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Novel injectable and *in situ* cross-linkable hydrogels of dextran methacrylate and scleroglucan derivatives: Preparation and characterization

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ABSTRACT

In this paper mixtures of two biocompatible polymers, dextran methacrylate (DEX-MA) with different molecular weights and scleroglucan (Scl), in its native form and as carboxymethyl derivative (Scl-CM), were tested as injectable and *in situ* cross-linkable systems. Rheological and texture analyses were carried out to better investigate the behavior of this kind of matrices. The combination of these polymers is able to assure adequate mechanical properties, suitable for biomedical applications. In addition swelling studies and *in vitro* release studies of three different biomolecules were also carried out.

1. Introduction

Tissue engineering represents a great challenge for biomedical research because it ranges from the repair of damaged tissue to the release of bioactive molecules (Langer, 1999; Langer & Karp, 2007; Langer & Tirrell, 2004). Among the numerous systems proposed in this field hydrogels seem to have the most attractive characteristics (Baroli, 2007; Drury, 2003). Hydrogels are crosslinked, three-dimensional polymeric networks able to swell in aqueous media without dissolving their structure. Formed by water-soluble polymers, these systems show very similar properties to leaving tissue ones, and as a consequence well accepted for implantation. In particular injectable and/or *in situ* forming hydrogels represent a promising approach in many pharmaceutical and biomedical applications, because they allow treating injured patients by using minimal invasive techniques (Kretlow, Klouda, & Mikos, 2007). Moreover, injectable systems provide the ability to take the shape of the cavity where they are placed, thus filling possible irregular defects. Different synthetic and natural polymers have been employed as injectable and/or *in situ* forming hydrogels, whose preparations and utilizations have been exhaustively revised (Nair & Laurencin, 2006). Polysaccharides and their derivatives find employment as starting materials for the preparation of hydrogels for pharmaceutical purposes because of their biocompatibility, non-toxicity and not immunogenic properties (Malafaya, Silva, & Reis, 2007). In particular dextran (DEX) is one of the most widely used polysaccharide. It consists of a linear chain of (1→6) linked α-D-glucopyranosyl units with few (1→2) (1→3) (1→4) α-D-glucopyranosyl ramifications. The reaction of dextran with glycidyl methacrylate produces a derivative (DEX-MA) able to form hydrogels by UV irradiation, through crosslinking of methacrylate moieties (Coviello et al., 2007; van Dijk-Wolthuis, Kettennes-van den Bosch, van der Kerk-van Hoof, & Hennink, 1997). Despite the numerous applications proposed for DEX-MA based hydrogels (Kim & Chu, 2000; Meyvis, De Smedt, Stubbe, Hennink, & Demeester, 2001), these systems show rheological properties unsuitable for tissue engineering applications because they are very brittle. In the literature it is reported that mixtures of polymers can lead to the formation of new materials having properties completely different with respect to the starting polymers ones (Bajpai, Shukla, Bhanu, & Kankane, 2008; Goycoolea, Morris, & Gidley, 1995). Therefore in order to overcome the drawbacks of DEX-MA hydrogels, mixtures of DEX-MA and calcium alginate have been recently proposed as interpenetrating polymer system (IPN) for biomedical applications (Matricardi, Pontoriero, Coviello, Casadei, & Alhaique, 2008; Pescosolido et al., 2009). Mechanical characterization confirmed

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the possibility to obtain a system able to be injected and crosslinked *in situ*. Now we decided to study new systems composed of DEX-MA and scleroglucan, in its native form and as carboxymethyl derivative (Scl-CM). Scleroglucan is a polysaccharide consisting of a backbone of (1→3)-β-linked glucopyranosyl residues substituted with a single (1→6)-β-glucose residue every third backbone units. It is already employed in pharmaceutical field (Coviello et al., 2005). We choose to use a carboxylated derivative of scleroglucan (Scl-CM) that is able to give physical hydrogels (Casadei, Matricardi, Fabrizi, Feeney, & Paolicelli, 2007; Corrente et al., 2009). Binary mixtures of DEX-MA and Scl or Scl-CM and their hydrogels were prepared and the mechanical properties of all the systems were investigated. At the same time the possibility to deliver molecules of different steric hindrance loaded inside, was studied.

2. Materials and methods

2.1. Materials

All used reagents were of analytical grade. Scleroglucan with $M_w = 1.4 \times 10^6$ as evaluated by viscosimetric measurements in 0.01 M NaOH, was provided by Carbomer. Dextran (DEX) from *Leuconostoc* ssp. (M_w 40,000 and 500,000), 4-dimethylaminopyridine (DMAP), anhydrous dimethylsulfoxide (DMSO), theophylline (THP), myoglobin (MGB), chloroacetic acid were purchased from Fluka (Switzerland). Glycidyl methacrylate (GMA), methacrylic acid (MA), vitamin B12 (VitB12), D_2O, DMSO-d_6, DOWEX 50WX4-50 ion-exchange resin, Irgacure 2959 (2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone) were purchased from Sigma–Aldrich (England). Dialysis tubes (cut-off 12,000–14,000) were purchased from Medicell International (UK).

2.2. Synthesis of dextran methacrylate (DEX-MA)

Dextran methacrylate was synthesized as already reported (van Dijk-Wolthuis et al., 1997). Briefly, to a solution of dextran (M_w 40,000, DEX_{40}, 5.0 g) in anhydrous DMSO (40 ml), DMAP (1.0 g) and GMA (1.50 g, 1 mol/3 mol of repetitive unit) were added. The solution was maintained under stirring at room temperature for 24 h. EtOH (200 ml) was added dropwise; the precipitated solid was recovered by filtration and dissolved in water (15 ml). The solution was neutralized with 0.1 M HCl and submitted to exhaustive dialysis against distilled water. After freeze-drying, the polymer was characterized by FT-IR, ^{13}C NMR and ^{1}H NMR. FT-IR spectra were recorded using KBr pellets with a PerkinElmer Paragon 1000 spectrophotometer in the range 4000–400 cm^{-1} (resolution of 1 cm^{-1}). ^{13}C NMR and ^{1}H NMR spectra were obtained in D_2O with a Bruker AC-400 instrument. The degree of derivatization (number of methacrylic groups for 100 glucopyranosyl residues, DD), calculated on the basis of the ^{1}H NMR spectrum, was 20 ± 1. DEX-MA with the same DD was obtained starting from DEX with M_w 500,000 (DEX_{500}) and applying the same procedure but adding 45 ml of anhydrous DMSO to 5.0 g of polymer.

2.3. Synthesis of carboxymethyl scleroglucan (Scl-CM)

Scleroglucan (1.0 g) was derivatized through reaction with $ClCH_2COOH$ in basic medium according to the method already described (Casadei et al., 2007). After elution through a DOWEX 50WX4-50 ion-exchange resin column previously treated with 2.0 M HCl, the freeze-dried polymer was characterized by FT-IR and submitted to potentiometer titration. The degree of derivatization (number of carboxymethyl groups for 100 repetitive units) was 80 ± 5.

2.4. Preparation of the polymeric solutions

The following binary mixtures were investigated:

- DEX_{40}-MA/Scl-CM
- DEX_{500}-MA/Scl-CM
- DEX_{500}-MA/Scl
- DEX_{500}-MA/$Scl_{0.2}$

After dissolution of Scl-CM (1.0%, w/v) or Scl (1.0%, w/v and 0.2%, w/v), DEX-MA (5.0%, w/v) with different molecular weight was added.

2.5. Hydrogels formation

All the polymeric mixtures (5 ml) were placed in Petri plates ($d = 7.0$ cm) previously treated with a polyethylene glycol solution (10%, w/v PEG 40,000 in phosphate buffer, pH 7.4), in order to easily remove the hydrogel after gelation (Kuijpers et al., 1999). A solution of the photoinitiator Irgacure 2959 (250 μl or 25 μl of a 20% (w/v) solution in N-methyl-pyrrolidone) was added just before the irradiation. The samples were irradiated with a UV lamp, G.R.E. 125 W Helios Italquarz for 5, 10 or 20 min. The hydrogels were analyzed after 24 h from the preparation in order to assure the complete crosslinking of the methacrylic groups.

2.6. Rheological measurements

Rheological experiments were performed with a Haake RheoStress 300 Rotational Rheometer (Germany) equipped with a Haake DC10 thermostat. Flow curves of all the polymeric solutions were obtained with a cone-plate geometry in the range of 0.01–100 Pa. Frequency sweep experiments were also performed on the same samples in the range 0.01–10 Hz in the linear viscoelastic region, assessed by preliminary stress sweep studies. Instead hydrogels (thickness of 1.0–3.0 mm) obtained after UV irradiation of the solutions, were submitted to oscillatory analyses using a serrated plate–plate geometry in the same conditions as before. All the experiments were carried out at least in triplicate at the temperature of 25.0 and 37.0 ± 0.1 °C.

2.7. Swelling studies

Swelling studies were performed on freeze-dried hydrogels in different aqueous media: HCl 0.1 M, phosphate buffer (PB, pH 7.4, ionic strength $I = 0.1$), bidistilled water and NaCl 0.1 M. Aliquots (30 mg) of the gel were placed in tarred 5.0 ml sintered glass filters (Ø 10 mm; porosity G3) and allowed to swell at 37.0 ± 0.5 °C after immersion of the filters in the swelling media. After 24 h the excess liquid was removed by percolation at atmospheric pressure. Then the filters were weighed.

The swelling degree (q) was expressed as:

$$q = \frac{W_s}{W_d}$$

where W_s and W_d are the weights of the swollen and dry hydrogels, respectively. Each experiment was performed in triplicate. The swelling ability was evaluated for the hydrogels DEX_{40}-MA/Scl-CM, DEX_{500}-MA/Scl-CM, DEX_{500}-MA/Scl and DEX_{500}-MA/$Scl_{0.2}$.

2.8. Determination of unreacted methacrylic groups

In order to investigate the amount of unreacted methacrylic groups present in the hydrogels after the photocrosslinking, about

3.5 g of gel was put to react in 10 ml of NaOH 5 N under magnetic stirring at room temperature. The mixture was kept into the dark to avoid the polymerization of free methacrylic acid. After 24 h, an aliquot of the solution was filtered with a glass filter; 0.2 ml of the filtrate were neutralized with HCl 2 N and analyzed. Determination of MA was carried out with an HPLC consisted of a Series 200 LC pump equipped with a 235 Diode Array (PerkinElmer, USA), using a Merck Hibar LiChrocart (250–4.5 μm) RP-18 column. A MeOH/CF_3COOH 10^{-2} M mixture (4:6) was used as an eluant, with a flow of 0.6 ml/min and MA concentration was determined monitoring at 210 nm. The experiments were carried out for 72 h, which is a sufficient time to ensure the complete hydrolysis of the gels. The percentage of unreacted MA was calculated with the formula:

$$\%MA = \frac{\text{moles of free MA}}{\text{total moles of MA}} \times 100$$

where the total moles of MA were equal to 20% of the polymer repetitive units.

In order to assure the validity of the method, the procedure of hydrolysis with NaOH 5 N was first applied to the polymeric solutions of DEX_{40}-MA and DEX_{500}-MA. The number of methacrylic groups calculated by ^1H NMR agreed exactly with that obtained by hydrolysis experiments. All the experiments were performed in triplicate.

2.9. Texture analyzer

A software-controlled dynamometer TA-XT2i Texture Analyzer (Stable Micro Systems, UK), with a 5-kg load cell, a force accuracy of 0.0025%, a trigger force of 0.005 N and a distance resolution of 0.0025 mm, was used for the mechanical characterization of the gels. The hydrogel resistance to penetration was obtained with an ebonite cylindrical probe having 10 mm diameter. The pre- and post-test speed was 2.0 mm/s and the test speed was 1.0 mm/s till a deformation of 80%. All measurements were carried out in triplicate.

2.10. Drug loading and release studies

Release studies were carried out to test the ability of the hydrogels to entrap and release biomolecules with different steric hindrance. THP [M_w 180, van der Waals radius 3.7 Å], VitB12 [M_w 1355, van der Waals radius 8.5 Å (Bajpai et al., 2008)] and MGB [M_w 17,800, van der Waals radius 21 Å (Meyvis et al., 2001)] were taken as model drugs. The polymeric solutions containing each type of molecule in a concentration of 1 mg/ml were transferred in Petri plates and irradiated according to the procedure described above. An irradiation time of 5 min was chosen for the preparation of drug-loaded hydrogels in order to limit the degradation due to the UV exposure.

Aliquots of the hydrogels (1.0 g) were placed in phosphate buffer pH 7.4 (500 ml for THP release, 100 ml for VitB12 and 20 ml for MGB) at 37.0 ± 0.1 °C under stirring (100 rpm). Aliquots of dissolution medium were withdrawn at fixed time intervals and replaced with the same volume of fresh medium. The amount of released THP and MGB was determined using a PerkinElmer UV–vis spectrophotometer at 272 and 410 nm, respectively, using quartz cells with a path length of 1.0 cm. Instead, the amount of VitB12 was analyzed using HPLC. A MeOH/CF_3COOH 0.0025% (v/v) mixture (4:6) was used as an eluant, with a flow of 0.6 ml/min and VitB12 concentration was determined at 361 nm. All the experiments were carried out in triplicate.

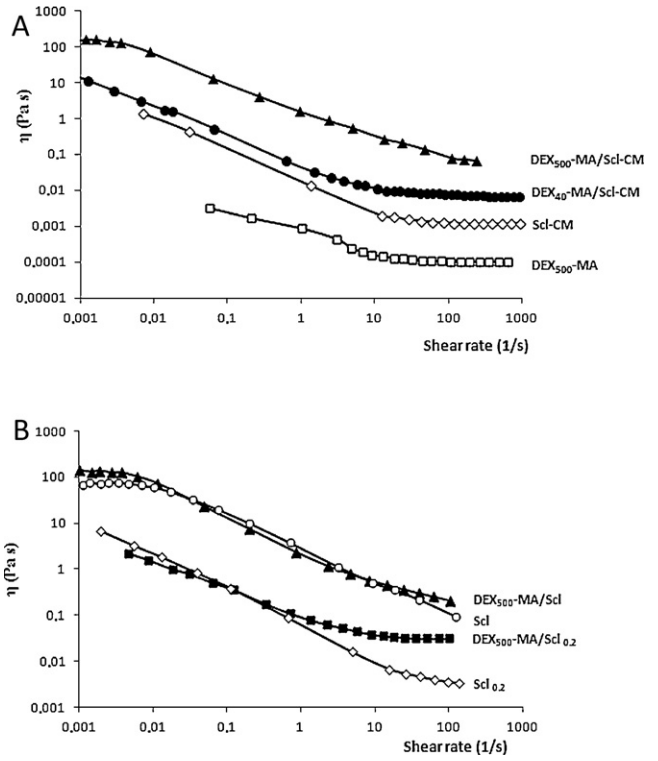

Fig. 1. Flow curves. (A) Flow curves of solutions of DEX_{40}-MA/Scl-CM (●), DEX_{500}-MA/Scl-CM (▲), Scl-CM (◊) and DEX_{500}-MA (□). (B) Flow curves of solutions of DEX_{500}-MA/Scl (▲), DEX_{500}-MA/$Scl_{0.2}$ (■), $Scl_{0.2}$ (◊) and Scl (○).

3. Results and discussion

3.1. Mechanical characterization of DEX-MA/Scl-CM binary mixtures

Scl-CM is a very versatile polymer, synthesized in our laboratory and already proposed for different pharmaceutical applications (Casadei et al., 2007; Corrente et al., 2009). The polyanionic characteristics of this derivative make it able to form, in the presence of divalent ions, physical hydrogels whose strength depends on both polymer and salt concentrations. Semi-IPN systems made of Scl-CM/Ca physical gels and DEX-MA were designed therefore with the aim to have a system viscous enough to be injected, fill a cavity and be cross-linked *in situ*. First of all the influence of Scl-CM on the rheological properties of solutions of DEX-MA, with derivatization degree of 20 ± 1 and two different molecular weights had been studied. Scl-CM was dissolved in water (1.0%, w/v) and DEX-MA (5.0%, w/v) was added. Flow curves recorded on blends of the two polymers (Fig. 1A) showed pseudo-plastic behavior, with the viscosity value that reduces as the shear stress increases.

This property is fundamental for injectable systems that should have a consistence allowing them to remain in the injection site. The increase of the M_w of DEX-MA did not hamper the possibility of the solution to be injected and this system, having higher viscosity, is able to better maintain the form in the injection site before the UV irradiation. It is also possible to observe an increasing in the viscosity of the mixture solutions of DEX-MA and Scl-CM compared to the polymers alone, without addition of any salts. It is likely that intermolecular interactions, such as hydrogen bonds between the carboxylic groups of Scl-CM and dextran chains, occur in addition to physical entanglements. Therefore in opposition to our first idea, no calcium ions were added to the polymeric mixtures and additional characterization of these systems were carried out without the addition of any salts. In order to verify the influence of

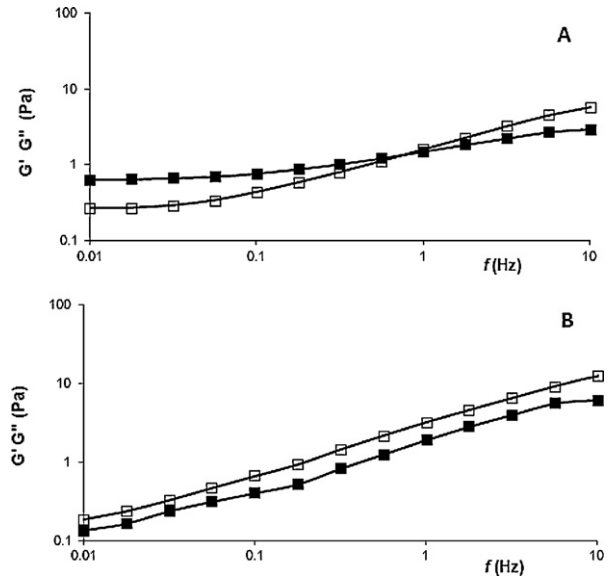

Fig. 2. Oscillatory frequency sweep. Mechanical spectra of the systems of (A) DEX_{500}-MA/Scl and (B) DEX_{500}-MA/Scl-CM (G' -■-; G'' -□-).

the carboxymethyl groups on the properties of the system, further studies with native Scl were performed. Because it is well known that native Scl has good properties as thicker and forms gels at concentration >0.23% (w/v), it was employed at concentration of 1.0% (w/v), like Scl-CM, and 0.2% (w/v) near the gel point. The rheological profiles of the two binary mixtures are reported in Fig. 1B.

DEX_{500}-MA/Scl and Scl alone show almost the same behavior whatever the concentration of scleroglucan used. No increase in viscosity was observed in both these cases. Anyway the system composed of Scl 0.2% (w/v) shows a viscosity value near to the polymers alone at low shear rate, but a slight difference can be observed when the samples were subjected to higher stress. Besides, at such low concentration the system loses the possibility to remain in the injection site.

Although no difference in the pseudoplastic behavior was observed between the systems of DEX500-MA/Scl-CM and DEX500-MA/Scl, oscillatory frequency sweep experiments were carried out in order to better understand their properties (Fig. 2). When DEX500-MA is combined with Scl-CM, the resulting system shows values of the modulus G'' higher than G' over all the frequency range, behaving like a solution. Instead the system containing Scl is a weak gel with storage and loss moduli strongly dependent on frequency and a cross-over between G' and G'' can be observed around 1 Hz.

3.2. Hydrogels formation and rheological experiments

After addition of Irgacure 2959, all the binary mixtures DEX-MA/Scl-CM and DEX-MA/Scl were submitted to UV irradiation in order to obtain the corresponding hydrogels. Preliminary experiments were carried out on DEX_{500}-MA/Scl-CM to identify the best experimental conditions for the hydrogel formation in terms of irradiation time and optimum amount of photoinitiator. It was observed that long irradiation times and high amounts of photoinitiator did not influence positively the mechanical properties of the gels (data not shown), therefore 5 min of UV irradiation and 0.1% (w/v) of photoinitiator were chosen for their formation.

Mechanical spectra were recorded at both 25 and 37 °C, but no changes were detected (data not shown), so the following experiments were performed at 37 °C. The mechanical spectra of all the systems are shown in Fig. 3.

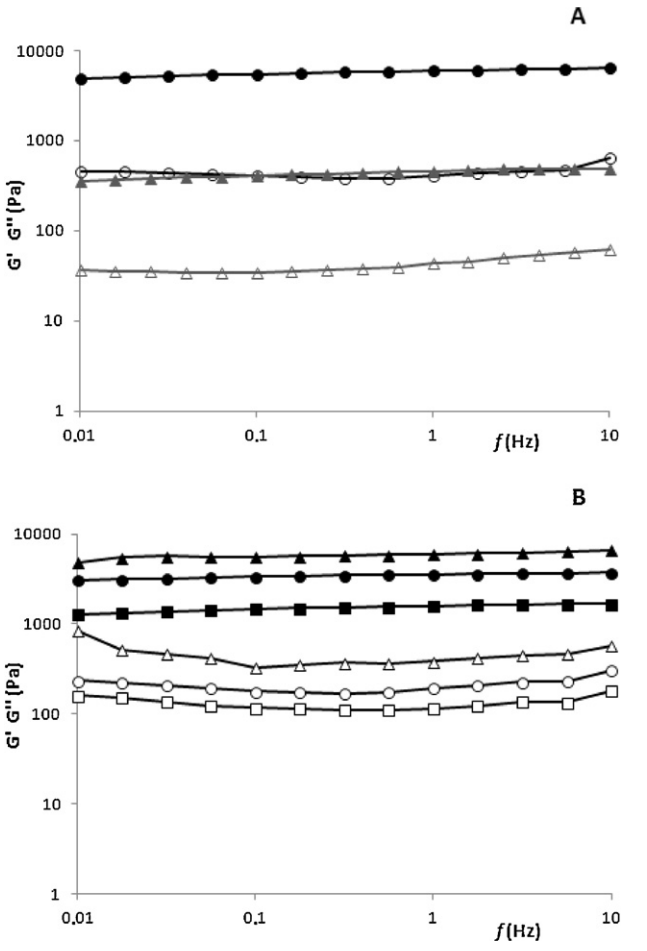

Fig. 3. Oscillatory frequency sweep. (A) Mechanical spectra recorded on DEX_{40}-MA/Scl-CM (G' -▲-, G'' -△-) and DEX_{500}-MA/Scl-CM (G' -●-; G'' -○-) hydrogels; (B) DEX_{500}-MA/Scl (G' -▲-, G''-△-), DEX_{500}-MA/$Scl_{0.2}$ (G' -●-; G'' -○-) and DEX_{500}-MA hydrogels (G' -■-; G'' -□-).

In all the samples the value of the elastic modulus G' is about an order of magnitude higher than the dissipative modulus G'', irrespective of the frequency. Hydrogels made of DEX_{500} showed G' and G'' values higher than DEX_{40} (Fig. 3A) probably due to the increasing chain entanglement between the two polymers. In fact, with an increase of the molecular weight, the density of physical entanglements is expected to rise because of the necessary accommodation between numerous, long flexible chains (Fetters, Lohse, Richter, Witten, & Zirkel, 1994). The presence of Scl-CM is able to increase the strength of the hydrogel composed of DEX-MA alone. Furthermore the strength of the gel is not deeply influenced by the derivatization of Scl; in fact, the concentration being the same, G' values of the hydrogels containing Scl or Scl-CM are almost coincident (Fig. 3A and B). On the contrary when the concentration of Scl decreases, a remarkable reduction of G' value was observed. This means that the gel strength can be easily modified changing Scl concentration.

3.3. Swelling studies

The ability of hydrogels to swell in aqueous media is a parameter that influences the mechanical properties as well as the release of drugs loaded inside. Swelling experiments were carried out on freeze-dried samples in different aqueous media, in order to evaluate their affinity toward biological fluids. The measurements were performed after 24 and 48 h of contact with the fluids, but no difference in the swelling ability was detected at the two time points,

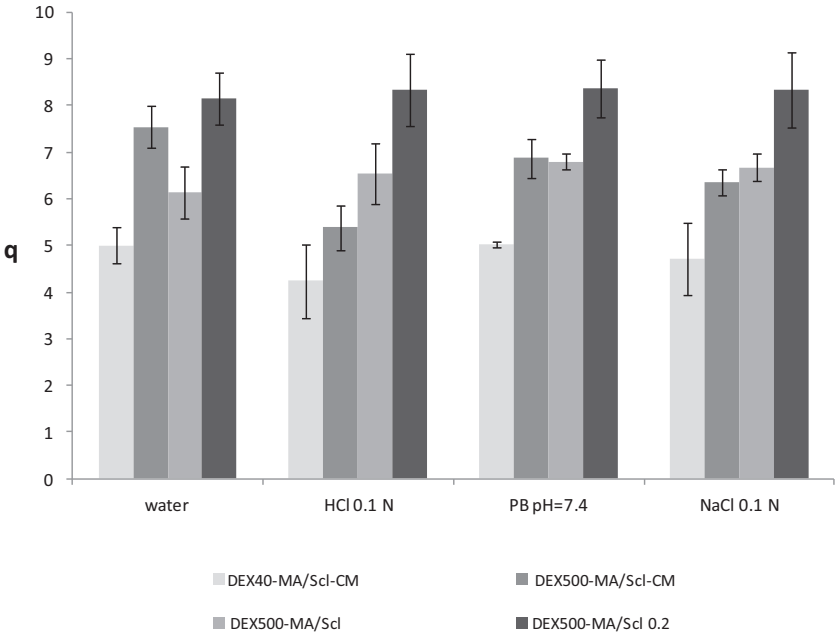

Fig. 4. Swelling studies. q Values of DEX_{40}-MA/Scl-CM, DEX_{500}-MA/Scl-CM, DEX_{500}-MA/Scl and DEX_{500}-MA/$Scl_{0.2}$ hydrogels at 24 h in different media at 37.0 ± 0.5 °C.

therefore only the swelling data obtained after 24 h are shown in Fig. 4.

The swelling degree is influenced by both the M_w of DEX and the nature of the medium.

The value of q increases with the M_w of the polymer. In fact, DEX-MA_{500}/Scl-CM hydrogels showed swelling degree values higher than DEX-MA_{40}/Scl-CM ones. In order to explain this behavior, hydrolysis reactions were performed on the hydrogels as reported in Section 2. The percentage of unreacted methacrylic groups, determined after 72 h of reaction, was 12.5 ± 2.5 for DEX_{40}-MA and 21.1 ± 0.1 for DEX_{500}-MA. These results can explain the difference in the swelling degree measured for hydrogels made of DEX-MA obtained from polymers at different molecular weight. It is likely that the higher viscosity of the solution of DEX_{500}-MA makes more difficult the approach of the chains and thus the cross-linking reaction, so that the network has larger pores and is able to absorb higher amount of aqueous media.

The systems containing Scl-CM showed pH-sensitive behavior. In fact, the carboxylic groups of the polymer are completely undissociated at pH 1, allowing the chains to get closer to each other and a small value of q results. At pH 7.4 the carboxylic groups are dissociated and the swelling degree of the matrix is higher because of the electrostatic repulsions among the chains. Furthermore the swelling degree of DEX-MA/Scl-CM hydrogels follows the trend H_2O > PB > NaCl, probably because the presence of salts shields the charges decreasing the electrostatic repulsions. Finally, molecular weight being equal, the q value is influenced by the amount of native scleroglucan. In fact, as the concentration of Scl within the hydrogel decreases, a higher swelling ability can be observed as a consequence of the smaller amount of the less soluble scleroglucan.

3.4. Texture analyzer

The profiles of penetration/withdrawal experiments performed on all the matrices are shown in Fig. 5.

Several mechanical parameters were extrapolated from these curves: (1) the system hardness, *i.e.* the maximum positive force registered while attaining the imposed deformation (F_{max}); (2) the work of cohesion (cohesiveness), proportional to the positive area under the force-time curve from zero to the maximum deformation imposed; (3) the work of adhesion (adhesiveness), proportional to the negative area under the force–time curve and (4) the Young modulus (E), obtained from the initial slope of the stress–strain curve at 10% of strain. All these parameters are reported in Table 1.

According to the results, DEX_{500}-MA hydrogel shows the smallest Young modulus and very low values of both hardness and cohesiveness. This is due to the intrinsic fragility of this kind of matrices and their lack of elasticity. Low mechanical strength of hydrogels usually results from inhomogeneities of polymeric topological structure created by cross-linking. A simple gel, even without any visible flaw, often breaks easily due to the large amount of defects and inhomogeneities in the network (Ikkai & Shibayama, 2005; Shibayama, 2012). Instead, the presence of Scl or its derivate gave a system with better mechanical properties. It is likely that the combination of the two polymers lead to a less inhomogeneous structure which results in an improvement of the mechanical strength. It is also interesting to observe that stronger hydrogels were obtained when native scleroglucan was mixed with DEX-MA maybe because of its rigid rod-like structure. Hardness and cohesiveness were also influenced by the different concentration of Scl used to prepare the hydrogel and this behavior is in accordance with the value of G' and G'' reported in the mechanical spectra showed before (Fig. 3B). For all the systems no adhesiveness was observed.

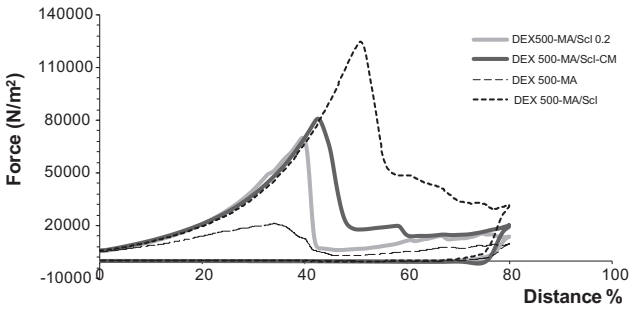

Fig. 5. Texture analysis. Stress–strain curves of DEX_{500}-MA, DEX_{500}-MA/Scl-CM, DEX_{500}-MA/Scl and DEX_{500}-MA/$Scl_{0.2}$.

Table 1
Mechanical parameters obtained from texture analyses carried out on DEX-MA, DEX-MA/Scl and DEX-MA/Scl-CM hydrogels.

Hydrogel	Hardness $\times 10^{-5}$ (N/m^2)	Cohesiveness $\times 10^{-5}$ (J/m^2)	Young modulus $E \times 10^{-3}$ (N/m^2)
DEX_{500}-MA	0.19 ± 0.03	0.12 ± 0.05	0.86 ± 0.03
DEX_{500}-MA/$Scl_{0.2}$	0.65 ± 0.19	0.38 ± 0.04	1.17 ± 0.07
DEX_{500}-MA/Scl	1.21 ± 0.06	0.51 ± 0.06	1.07 ± 0.02
DEX_{500}-MA/Scl-CM	0.74 ± 0.10	0.33 ± 0.02	1.15 ± 0.02

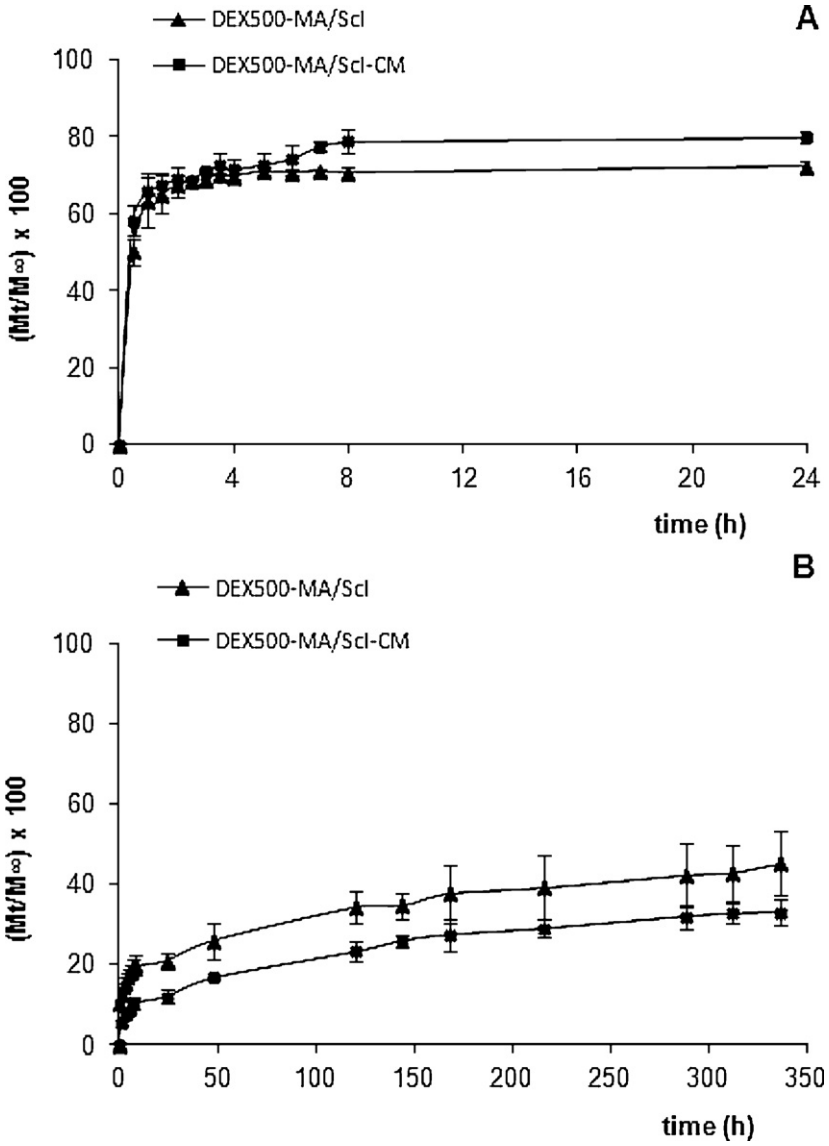

Fig. 6. Release studies. Release profiles $[(M_t/M_\infty) \times 100]$ of vitamin B12 (A) and myoglobin (B) from DEX_{500}-MA/Scl-CM and DEX_{500}-MA/Scl hydrogels in PB (pH 7.4) at 37.0 ± 0.1 °C.

3.5. Release studies

Three model biomolecules of different molecular weight and dimensions were added to the solutions of DEX_{500}-MA/Scl-CM and DEX_{500}-MA/Scl and exposed to UV irradiation. These two systems were chosen because showed the most suitable mechanical properties. The release studies were performed in PB (pH 7.4) at 37.0 ± 0.1 °C. The systems were unable to maintain THP inside, which quickly diffused out from the two matrices and was quantitatively released within 2 h (data not shown).

The release profiles of VitB12 and MGB are shown in Fig. 6.

The release rate was related to the dimensions of the loaded molecules [THP: M_w 180, van der Waals radius 3.7 Å; VitB12: M_w 1355, van der Waals radius 8.5 Å (Bajpai et al., 2008) and MGB: M_w 17,800, van der Waals radius 21 Å (Meyvis et al., 2001)], so that about 80% of the vitamin was released in 24 h from both the systems, with no important differences in behavior. Instead, the model protein MGB was slowly released from the hydrogels and more than 60% of the macromolecule remained into the matrices after 2 weeks.

4. Conclusions

Novel injectable and *in situ* cross-linkable hydrogels were synthesized. They were made up of two different polysaccharides

DEX-MA and Scl, in its native form or carboxymethylated. Both unmodified and modified Scl were able to improve the mechanical properties of DEX-MA hydrogels that became harder, but also more elastic and easy to work with. The functionalization of Scl with carboxymethyl groups resulted in a synergistic interaction with DEX-MA and gave systems of higher viscosity than the polymers alone without the addition of salts. All the hydrogels were able to swell in contact with biological fluids showing a consistence similar to natural tissues. In addition the systems containing Scl-CM showed pH-sensitive behavior leading to higher q value at pH 7.4 where the repulsion among carboxymethyl groups occurs. Drug delivery properties were also tested on the hydrogels DEX_{500}-MA/Scl and DEX_{500}-MA/Scl-CM. The systems released very fast small molecules, such as THP, but were able to modulate the release of VitB12 and to behave as depot delivery systems for MGB.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.10.018.

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