0 ± 0.1°C. The release profiles are reported in Fig. 9. The drug release rate of acyclovir is strictly related to the temperature: increasing the temperature the release rate increases. The results are in accordance with the rheological behaviour of the hydrogel: when the temperature increases the strength of the gel decreases and the drug diffusion is enhanced. The overall observed behaviour could be related to a different molecular organization of the polymer chains in the network. The drug seems to be able to influence the structure of the hydrogel, and in particular of the hydrogel with the lower content of Ca^{+2}, i.e. the looser network. When the temperature raises this loose network is weakened and this effect is enhanced by the presence of the drug. The net result is that the drug is carried out by a soft hydrogel and can be released faster at body temperature.

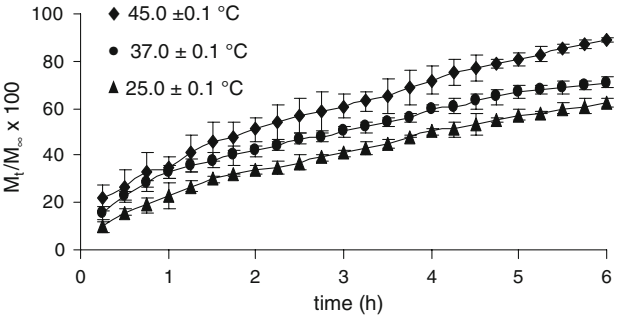

Fig. 9 Release profiles [(M_t/M_∞) × 100] of acyclovir from hydrogels of Scl-CM (1% w/v) and $CaCl_2$ 0.10 M, maintained in solution at pH = 7.4 at different temperatures for 6 h

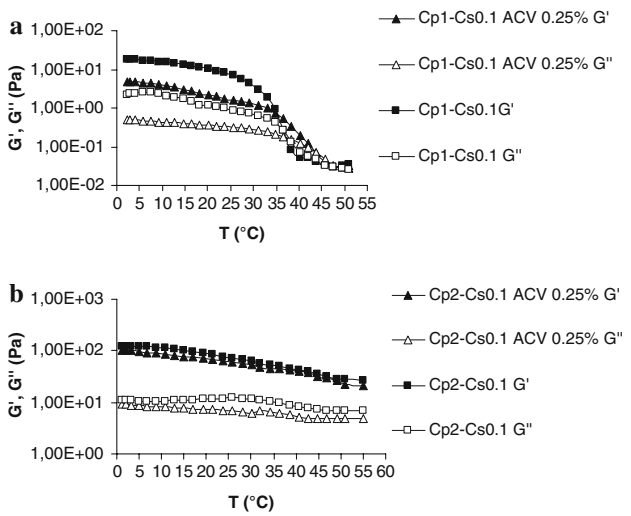

Fig. 8 Temperature sweeps of samples of Scl-CM **a** $C_p1 - C_s0.1$ and **b** $C_p2 - C_s0.1$ with and without the presence of acyclovir (ACV)

4 Conclusions

The rheological behaviour of a carboxymethyl derivative of scleroglucan (Scl-CM) was studied and compared with that of the starting polymer (native Scl). The presence of charged groups along polymeric chains determine an electrostatic repulsion among the scleroglucan chains that avoids the interchain association responsible for the weak gel behaviour of Scl even at low concentration values. Adding $CaCl_2$ to aqueous solutions of Scl-CM (1.0 and 2.0% w/v) the system evolves from a solution or near gel state to a hydrogel state. The strength of the hydrogels obtained depends on the polymer and salt concentrations and, for a particular molar ratio range between carboxylic groups and Ca^{+2}, the matrices show a thermosensitive behaviour that could be exploited in topical application in drug release field; in this respect acyclovir has been successfully tested as a model drug for topical applications.

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