Carbohydrate Polymers 112 (2014) 525–531

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Influence of both cation and alginate nature on the rheological behavior of transition metal alginate gels

Pierre Agulhon[a], Mike Robitzer[a], Jean-Pierre Habas[b,*], Françoise Quignard[a,**]

[a] Institut Charles Gerhardt Montpellier UMR 5253 CNRS-UMII-ENSCM-UMI, Équipe Matériaux Avancés pour la Catalyse et la Santé, 8 rue de l'École Normale, 34296 Montpellier Cedex 5, France
[b] Institut Charles Gerhardt Montpellier UMR 5253 CNRS-UMII-ENSCM-UMI, Equipe Ingénierie et Architectures Macromoléculaires, Université Montpellier II, Place Eugène Bataillon, CC 17002, 34095 Montpellier Cedex 5, France

ARTICLE INFO

Article history:
Received 21 March 2014
Received in revised form 27 May 2014
Accepted 28 May 2014
Available online 10 June 2014

Keywords:
Alginate
Hydrogels
Relaxation time
Rheological properties
Spectromechanical behavior
Metallic cations

ABSTRACT

The rheological properties of several ionotropic alginate hydrogels were investigated according to the nature of the divalent cation (Mn^{2+}, Co^{2+}, Cu^{2+}) and the guluronic fraction of the alginate (HG and LG for "high G-content" and "low G-content"). Six hydrogels (Mn-LG, Mn-HG, Co-LG, Co-HG, Cu-LG and Cu-HG) were synthesized and studied by spectromechanical analyses. On one hand, Cu-HG, Cu-LG and Co-HG behaved as viscoelastic solids: the elastic contribution was higher than the dissipative component in all the frequency range studied ($G' > G''$). No flow zone ($G'' > G'$) was detected even at very low values of the shearing frequency. On the other, Mn-HG, Mn-LG and Co-LG presented a spectromechanical behavior that resembled that observed classically for entangled polymers. Indeed, at high frequency, these latter materials could be compared to a viscoelastic solid but at low frequency, the flow zone was described and the viscous character became prevalent with finite relaxation time. Very good correlations with the microscopic structurations of the network were evidenced (rubbery vs. flow zone and fibrillar vs. complex morphology respectively).

1. Introduction

Alginates are natural polysaccharides that are produced by brown algae. According to the exact nature of the seaweed species, they represent 10–45 w% of dry matter. Their high bioavailability and easy extraction process explain their low cost. They are linear block copolymers based on α-L-guluronate and β-D-mannuronate monomers (respectively named as G and M units). Alginates are widely used in many different applications due to their versatile properties that can be monitored according to different stimuli (concentration, temperature, pH...). In aqueous solution, alginates are usually employed as thickening agents to increase the viscosity of the medium. In the presence of cations (usually divalent or trivalent), they can produce a hydrogel according to a complexation mechanism. The transformation is known under the designation "ionotropic gelation". Due to its biocompatibility, Ca^{2+} is the most applied and studied gelling agent. In this latter case, the gelation is mainly attributed to the specific interaction of this cation with guluronate blocks in a so-called "egg-box" model (Grant, Morris, Rees, Smith, & Thom, 1973). A growing body of experimental (Benli et al., 2011; DeRamos, Irwin, Nauss, & Stout, 1997; Fang et al., 2007; Li, Fang, Vreeker, & Appelqvist, 2007; Sikorski, Mo, Skjak-Braek, & Stokke, 2007; Yuguchi, Urakawa, Kajiwara, Draget, & Stokke, 2000) and theoretical (Braccini & Perez, 2001; Peric, Pereira, Perez, & Huenenberger, 2008; Plazinski, 2011) proofs contributed to confirm and improve this model. In particular, rheological analyses were conducted to evidence the egg-box model at low calcium concentrations. Kinetics experiments clearly showed that the interaction between G-blocks and calcium cations does not provoke immediately the gelation of the medium. Indeed, the gelation rate depends on the Ca^{2+} source and its concentration in the aqueous solution. Until the gel point, growing junction zones attributed to the cation mediated G-block stacking were evidenced (Lu, Liu, Dai, & Tong, 2005). Beyond this critical point, the samples viscoelastic properties become independent of the shearing frequency, which is characteristic of the rheological behavior of classical gels (Stokke et al., 2000). Moreover, the response observed at high strain behavior seems coherent with an interconnected rod-like structure (Webber

* Corresponding author. Tel.: +33 467143780; fax: +33 467144028.
** Corresponding author. Tel.: +33 467163460; fax: +33 467163470.
E-mail addresses: pierre.aguhlon@enscm.fr (P. Agulhon), mike.robitzer@enscm.fr (M. Robitzer), jean-pierre.habas@univ-montp2.fr (J.-P. Habas), francoise.quignard@enscm.fr (F. Quignard).

http://dx.doi.org/10.1016/j.carbpol.2014.05.097
0144-8617/

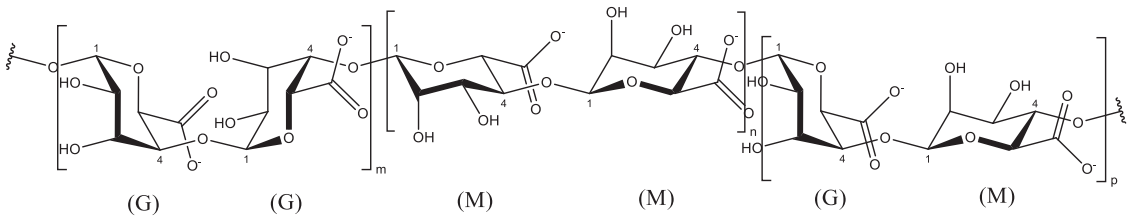

Fig. 1. Schematic representation of chemical structure of alginate. M, mannuronate unit; G, guluronate unit.

& Shull, 2004; Zhang, Daubert, & Foegeding, 2007). Actually, egg-box model is usually extended to other alkaline-earth cations even if slight differences are observed in their interaction with various alginate blocks (Morch, Donati, Strand, & Skjak-Braek, 2006) (Fig. 1).

Besides, a very low concentration of copper, cobalt or manganese divalent cations can provoke the sol–gel transition at a shorter time compared to calcium (Wang, Zhang, Konno, & Saito, 1994). The gel forming ability follows the order $Cu^{2+} > Ca^{2+} > Co^{2+} > Mn^{2+}$ but this hierarchy seems to be temperature-dependent (Zheng, 1997). This evolution is justified by the intrinsic nature of the mechanisms involved. Indeed, at 5 °C intra-chain cross-linking predominates but is irreversibly converted into inter-chain interaction by heating up to 50 °C. Most of the researches published about the interaction of divalent cations with alginates were performed at low concentration of gelling cation that is say with a molar ratio cation/uronate lower than 0.2. In this scientific context, magnesium ions were long considered as non-gelling ions for alginates. But, a recent work showed the contrary by the conduction of experiments at much higher cation content (Topuz, Henke, Richtering, & Groll, 2012). The use of an excess of gelling cation seems to be an interesting route to investigate new properties because it makes possible the contribution of all uronate units (G and M) in the complexation mechanism (Agulhon, Robitzer, David, & Quignard, 2012).

Most of the rheological studies found in the literature with divalent cations, except Ca^{2+}, are often restricted to description of the viscosimetric behavior up to the gel point (Benli et al., 2011; Florian-Algarin & Acevedo-Rullan, 2008; Hernandez, Sacristan, & Mijangos, 2010). In other words, little experimental data are reported about the complete viscoelastic behavior of gels produced from the interaction of transition metal with alginates. This may appear somewhat surprising since dynamic rheometry is usually identified in polymer science as a technique able to provide more detailed information than simple viscosimetry. Spectromechanical tests are sometimes proposed but are often restricted to narrow frequency range (Nickerson & Paulson, 2004). Moreover, in some studies, confusion is made between rheometry and mechanics. For instance, Ouwerx's research is declared as a study devoted to the influence of the divalent cation type on the rheological properties of alginate-based gels. But, in fact, this work is focused only on compressive mechanical strength (Ouwerx, Velings, Mestdagh, & Axelos, 1998). To our knowledge, no study has yet been published about the viscoelastic behavior of gels produced by the interaction of several metallic divalent cations in excess with different kinds of alginate.

By DFT theoretical calculations, we have already demonstrated in a recent paper the intrinsic difference between alkaline-earth and transition metal cations interacting with alginates (Agulhon, Markova, Robitzer, Quignard, & Mineva, 2012). The computed bond distances, cation interaction energies and molecular orbital composition analysis revealed that the complexation of the transition metal ions to the disaccharides occurs via the formation of strong coordination-covalent bonds. Conversely, the alkaline earth cations form ionic bonds with the uronates. These discrepancies at the local scale should be responsible of experimental differences. They were already reported by others using spectroscopic techniques such as infrared (Papageorgiou et al., 2010), Raman (Hernandez et al., 2010) ^{13}C NMR (Emmerichs, Wingender, Flemming, & Mayer, 2004; Wang, Zhang, Konno, & Saito, 1993a; Wang, Zhang, Konno, & Saito, 1993b) but also circular dichroism (Thom, Grant, Morris, & Rees, 1982). At the secondary structure scale, Small Angle X-ray Scattering (SAXS) technique does not seem to discriminate these two cation families but reveals other main differences considering the structural regime (Agulhon, Robitzer, et al., 2012). With M or G rich alginates, copper gels show a monodisperse rod-like secondary structure as already stated for calcium (Stokke et al., 2000). This model is not convenient with manganese cation that always induces gels structures with various junction zone multiplicities, like acidic gels (Draget, Stokke, Yuguchi, Urakawa, & Kajiwara, 2003). In the case of cobalt and zinc, the morphology depends on the M/G ratio in the alginate: G-rich sample leads to the rod-like structure whereas M-rich sample leads to the more complex structure (Agulhon, Robitzer, et al., 2012).

It is now clear that the structure of the hydrogel depends on the respective nature of both alginate and transition metal cations used in excess. But, a question remains about the influence of this structure on the macroscopic properties of the hydrogel. Then, this paper aims to investigate more particularly the effects of the structural regime on the rheological characteristics of each system. To build reliable "structure-properties" relationships, two different alginates with similar molecular weight but distinct structures were used (respectively 33% or 63% of G units). In reference to the SAXS results (Agulhon, Robitzer, et al., 2012), copper, cobalt and manganese were chosen as representative cations of the three behaviors at the secondary structure scale.

2. Materials and syntheses

2.1. Raw materials

Two alginate samples were supplied by FMC Biopolymer (Norway). Their chemical characterization by ^{1}H NMR was performed following the methodology already published by Grasdalen (Grasdalen, 1983; Grasdalen, Larsen, & Smidsrod, 1979). Different details about their composition are presented in Table 1 such as the respective fraction of mannuronate (F_M) and guluronate (F_G) in the alginate backbone but also the η parameter which describes the alternation of M and G groups ($0 < \eta < 1$: sequenced block copolymer, $\eta = 1$ statistical polymer, $1 < \eta < 2$: alternated polymer). The N_G and $N_{G>1}$ parameters that represent the average number of G units in G-blocks and in G-blocks of more than one G unit, respectively and the fractions of dyad sequences are also reported. According to their respective guluronate content, alginates were respectively designated as HG and LG for "High G-content" and "Low G-content". The corresponding percents of guluronate units in the alginate backbone were 63% and 33% respectively. The respective molecular weight, determined by viscosimetry according to a methodology previously described in literature, were 155 kDa and 95 kDa (Martinsen, Skjakbraek, Smidsrod, Zanetti, & Paoletti,

Table 1
Chemical composition of alginate samples. "F" symbol represents the molar fraction of subscripted sequence.

Sample designation	F_M	F_G	F_{MM}	F_{MG} F_{GM}	F_{GG}	F_{GGG}	F_{GGM} F_{MGG}	F_{MGM}	η	N_M	N_G	$N_{G>1}$
HG	0.37	0.63	0.26	0.10	0.53	0.49	0.05	0.09	0.45	4	6	11
LG	0.67	0.33	0.54	0.13	0.21	0.18	0.02	0.12	0.57	5	3	9

1991; Renaud, Belgacem, & Rinaudo, 2005; Vold, Kristiansen, & Christensen, 2006; Vold, Kristiansen, & Christensen, 2007). The chloride salts of transition metals were supplied by Aldrich.

2.2. Samples preparation

Hydrogels samples were prepared according to a method derived from a methodology described in literature (Bracher, Gupta, Mack, & Whitesides, 2009; Bracher, Gupta, & Whitesides, 2009). First, a paper sheet commonly used for chromatography (Whatman no. 1) was impregnated with an aqueous solution of gelling cations (0.1 mol L^{-1}) and set after draining in a Petri dish with the same size as the paper disk. Then, 6 mL of sodium alginate aqueous solution (2% w:w) were poured in the same container and covered by another cation impregnated paper disk. Finally this system is gently covered with about 5 mm of the cation solution. After a period of 60 min, the gelation at the surfaces made possible the removal of each paper cover. The sample was consecutively set in a new bath containing complexing cations in excess (still 0.1 mol L^{-1}). After a maturation time of 24 h, the film was washed in several baths of distilled water and retained in a solution containing the gelling cations at a concentration of 10^{-3} mol L^{-1}. The different gels prepared according to this procedure seemed perfectly homogeneous and transparent. Their color was given by the nature of the complexing cation. Ultimately, the sampling of small plates with a diameter of 25 mm was operated using a cutting shape just before the rheological tests.

2.3. Rheological experiments

Viscoelastic properties of alginate gels were registered using a stress-controlled dynamic rheometer (MCR 301 from Anton Paar®) equipped with parallel plates geometry (diameter of 25 mm). All experiments were performed at a fixed temperature (T = 25 °C) using a Peltier heating system including a moisture-saturated chamber to prevent samples drying during the rheological test. As all samples were under preformed gels form, the control of the normal force applied to the material was judged of first importance to achieve beneficial research results. Indeed, as no creep was possible, the following procedure was strictly respected. After disposition of the sample on the lowest plate of the rheometer, the upper plate was lowered until contact with the sample. Then, its vertical position was adjusted to obtain a normal force close to zero, that is to say able to insure a good mechanical contact but without compression in order to prevent any exudation phenomenon.

The spectromechanical analyses consisted in registering the complex shear modulus $G^* = G' + j\,G''$ as a function of the shearing angular frequency ω ranging from 10^{-3} to 100 rad s^{-1} in decreasing mode and under control of the normal force close to zero value. The component G', called "storage modulus", represents the mechanical rigidity of the sample (i.e. its elastic contribution) whereas the loss modulus G'' relates to the dissipated mechanical energy. Each experiment was duplicated on different samples to check the reproducibility and the coherence of registered data.

3. Results and discussion

3.1. Investigation of the linear regime

The complex shear modulus is defined by Hooke's law, which postulates that complex shear stress σ^* and strain γ^* in a sample are related with an affine function i.e. $\sigma^* = G^*\gamma^*$. The same relationship implies that G^* must be independent of the applied stress (resp. the strain) to be meaningful. Then, first rheological experiments consisted in measuring the evolution of G' and G'' moduli as a function of the imposed stress value σ at constant temperature and fixed angular frequency. Fig. 2 shows as an example two series of results obtained with the hydrogel obtained from the interaction of HG alginate with cobalt cation (Co-HG). Each experiment was performed at specific values of ω (respectively 1 and 100 rad s^{-1}). In each analysis, both moduli values were found to be almost constant for applied stress values lower than 50 Pa. In rheology, this peculiarity is characteristic of the so-called "linear domain" of the studied sample (Wloka, Rehage, Flemming, & Wingender, 2004). From a practical point of view, these experiments define in our research the stress range where G' and G'' are meaningful (Funami et al., 2009).

For σ > 100 Pa, the rheological response of Co-HG gel becomes non linear and this effect is likely increased by the sample slipping on the rheometer measurement plates (Moresi, Bruno, & Parente, 2004). Then, keeping a safety margin, the shear stress value retained for the consecutive spectromechanical tests was fixed at 20 Pa. Same methodology was applied for all hydrogels produced by the interaction of the others cations (Co^{2+}, Cu^{2+} or Mn^{2+}) with alginates (HG or LG) prior their spectromechanical characterization to insure the scientific validity of the measurements.

3.2. Spectromechanical analyses

Fig. 3 shows the influence of the shearing frequency on the viscoelastic response of hydrogels formed by complexation of HG and LG alginates with the divalent cation Mn^{2+}. In both cases, the behavior described resembles that classically observed with entangled polymers. Indeed, in the high frequency zone, the material can be

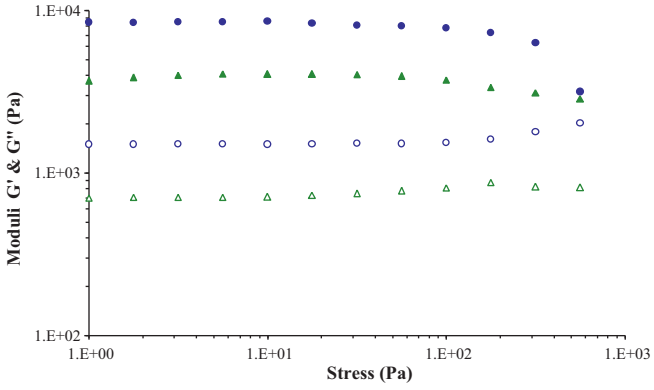

Fig. 2. Investigation of the linear regime by stress sweep experiments performed on Co-HG alginate system at 25 °C – G' (full symbols), G'' (empty symbols) and ω = 100 rad s^{-1} (●, ○); ω = 1 rad s^{-1} (▲, △).

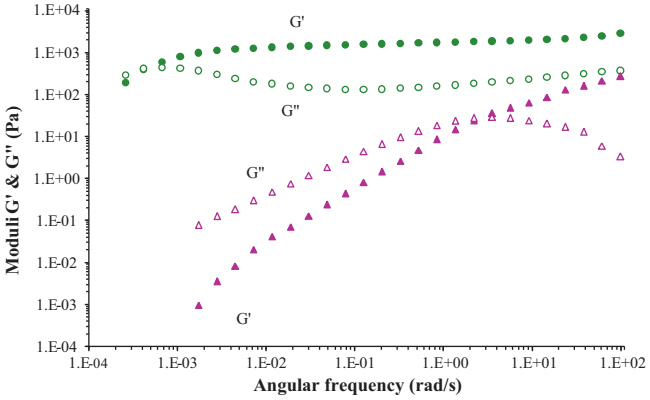

Fig. 3. Spectromechanical properties of Mn-HG (●/○) and Mn-LG (▲/△) alginates G′ (filled symbols) and G″ (open symbols) – T = 25 °C; σ_0 = 20 Pa.

compared to a viscoelastic solid because the elastic contribution is predominant ($G' > G''$).

But, at a critical value ω_c of the shear angular frequency, G' and G'' intersect. Then, the terminal relaxation time τ of each system can be evaluated as $\tau = 1/\omega_c$ (Pannetier, Habas, Peyrelasse, & Francois, 1999; Perreur, Habas, Peyrelasse, Francois, & Lapp, 2001). Then, for $\omega < \omega_c$, the flow zone is described and the viscous character becomes prevalent ($G'' > G'$).

These results lead to important information. Firstly, none of these systems should be named "gel" due to the existence of a finite relaxation time in both cases (Habas, Pavie, Lapp, & Peyrelasse, 2004). Secondly, the exact nature of the alginate strongly influences the dynamic mechanical properties of the material produced after interaction with manganese cation. For instance, the use of HG alginate leads to a material that is characterized by a higher rubbery rigidity. Moreover, its terminal relaxation time close to 40 min is also much higher than that registered with Mn-LG system (τ = 0.45 s). In other words, the systems produced by the interaction of Mn^{2+} with HG and LG alginates seem to be based on the same structure but with higher cohesion energy in the case of the Mn-HG material. Moreover, the flow zone of Mn-HG system is quite interesting to examine the molecular weight distribution of the alginate chains through their interactions mediated by Mn^{2+}. Indeed, even if in the very low frequency region G' and G'' moduli scale respectively as ω^2 and ω, it is clear that the Mn-HG system does not behaves as a typical Maxwell fluid. The rheological profile reveals the presence of additional modes of stress relaxation. The combination of two Maxwellian distributions can be used to describe in a reasonable way the large-scale viscoelastic response (Fig. 4).

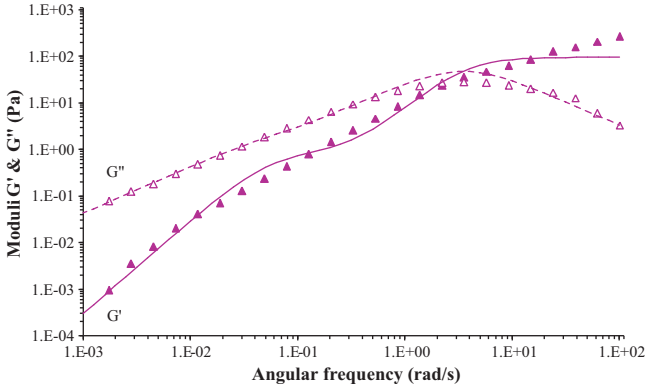

Fig. 4. Description of the flow zone characteristic of Mn-LG (▲/△) alginate G′ (▲) and G″ (△) with a model based upon the combination of two Maxwellian distributions (continuous and dot lines).

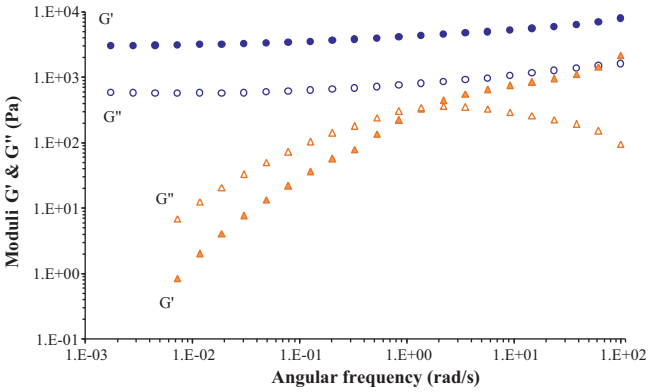

Fig. 5. Influence of the shearing frequency on the viscoelastic properties of Co-HG (●/○) and Co-LG (▲/△) alginates. G′ (filled symbols) and G″ (open symbols) – T = 25 °C; σ_0 = 20 Pa.

The corresponding equations for G' and G'' moduli are defined as:

$$G'(\omega) = \sum_{i=1}^{2} \frac{G_i \theta_i^2 \omega^2}{1 + \theta_i^2 \omega^2} \quad G''(\omega) = \sum_{i=1}^{2} \frac{G_i \theta_i \omega}{1 + \theta_i^2 \omega^2}$$

where G_i are the limit rigidity values and θ_i the attached characteristic times (Ferry, 1980).

The alginate nature was found to influence in a similar way the viscoelastic behavior of the materials produced by complexation with cobalt cation. Fig. 5 shows the spectromechanical properties of both hydrogels measured at 25 °C. The profile characteristic of the Co-LG material resembles that observed previously with the same alginate "gelled" with Mn^{2+}. However, in the present case the interaction between the alginate and the cobalt cation seems slightly stronger because the crossover point $G' = G''$ is observed for ω_c = 1.3 rad s^{-1} that is to say at a higher characteristic relaxation time (τ = 0.76 s). The stronger cohesion in the cobalt alginate gel is also observed with the HG-based hydrogel. Indeed, the elastic contribution is higher than the dissipative component throughout the frequency range studied ($G' > G''$). No crossover point can be detected even at very low ω values. The rheological behavior resembles that of a physical or chemical gel. The value of the rubbery plateau that is three times higher with cobalt than manganese cation also supports the idea that the network formed with Co-HG is stronger.

The study of the materials formed by complexation of HG and LG alginates with the divalent cation Cu^{2+} brought new observations. The spectromechanical properties of these materials are shown in Fig. 6. In both cases, the elastic contribution is higher

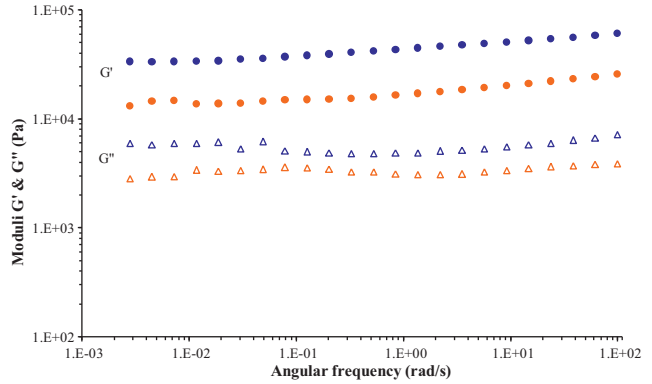

Fig. 6. Spectromechanical properties of Cu-HG (●/○) and Cu-LG (▲/△) alginates. G′ (filled symbols) and G″ (open symbols) – T = 25 °C; σ_0 = 20 Pa.

Table 2
Critical data from the spectromechanical analyses of the different alginate hydrogels.

Hydrogel	Mn-HG	Mn-LG	Co-HG	Co-LG	Cu-HG	Cu-LG
Rubbery plateau	√	√	√	√	√	√
Flow zone	√	√	×	√	×	×
G′ (Pa) at $\omega = 10\ rad\ s^{-1}$	2000	64	5400	770	51,000	21,000
τ	40 min	0.45 s	∞	0.75 s	∞	∞

√, observed; ×, not observed; ∞, infinite.

than the dissipative component throughout the frequency range studied ($G' > G''$). No flow zone ($G'' > G'$) is detected even at very low ω values. This means that these hydrogels behave as viscoelastic solids. Their mechanical rigidities are always higher than 10^4 Pa revealing that the interaction of LG and HG alginates with Cu^{2+} is much stronger than with the other divalent cations studied (Mn^{2+} and Co^{2+}).

The copper cation makes possible the production of polymeric networks with very high cohesion whatever the alginate used in our research. The crosslinks are the direct result of intense interactions between the carboxylate groups of the polysaccharide chain and the metallic cations. Similar mechanism was already proposed in literature with other cations (Hernandez et al., 2010). Considering the similarity of their spectromechanical behaviors, Cu-LG and Cu-HG hydrogels seem be based on the same structure even if the latter appears to be more rigid.

The low frequency-dependence of the spectromechanical behaviors of Cu-based hydrogels resembles a situation already described in literature by Wloka et al. but with calcium as divalent cation (Wloka et al., 2004). However, in this case, the rubbery modulus value was found ten thousand times lower than that registered with our hydrogels. In fact, the gel strength is directly dependent on the Ca^{2+} concentration (Stokke et al., 2000; Webber & Shull, 2004). Using higher Ca^{2+} content, Yang and co-workers could prepare hydrogels with a mechanical rigidity comparable to that characteristic of our Cu-based systems. These systems were prepared with an alginate concentration similar to that used in our research but required a much lower concentration of divalent cation, i.e. 4 mmol/L (Yang, Campanella, Hamaker, Zhang, & Gu, 2013). Similar viscoelastic behavior was also observed with hydrophobic modified alginates (Choudhary & Bhatia, 2012). Their modification consisted in the grafting of alkyl chains upon the alginate skeleton, which caused strong repulsions and increased the mechanical properties in comparison with "classical" alginates.

Table 2 presents the main critical information registered from previous spectromechanical analyses for each couple "alginate-cation". As the size of the rubbery plateau is directly dependent on the value of the terminal relaxation time, we chose to take the G' value at $\omega = 10\ rad\ s^{-1}$ as representative of the hydrogel elasticity because at this angular frequency $G' > G''$ for all hydrogels studied. In the case of the systems for which the flow zone was detected, the value of the terminal relaxation time is also specified for a better discussion. The careful examination of these data clearly shows that the cation nature has a strong influence on the properties of the alginate hydrogel.

On one hand, the use of the copper ions gives materials characterized by a high rubbery rigidity and with no apparent terminal relaxation time whatever the alginate nature. On the other hand, manganese and cobalt cations have an action that differs according to the alginate nature. They lead to the production of hydrogels with lower performances compared to that observed with Cu^{2+}. The lowest complexing power is observed with manganese.

As regards the influence of the alginate nature, the HG-based hydrogels (i.e. rich in G units) are characterized by higher mechanical rigidity and terminal relaxation time in comparison with those obtained with LG-derived formulations (i.e. rich in M units). This observation is consistent with the influence of alginate composition on the mechanical rigidity of hydrogels as previously reported in literature (Draget, Smidsrod, & Skjak-Braek, 2002). This behavior also agrees with the data characteristic of hydrogels obtained by interaction of alginates with calcium (Mancini, Moresi, & Rancini, 1999) and with the higher affinity of G-rich alginates for divalent metal ions (Kawai, Matsumoto, & Masuda, 1994). The higher stiffness of guluronate sequences is well known as being due to their axial linkages that provoke greater restriction of rotation about the glycosidic bond while mannuronate parts are characterized by less restricted equatorial linkages. Similar hierarchy is also supported by the results of experiments with different sodium alginate systems (Kakita & Kamishima, 2008). However, it should be noted that Karakasyan et al. proposed an opposite hierarchy considering the cold gelation of alginate species in the presence of potassium salts (Karakasyan et al., 2010). In our study, the most noticeable case is that observed with the hydrogels by complexation with the cobalt cation. Indeed, they show spectromechanical characteristics that are radically different according to the richness in G or M units. The Co-HG system seems to be based on a rigid network based on strong interactions between the alginate units and the cobalt cation whereas the Co-LG presents a viscoelastic behavior currently observed with an entangled polymer but that is able to flow with a low relaxation time.

All these results seem to show that the dynamic rheometry is suitable to evaluate the intensity of the interactions between the alginate units and the divalent cations. The discrimination between the different hydrogels is possible using the respective values of the elastic modulus in the rubbery zone and the terminal relaxation time obtained before flow. This combination of parameters is essential to evaluate the real difference between the hydrogels. But, it is necessary to perform spectromechanical experiments over a larger frequency range than that "classically" used in literature (Choudhary & Bhatia, 2012; Draget et al., 2003; Hernandez et al., 2010; Moresi et al., 2004). Indeed, in our research, some hydrogels presented a similar rubbery rigidity but with distinct relaxation times. This observation reveals the limits of an experimental approach based only on characterization of the hydrogel mechanical rigidity. Able to measure the viscoelastic behavior on a large range of frequency (and consequently of solicitation time), the spectromechanical analyses offer a more complete description of the hydrogel using a multi-scale approach.

One might wonder in the case of the most rigid hydrogels if it would be possible to measure a finite relaxation time at very low values of shearing frequency, that is to say below $\omega < 10^{-4}\ rad\ s^{-1}$. This question remains an open point with both compounds obtained after complexation with the copper cation. Indeed, the progressive increase of both moduli in the low frequency domain suggested water evaporation from the hydrogel substrates during the rheological analysis. Moreover, the non-reproducibility of the experiment on the same sample assessed the occurrence of this undesirable phenomenon what prohibited measurements during very long period despite the use of the chamber

saturated with moisture. Similar situation was observed with the Co-HG hydrogel.

We will now consider two families of hydrogels. On one hand, there are those that are characterized by an infinite terminal relaxation time (or at least away from the accessible frequency range) and on the other hand, those that present a flow zone i.e. a finite terminal relaxation time. Spectromechanical tests seem more adequate than kinetics tests to discriminate hydrogels (Yang et al., 2013).

In a previous paper, the structure of these hydrogels was investigated using SAXS (Agulhon, Robitzer, et al., 2012) and revealed different nanostructures depending both on the alginate structure and cation nature. According to SAXS experiments, the hydrogels obtained after complexation with copper cation, which were previously identified in rheology as being characterized by a high rubbery rigidity and an apparent infinite relaxation time, are characterized by a fibrillar structure like calcium-based hydrogels (Maki et al., 2011). Conversely, other cations such as manganese lead to the formation of materials with more complex structure with both kinds of alginate studied here (LG or HG). At the same time, their rheological profiles were found to be similar to that observed for entangled polymers characterized by a finite relaxation time. Finally, other divalent cations such as cobalt are able to produce hydrogel in each category depending on alginate nature. Indeed, when HG is used, the corresponding hydrogel presents a fibrillar structure whereas a complex morphology is observed with LG alginate. Once more, it is remarkable to note that this differentiation is also supported by rheometry since Co-HG behaves as a gel while Co-LG is able to flow.

4. Conclusions

The experimental approach based on dynamic spectromechanical analyses proposed in this paper clearly brought interesting information as regards the rheological properties of several ionotropic alginate hydrogels. The rheological profile was observed to be clearly dependent on both nature of the divalent cation and guluronate fraction of the alginate. Some of the hydrogels behaved as viscoelastic solids with mechanical rigidities higher than 5 kPa at ambient temperature. No flow zone ($G'' > G'$) could be detected even at very low values of the shearing frequency. Other hydrogels presented spectromechanical properties similar to those of entangled coils. At high frequency, the material could be compared to a viscoelastic solid whereas at low frequency the flow zone was clearly shown with the existence of a relaxation time lower than 40 min. These macroscopic properties could be explained in terms of specific microscopic structures in the network (rubbery vs. flow zone and fibrillar vs. complex morphology respectively).

Traditional applications of alginate hydrogels were developed in food, agricultural, pharmaceutical, and chemical industries especially with calcium. The use of transition metal ions as gelling cations opens new area fields in material science such as production of substrates for catalysis, optical and magnetism, etc. The mechanical behavior of the gel as an indication of the lifetime of the material will help the choice of the proper combination of alginate and cation.

References

Agulhon, P., Markova, V., Robitzer, M., Quignard, F., & Mineva, T. (2012). Structure of alginate gels: Interaction of diuronate units with divalent cations from density functional calculations. *Biomacromolecules*, *13*(6), 1899–1907.

Agulhon, P., Robitzer, M., David, L., & Quignard, F. (2012). Structural regime identification in ionotropic alginate gels: Influence of the cation nature and alginate structure. *Biomacromolecules*, *13*(1), 215–220.

Benli, B., Boylu, F., Can, M. F., Karakas, F., Cinku, K., & Ersever, G. (2011). Rheological, electrokinetic, and morphological characterization of alginate–bentonite biocomposites. *Journal of Applied Polymer Science*, *122*(1), 19–28.

Braccini, I., & Perez, S. (2001). Molecular basis of Ca^{2+}-induced gelation in alginates and pectins: The egg-box model revisited. *Biomacromolecules*, *2*(4), 1089–1096.

Bracher, P. J., Gupta, M., Mack, E. T., & Whitesides, G. M. (2009). Heterogeneous films of ionotropic hydrogels fabricated from delivery templates of patterned paper. *ACS Applied Materials & Interfaces*, *1*(8), 1807–1812.

Bracher, P. J., Gupta, M., & Whitesides, G. M. (2009). Shaped films of ionotropic hydrogels fabricated using templates of patterned paper. *Advanced Materials*, *21*(4), 445–450.

Choudhary, S., & Bhatia, S. R. (2012). Rheology and nanostructure of hydrophobically modified alginate (HMA) gels and solutions. *Carbohydrate Polymers*, *87*(1), 524–530.

DeRamos, C. M., Irwin, A. E., Nauss, J. L., & Stout, B. E. (1997). ^{13}C NMR and molecular modeling studies of alginic acid binding with alkaline earth and lanthanide metal ions. *Inorganica Chimica Acta*, *256*(1), 69–75.

Draget, K. I., Smidsrod, O., & Skjak-Braek, G. (2002). Alginates from Algae. In E. J. Vandamme, S. De Baets, & A. Steinbüchel (Eds.), *Biopolymers* (pp. 215–244). Weinheim: Wiley-VCH.

Draget, K. I., Stokke, B. T., Yuguchi, Y., Urakawa, H., & Kajiwara, K. (2003). Small-angle X-ray scattering and rheological characterization of alginate gels. 3. Alginic acid gels. *Biomacromolecules*, *4*(6), 1661–1668.

Emmerichs, N., Wingender, J., Flemming, H. C., & Mayer, C. (2004). Interaction between alginates and manganese cations: Identification of preferred cation binding sites. *International Journal of Biological Macromolecules*, *34*(1-2), 73–79.

Fang, Y., Al-Assaf, S., Phillips, G. O., Nishinari, K., Funami, T., Williams, P. A., et al. (2007). Multiple steps and critical behaviors of the binding of calcium to alginate. *Journal of Physical Chemistry B*, *111*(10), 2456–2462.

Ferry, J. D. (1980). *Viscoelastic Properties of Polymers*. Wiley: New York.

Florian-Algarin, V., & Acevedo-Rullan, A. (2008). Rheology and gelation temperature of aqueous gelatin and sodium alginate solutions. In A. Co, L. G. Leal, R. H. Colby, & A. J. Giacomin (Eds.), *XVth International Congress on Rheology – the Society of Rheology 80th Annual Meeting, Pts 1 and 2* (pp. 618–620).

Funami, T., Fang, Y., Noda, S., Ishihara, S., Nakauma, M., Draget, K. I., et al. (2009). Rheological properties of sodium alginate in an aqueous system during gelation in relation to supermolecular structures and Ca(2+) binding. *Food Hydrocolloids*, *23*(7), 1746–1755.

Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C., & Thom, D. (1973). Biological interactions between polysaccharides and divalent cations – Egg-box model. *FEBS Letters*, *32*(1), 195–198.

Grasdalen, H. (1983). High field, H-1-NMR spectroscopy of alginate – Sequential structure and linkage conformations. *Carbohydrate Research*, *118*(July), 255–260.

Grasdalen, H., Larsen, B., & Smidsrod, O. (1979). PMR study of the composition and sequence of uronate residues in alginates. *Carbohydrate Research*, *68*(1), 23–31.

Habas, J. P., Pavie, E., Lapp, A., & Peyrelasse, J. (2004). Understanding the complex rheological behavior of PEO-PPO-PEO copolymers in aqueous solution. *Journal of Rheology*, *48*(1), 1–21.

Hernandez, R., Sacristan, J., & Mijangos, C. (2010). Sol/gel transition of aqueous alginate solutions induced by Fe^{2+} cations. *Macromolecular Chemistry and Physics*, *211*(11), 1254–1260.

Kakita, H., & Kamishima, H. (2008). Some properties of alginate gels derived from algal sodium alginate. *Journal of Applied Phycology*, *20*(5), 543–549.

Karakasyan, C., Legros, M., Lack, S., Brunel, F., Maingault, P., Ducouret, G., et al. (2010). Cold gelation of alginates induced by monovalent cations. *Biomacromolecules*, *11*(11), 2966–2975.

Kawai, M., Matsumoto, T., & Masuda, T. (1994). Rheological properties of alginate gel. *Nippon Kagaku Kaishi*, *2*, 108–114.

Li, L., Fang, Y., Vreeker, R., & Appelqvist, I. (2007). Reexamining the egg-box model in calcium-alginate gels with X-ray diffraction. *Biomacromolecules*, *8*(2), 464–468.

Lu, L., Liu, X. X., Dai, L., & Tong, Z. (2005). Difference in concentration dependence of relaxation critical exponent *n* for alginate solutions at sol–gel transition induced by calcium cations. *Biomacromolecules*, *6*(4), 2150–2156.

Maki, Y., Ito, K., Hosoya, N., Yoneyama, C., Furusawa, K., Yamamoto, T., et al. (2011). Anisotropic structure of calcium-induced alginate gels by optical and small-angle X-ray scattering measurements. *Biomacromolecules*, *12*(6), 2145–2152.

Mancini, M., Moresi, M., & Rancini, R. (1999). Mechanical properties of alginate gels: Empirical characterisation. *Journal of Food Engineering*, *39*(4), 369–378.

Martinsen, A., Skjakbraek, G., Smidsrod, O., Zanetti, F., & Paoletti, S. (1991). Comparison of different methods for determination of molecular-weight and molecular-weight distribution of alginates. *Carbohydrate Polymers*, *15*(2), 171–193.

Morch, Y. A., Donati, I., Strand, B. L., & Skjak-Braek, G. (2006). Effect of Ca^{2+}, Ba^{2+}, and Sr^{2+} on alginate microbeads. *Biomacromolecules*, *7*(5), 1471–1480.

Moresi, M., Bruno, M., & Parente, E. (2004). Viscoelastic properties of microbial alginate gels by oscillatory dynamic tests. *Journal of Food Engineering*, *64*(2), 179–186.

Nickerson, M. T., & Paulson, A. T. (2004). Rheological properties of gellan, kappa-carrageenan and alginate polysaccharides: Effect of potassium and calcium ions on macrostructure assemblages. *Carbohydrate Polymers*, *58*(1), 15–24.

Ouwerx, C., Velings, N., Mestdagh, M. M., & Axelos, M. A. V. (1998). Physico-chemical properties and rheology of alginate gel beads formed with various divalent cations. *Polymer Gels and Networks*, *6*(5), 393–408.

Pannetier, N., Habas, J. P., Peyrelasse, J., & Francois, J. (1999). Rheological properties of the system lime/sucrose/water. *Rheologica Acta*, *38*(3), 241–250.

Papageorgiou, S. K., Kouvelos, E. P., Favvas, E. P., Sapalidis, A. A., Romanos, G. E., & Katsaros, F. K. (2010). Metal-carboxylate interactions in metal-alginate complexes studied with FTIR spectroscopy. *Carbohydrate Research*, *345*(4), 469–473.

Peric, L., Pereira, C. S., Perez, S., & Huenenberger, P. H. (2008). Conformation, dynamics and ion-binding properties of single-chain polyuronates: A molecular dynamics study. *Molecular Simulation*, *34*(4), 421–446.

Perreur, C., Habas, J. P., Peyrelasse, J., Francois, J., & Lapp, A. (2001). Rheological and small-angle neutron scattering studies of aqueous solutions of branched PEO-PPO-PEO copolymers. *Physical Review E*, *63*(3).

Plazinski, W. (2011). Molecular basis of calcium binding by polyguluronate chains. Revising the egg-box model. *Journal of Computational Chemistry*, *32*(14), 2988–2995.

Renaud, M., Belgacem, M. N., & Rinaudo, M. (2005). Rheological behaviour of polysaccharide aqueous solutions. *Polymer*, *46*(26), 12348–12358.

Sikorski, P., Mo, F., Skjak-Braek, G., & Stokke, B. T. (2007). Evidence for egg-box-compatible interactions in calcium-alginate gels from fiber X-ray diffraction. *Biomacromolecules*, *8*(7), 2098–2103.

Stokke, B. T., Draget, K. I., Smidsrod, O., Yuguchi, Y., Urakawa, H., & Kajiwara, K. (2000). Small-angle X-ray scattering and rheological characterization of alginate gels. 1. Ca-alginate gels. *Macromolecules*, *33*(5), 1853–1863.

Thom, D., Grant, G. T., Morris, E. R., & Rees, D. A. (1982). Characterization of cation binding and gelation of polyuronates by circular-dichroism. *Carbohydrate Research*, *100*, 29L 42. MAR.

Topuz, F., Henke, A., Richtering, W., & Groll, J. (2012). Magnesium ions and alginate do form hydrogels: A rheological study. *Soft Matter*, *8*(18), 4877–4881.

Vold, I. M. N., Kristiansen, K. A., & Christensen, B. E. (2006). A study of the chain stiffness and extension of alginates, in vitro epimerized alginates, and periodate-oxidized alginates using size-exclusion chromatography combined with light scattering and viscosity detectors. *Biomacromolecules*, *7*(7), 2136–2146.

Vold, I. M. N., Kristiansen, K. A., & Christensen, B. E. (2007). A study of the chain stiffness and extension of alginates, in vitro epimerized alginates, and periodate-oxidized alginates using size-exclusion chromatography combined with light scattering and viscosity detectors (vol 7, pg 2136, 2006). *Biomacromolecules*, *8*(8), 2627–2627.

Wang, Z. Y., Zhang, Q. Z., Konno, M., & Saito, S. (1993a). Sol–gel transition of alginate solution by addition of calcium-ions – Alginate concentration-dependence of gel point. *Journal De Physique Ii*, *3*(1), 1–7.

Wang, Z. Y., Zhang, Q. Z., Konno, M., & Saito, S. (1993b). Sol–gel transition of alginate solution by the addition of various divalent-cations – ^{13}C-NMR spectroscopic study. *Biopolymers*, *33*(4), 703–711.

Wang, Z. Y., Zhang, Q. Z., Konno, M., & Saito, S. (1994). Sol–gel transition of alginate solution by the addition of various divalent-cations – A rheological study. *Biopolymers*, *34*(6), 737–746.

Webber, R. E., & Shull, K. R. (2004). Strain dependence of the viscoelastic properties of alginate hydrogels. *Macromolecules*, *37*(16), 6153–6160.

Wloka, M., Rehage, H., Flemming, H. C., & Wingender, J. (2004). Rheological properties of viscoelastic biofilm extracellular polymeric substances and comparison to the behavior of calcium alginate gels. *Colloid and Polymer Science*, *282*(10), 1067–1076.

Yang, Y., Campanella, O. H., Hamaker, B. R., Zhang, G., & Gu, Z. (2013). Rheological investigation of alginate chain interactions induced by concentrating calcium cations. *Food Hydrocolloids*, *30*(1), 26–32.

Yuguchi, Y., Urakawa, H., Kajiwara, K., Draget, K. I., & Stokke, B. T. (2000). Small-angle X-ray scattering and rheological characterization of alginate gels. 2. Time-resolved studies on ionotropic gels. *Journal of Molecular Structure*, *554*(1), 21–34.

Zhang, J., Daubert, C. R., & Foegeding, E. A. (2007). A proposed strain-hardening mechanism for alginate gels. *Journal of Food Engineering*, *80*(1), 157–165.

Zheng, H. H. (1997). Interaction mechanism in sol–gel transition of alginate solutions by addition of divalent cations. *Carbohydrate Research*, *302*(1–2), 97–101.