Cationic cellulose and its interaction with chondroitin sulfate. Rheological properties of the polyelectrolyte complex

Karina L. Bierbrauer [a,b,1], Roxana V. Alasino [a,b,1], Miriam C. Strumia [b,c,*], Dante M. Beltramo [a,b,*]

[a] Centro de Excelencia en Productos y Procesos de Córdoba, Gobierno de la Provincia de Córdoba, Pabellón CEPROCOR, CP 5164 Santa María de Punilla, Córdoba, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, Argentina
[c] Departamento de Química Orgánica, Facultad de Ciencias Químicas, IMBIV, Universidad Nacional de Córdoba, Ciudad Universitaria, CP 5000 Córdoba, Argentina

A R T I C L E   I N F O

A B S T R A C T

This work describes the polyelectrolyte complexation between polyquaternium cellulose (PQ-10) and chondroitin 4-sulfate (C-4S), an important structural component of cartilages. The complex shows different behaviors with increasing concentrations of the polyanion. Initially, PQ-10/C-4S interaction forms a soluble, translucent hydrogel that reaches maximum viscosity at a sulfate carboxylate/quaternary ammonium molar ratio lower than 1. After that, the complex begins to aggregate until complete precipitation, and finally, at higher concentrations of C-4S, the resuspension of the aggregate is observed. Further addition of PQ-10 initiates new cycles of precipitation/resuspension for at least seven times.

C-4S contains sulfates and carboxylates able to interact with quaternary ammonium of PQ-10. The results of a comparative study with polymers containing only carboxylate or sulfate to evaluate the influence of each group suggest that the phenomena observed involved, essentially, carboxylate groups.

1. Introduction

Polyelectrolyte complexes have been subject of thorough research during the last decades. These complexes have huge potential for many practical applications such as additives thickener, membranes with selective retention ability in multilayer assembly or as a model to study complex living systems in which biomacromolecules are ionically associated [1–5]. Their biocompatibility, biodegradability and natural availability are also particularly interesting in many subjects. An example of these involves polyelectrolyte complexes of chitosan with anionic polymers such as dextran sulfate [6], polygalaturonic acid [7] or poly(acrylic acid) [8], chondroitin sulfate [9–11], hyaluronate [9], and alginate [12] which have been far characterized. These hydrogels exhibit pH responsiveness, especially important for developing "rheologically smart" fluids or carriers for the release of pharmacologically active compounds. Other polysacharides such as policationic celluloses have been studied to a lesser extend in combination with polyanions as carbopol [13] or with different glycosaminoglycans, mainly for personal care and medical applications [14,15].

It is known that the formation of polyelectrolyte complexes is governed by the characteristics of the individual components (e.g., properties and position of ionic sites, charge density, macromolecular chain rigidity) and of the chemical environment (e.g., solvent, ionic strength, pH and temperature). Usually, the formation of the complex is possible in a narrow pH range, especially when weak polyelectrolytes are used, and in a way similar to the cooperative binding process of biomacromolecules. This process does not occur with low-molecular-weight substances,

* Corresponding authors. Address: Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, Argentina. Tel.: +54 351 4334170/73x120 (M.C. Strumia). Address: Centro de Excelencia en Productos y Procesos de Córdoba, Gobierno de la Provincia de Córdoba, Pabellón CEPROCOR, CP 5164 Santa María de Punilla, Córdoba, Argentina. Tel.: +54 3541 489651/53x143; fax: +54 3541 489651/53x129 (D.M. Beltramo).
E-mail addresses: mcs@fcq.unc.edu.ar (M.C. Strumia), dbeltramo@ceprocor.uncor.edu (D.M. Beltramo).
[1] These are both first authors.

suggesting that the cause of exchange reactions between oppositely charged polymers lies in the cooperative interaction of sufficiently long sequences of functional groups attached to a chain. The initial contact between two complementary macromolecules takes place at some point along the polymer chains. Further interaction proceeds between ionic groups adjacent to the recently formed links, since these groups are sufficiently close to each other. As a result of such a process, a zipped or double-stranded structure is formed. Previous studies conducted in our laboratory [16], mean field equations were applied to the simulated density profiles in order to assess the entropic contributions. It was found that these are the driving force for the phenomenon, which stems from the release of counterions from the double layers of the polyelectrolyte and the charged surface.

In view of this, the aim of this study is the characterization of the interaction between polyquaternium-10 (PQ-10), more specifically celquat SC-230, a polysaccharide of high molecular weight containing quaternary ammonium groups and having hydrophilicity, biocompatibility and mucoadhesivity [17–20], with oppositely charged polymer involved in the constitution of the intercellular matrix such as chondroitin sulfate (C-4S). The C-4S polymer is a sulfated and carboxylated glycosaminoglycan (GAG) composed of a chain of alternating sugars (N-acetylgalactosamine and glucuronic acid). It is a major component of cartilage, usually attached to proteins as part of a proteoglycan. The tightly packed and highly charged sulfate groups of chondroitin sulfate generate electrostatic repulsion that provides much of the resistance of cartilage to compression [21]. Loss of chondroitin sulfate from the cartilage is a major cause of osteoarthritis. Because C-4S has two kinds of negative groups able to interact with PQ-10 (sulfates and carboxylates), we also studied polymers containing only one, carboxylates (alginates) or sulfates (carrageenans), in order to evaluate the relative contribution of each anionic group to the interaction.

2. Materials and methods

2.1. Materials

High-viscosity cationic hydroxyethylcellulose, Polyquaternium-10 (PQ-10), also known as Celquat SC-230, was kindly provided by The National Starch and Chemical Co., (Bridgewater, NJ), MW 1.700 kDa. Chondroitin sulfate (C-4S) in the sodium salt formed was provided by BiotechLab S.R.L (MW 17 kDa). Protanal KF 200 S Potassium Alginate (ALG), with a relation of manuronic acid/guluronic acid (M/G) of 1.6, was supplied by Colorcon S.A. (MW 120 kDa). Carrageenan type I or *kappa* carrageenan (CAR κ), carrageenan type II or *iota* carrageenan (CAR ι), carrageenan type IV or *lambda* carrageenan (CAR λ) were supplied by SIGMA (MW 300–400 kDa). All these polymers were used as received with no further purification. All other reagents were of analytical grade.

Purified water by reverse osmosis (MilliQ®, Millipore Spain) with resistivity >1.82 MΩ cm was used.

2.2. Preparation of cationic cellulose/polyanion complexes

2.2.1. Dilute dispersions

Samples were prepared by addition of increasing concentrations of water solutions of each polyanion (C-4S from 0% to 0.8%, ALG from 0% to 0.17%, or CAR κ, ι, λ from 0% to 0.33% w/v) into 0.2% w/v of PQ-10 solutions vigorously shaken. Finally, the system was left to stand for 24 h at 4 °C. The pH of the resultants solutions were between 6.5 and 7.

The zero shear viscosities ($\eta°$) of these mixtures were obtained from shear rate curve dependence of the viscosity using a rheometer and through concentrated dispersions explained in the paragraph below. These samples were characterized by FTIR, zeta potential, particle size, microscopic and turbidity measurements.

2.2.2. Concentrated dispersions

Similarly, complex of 1% w/v PQ-10 with C-4S, ALG, or CAR ι at 1:0.5, 1:1.5 and 1:5.5 sulfate carboxylate/ammonium group molar ratios was prepared.

The rheological behavior of these samples was evaluated in triplicate at 25 °C in a Physica Rheometer Rheoplus/32, Anton Paar, Sanico equipped with an AR2500 data analyzer, fitted with a Peltier temperature control. A 5 cm cone-plate measuring geometry was used. Oscillatory shear responses (G' or storage modulus, and G'' or loss modulus) were determined at 0.1 Pa over the frequency range of 0.1–100 rad s^{-1}. The test conditions were within the linearity range of the viscoelastic properties.

2.3. Estimation of the quaternary ammonium groups of PQ-10

The quaternary ammonium groups in the PQ-10 were inferred from the determination of chloride through *Standard Method for the examination of water and wastewater 4500-Cl B, Ed. 19* by the quantification of PQ-10 chloride groups.

2.4. FTIR measurements

IR Spectra were recorded at ambient temperature on a Shimadzu FTIR spectrometer using an accumulation of 16 runs in each sample in the diffuse-reflectance mode.
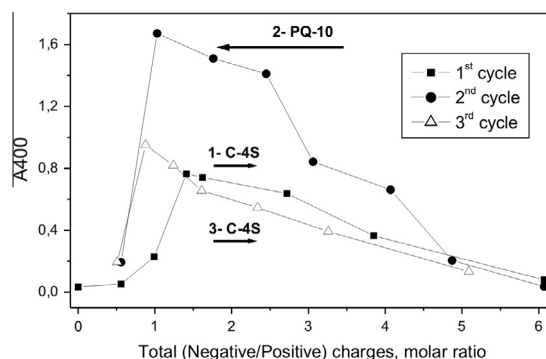

Fig. 1. Turbidity measurements at 400 nm of PQ-10/C-4S complex at different charge molar ratio. 1st and 3rd cycle: constant PQ-10 and increasing C-4S; 2nd cycle: constant C-4S and increasing PQ-10.
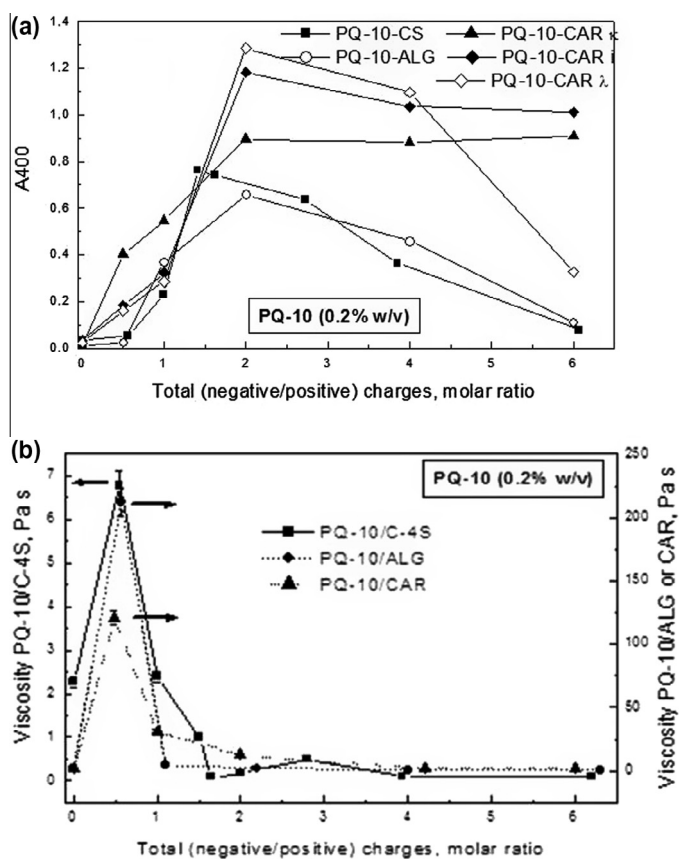
Fig. 2. (a) Turbidity measurements at 400 nm of PQ-10–C-4S, PQ-10–ALG and PQ-10-CAR κ, ι and λ, with constant PQ-10 (0.2% w/v) and increasing polyanion concentration. (b) The zero shear viscosity ($\eta°$) as a function of constant PQ-10 (0.2% w/v) and increasing polyanion concentration (mean ± SD, $n = 3$).

2.5. Turbidity measurements

The turbidity of PQ-10–polyanion dispersions at the different proportions and at different ionic strength was determined by measuring absorbance at 400 nm (Shimadzu UV-240) against a distilled water blank.

2.6. Light scattering and zeta-potential measurements

Particle size measurements and zeta-potential of PQ-10/C-4S dispersions were performed using Delsa™ Nano C Particle Analyzer Beckman Coulter. In the plots, we show the averaged mode of several particle size distribution measurements.

3. Results and discussion

3.1. Characterization of the interaction between PQ-10 and chondroitin 4-sulfate

3.1.1. Turbidity measurements

PQ-10 is a linear cationic hydroxyethyl cellulose containing quaternary ammonium groups (7.2×10^{-4} mmol ammonium groups/mg of polymer) potentially able to interact electrostatically with anionic carboxylate and/or
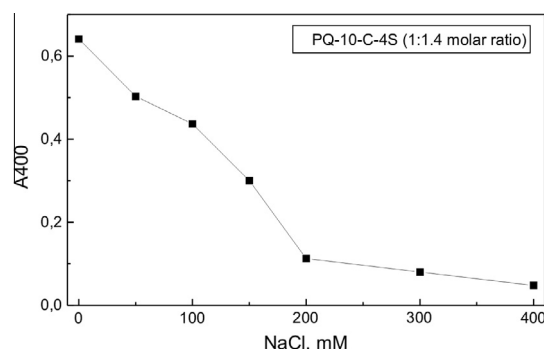

Fig. 3. Effect of ionic strength on the stability of PQ-10–C-4S complex (1:1.4 M ratio). NaCl was added after complex formation.

sulfate groups of polyanion. To elucidate the nature of the interactions, stock diluted solutions of 0.2% w/v PQ-10 were mixed with increasing quantities of C-4S.

Fig. 1 shows the turbidity measurements of PQ-10/C-4S complex at a different proportion of total positive/negative charge. After the addition of C-4S, the turbidity of the complex rise with the increase of the positive/negative molar ratio to peak to 1:1.4 where precipitation occurs. The apparent
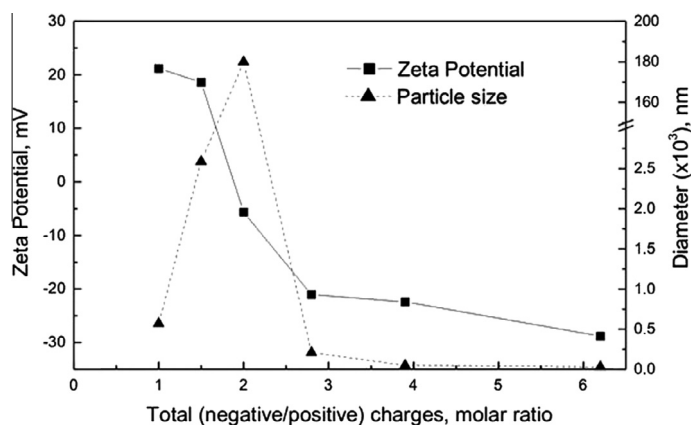
Fig. 4. Size and the zeta potential of PQ-10–C-4S particles in terms of the total positive/negative charge molar ratio.
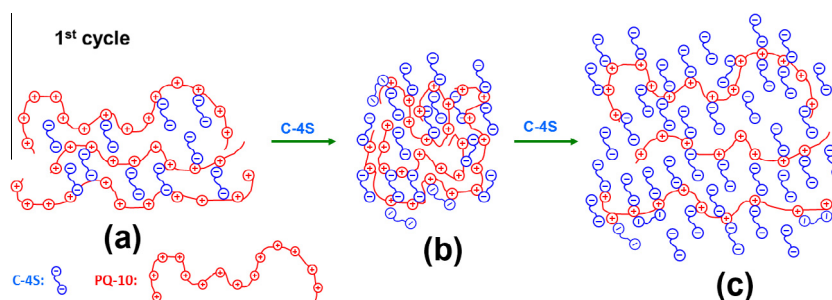

Fig. 5. Effect of the addition of C-4S on the conformation of cationic cellulose chains in aqueous solution at: (a) maximum viscosity, (b) maximum precipitation, and (c) great excess of polyanion.
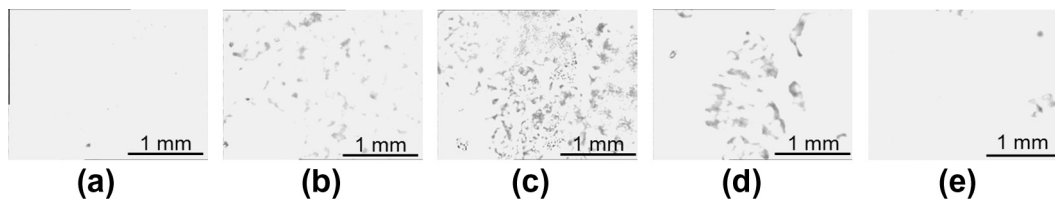

Fig. 6. Optic microscopy of: (a) soluble PQ-10 0.2% (w/v); (b–d) flocculation of PQ-10/C-4S particles at 1:1.5; 1:2; 1:4 positive/negative charge molar ratio, respectively; (e) dissolution of PQ-10/C-4S particles at 1:6.2 positive/negative charge molar ratio.

excess of negative charge present at the maximum precipitation of the complex, it is probably due to a change of the structure where the quaternary ammonium groups are occluded inside. Further addition of C-4S produces a gradual dissolution of the aggregates until almost complete solubilization at 1:6 positive/negative charge molar ratio.

A second aggregation–dissolution cycle on the 1:6 positive/negative charge molar ratio complex was afterwards studied by the addition of increasing amounts of PQ-10 and by keeping constant the amount of C-4S. In this case, we observe a maximum turbidity at 1:1 charge molar ratio. This value could respond to structural change of PQ-10 that allowed a better accessibility to the cationic sites of the polymer. Finally, this sample was subjected to a third cycle in which the same phenomenon of aggregation and

dissolution was observed. Following the same procedure, seven cycles were tested qualitatively in order to probe the reversibility of the phenomenon after the third cycle.

All these results reveal the presence of a cationic–anionic polyelectrolyte system with a reversible dissolution–precipitation–dissolution behavior able to occur in either direction by the addition of polycation or polyanion.

We also measured the turbidity of the complex PQ-10/C-4S 1:1.4 charge molar ratio at 37 °C, simulating physiological conditions, in order to evaluate the stability of it at higher temperature. We observed that complex formation was not affected by heating since absorbance value decreased only 5% respect to absorbance at 25 °C and the same molar ratio ($A_{400nm, \, 37 \, °C} = 0.72$; $A_{400 \, nm, \, 25 \, °C} = 0.76$ relative units).
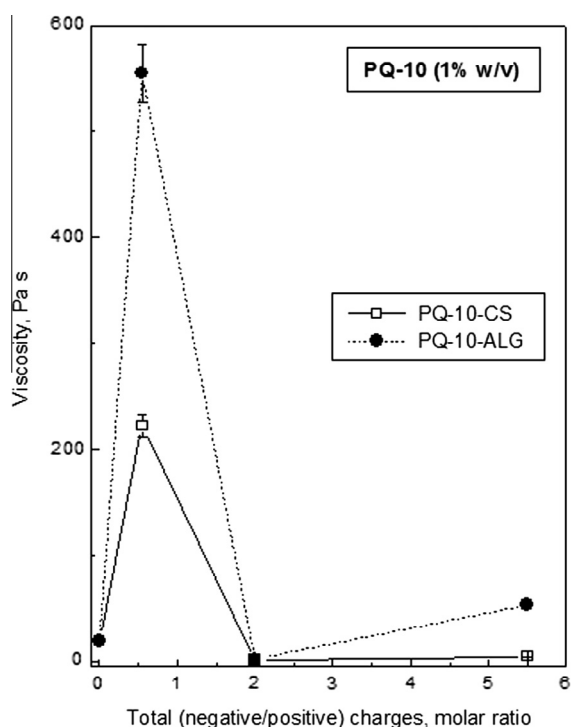
Fig. 7. Effect of the addition of polyanion C-4S or ALG on the zero shear viscosity ($\eta°$) of PQ-10 (1% w/v) at 25 °C (mean ± SD, $n = 3$).

Moreover, since C-4S contains two types of anionic functional groups (sulfates and carboxylates) able to interact with the ammonium groups, we studied the influence of each one with polymers containing carboxylate (alginate) or sulfates (carrageenans) only. Alginate has two carboxylates per disaccharide while the carrageenan family shows different contents of sulfate per disaccharide (*kappa* (κ) $1 -OSO_3^-$, *iota* (ι) $2 -OSO_3^-$ or *lambda* (λ) with $3 -OSO_3^-$).

To clarify the effect of each group as well as the charge density on polyanion-PQ-10 interaction, we analyze the turbidity of the complex formed by PQ-10 with increasing concentrations of C-4S, ALG and PQ-10/CAR κ, ι, λ (Fig. 2a).

The results show that C-4S and ALG display maximum turbidity at 1:1.4 PQ-10/C-4S and 1:2 PQ-10/ALG molar ratio, followed by a decrease when an excess of polyanion is added. In the case of CAR, the turbidity increases until reaching a plateau in the *kappa* and *iota* types; although decreases in the *lambda* type. These results clearly show that only CAR λ, with three sulfate groups by disaccharide, is able to resolubilize the complex by charge reversal, as ALG or C-4S. These results show that all polyanions studied are able to interact with PQ-10 through their carboxylate and/or sulfate groups; however, reversibility takes place essentially through carboxylate groups.

We also study the variation of the viscosity of these complexes and we observed that at low amounts of any of the polymers, C-4S, ALG or CAR, we find a clear hydrogel, with a maximum viscosity at 1:0.5 positive/negative charge molar ratio (Fig. 2b) independently of the molecular weight of the polyanion. After that, a rapid decrease of the viscosity is observed and remains low throughout this molar ratio. It is important to note that the decrease in

the viscosity is coincident with the appearance of aggregates as it was shown in Fig. 2a.

3.1.2. Effect of ionic strength on the formation of the complex

In order to gain a better understanding of the nature of PQ-10/C-4S interaction, we studied the effect of ionic strength on the formation of the complex. Therefore, we evaluated the turbidity of the 1:1.4 molar ratio PQ-10/C-4S complex (maximum cloudiness) next to the addition of increasing quantities of NaCl (Fig. 3). The results show a gradual interference of the interaction with increasing ionic strength and finally, the complete inhibition of the interaction occurs above 200 mM NaCl. This behavior demonstrates that electrostatic forces are involved at least in the first approach of PQ-10/C-4S complex interaction.

3.1.3. FTIR analyses of PQ-10/polyanionic complex

The FTIR characterization of the PQ-10/C-4S, PQ-10/ALG and PQ-10/CAR κ complexes presents similar spectra to those obtained from physical mixtures of an equivalent amount of the two polymers involved (data not shown). There are no shifts in the bands of carboxylate 1610 and 1410 cm^{-1} and sulfate groups that appear at 1250 cm^{-1} in PQ-10/C-4S (both bands) [9,10], PQ-10/ALG (only carboxylate) [12] or PQ-10/CAR κ (only sulfate) [22] complexes. We agree with previous works [9,12] that it is not possible to determine reliably the presence of interaction between the groups using this technique only. Because we could not evidence the interaction by FTIR, other techniques like light scattering, zeta-potential of particle surface, microscopy and rheological assays were required to characterize it.

3.1.4. Light scattering of PQ-10/C-4S complex

The particle size of PQ-10/C-4S dispersions was determined by means of dynamic light scattering. Significant changes in the particle size of PQ-10/C-4S complex of different proportions were observed (Fig. 4). At first, there was a gradual increase in particle size with the rising of C-4S proportion in the PQ-10/C-4S complex, reaching a maximum of about 0.18 mm at a 1:2 PQ-10/C-4S molar ratio (Figs. 4 and 6c), given by the flocculation of the particles that form large clumps. For molar ratios above 1:2 were observed signals at the detection limit of the instrument, that is because the excess of negative charge in this part of the cycle allowed the dissolution of the complex [20].

These observations agree with turbidity measurements (Fig. 1) since the maximum of turbidity is found at PQ-10/C-4S 1:1.4 molar ratio at which the biggest particles are observed before binding together to form large floccules, then diminishes gradually as they dissolve.

3.1.5. Measurements of the zeta-potential of particle surface

For a general understanding of the interaction of C-4S with PQ-10, the zeta potential of PQ-10/C-4S dispersions was determined. The zeta potential of the surface of complex particles at positive/negative charge molar ratio lower than 1:2 was positive, even in the presence of an excess of negative charge (Fig. 4). Around 1:2 positive/negative charge molar ratio shows the neutralization of the particles which coincides with the peak of formation of aggregates.
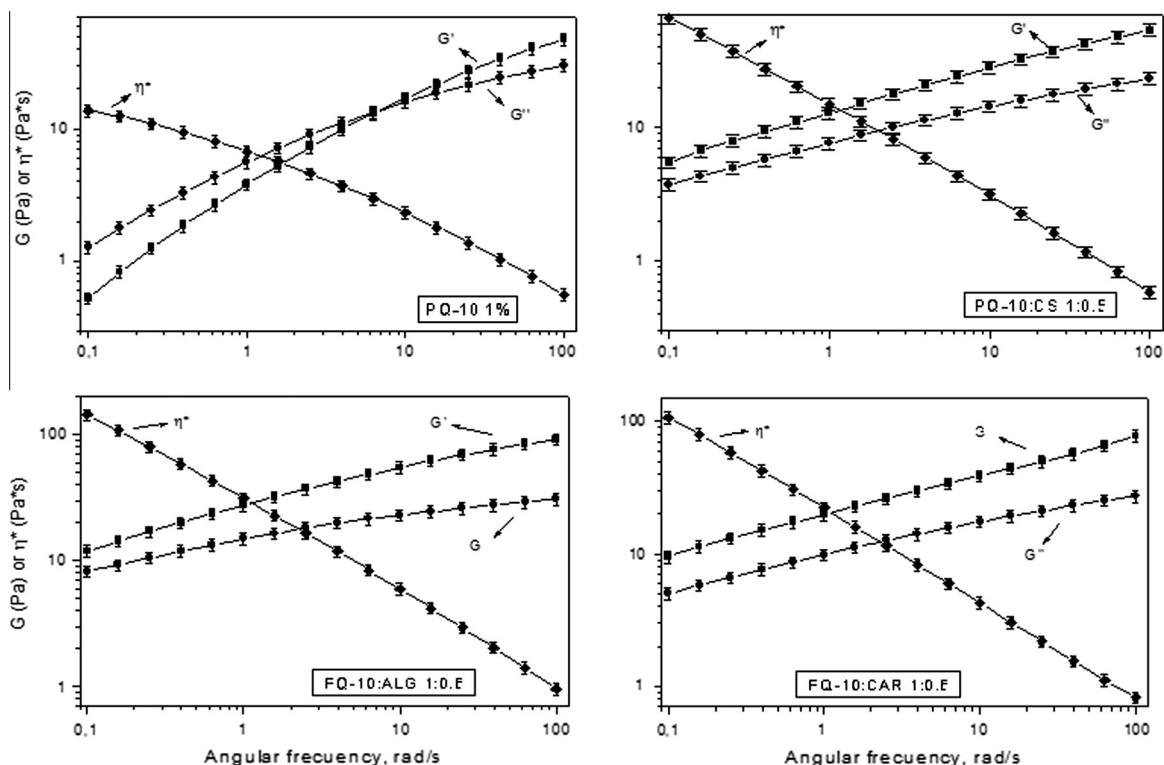
Fig. 8. Viscoelastic behavior of PQ-10 (1% w/v) and the PQ-10–polyanion complexes 1:0.5 M ratio at 25 °C (mean ± SD, $n = 3$).

From this point, particles are negatively charged and their size decreases. These results allow speculating on the fact that the impediment to the accessibility of the opposite charge in the structure of the polymer is attributed to the presence of intra-chain loops connected by PQ-10 molecules. Fig. 5 shows the association mechanism proposed.

3.1.6. Microscopic observations

The results obtained using the previously discussed methods were supported by observations with an optical microscope. Fig. 6 shows the optical microscopy images for PQ-10 0.2% (w/v) or PQ-10/C-4S complexes at 1:1.5, 1:2, 1:4, and 1:6.2 positive/negative charge molar ratios. As expected, no particles were observed for PQ-10 alone (Fig. 6a). At 1:2 positive/negative charge molar ratios, where the zeta potential is about zero, large aggregates can be seen joined by "bridges" of polyelectrolyte complex (Fig. 6c); however, at 1:6 positive/negative charge molar ratios, the high electronegative charge produces a repulsion of particles, thus the dissolution of aggregates occurs and the particles disappear almost completely, leaving very few aggregates in suspension (Fig. 6d). Images of intermediate charge molar ratio are also included in order to show the dependence of the process with complex composition.

3.2. Rheological behavior of PQ-10–Polyanion complexes

In order to acquire a deeper understanding of the behavior of the hydrogels formed, zero shear viscosities

($\eta°$), complex viscosities (η^*), storage (G') and loss (G'') modulus were determined for the concentrated dispersions of PQ-10 (1% w/v) with C-4S with a view to a possible application of the complex. In this study we also include a comparative analysis concerning those polymers that contain only sulfate or carboxylate groups. For sulfated polymers, we use only CAR ι for having two charges per disaccharide like the other polyanions studied.

Fig. 7 shows the viscosity patterns observed for both hydrogels of PQ-10/C-4S or PQ-10/ALG at similar points of positive/negative molar charge ratio, where the most significant changes take place, i.e., 1:0, 1:0.5, 1:2 and 1:5.5 (these points correspond to PQ-10 alone, the maximum viscosity, the maximum precipitation and the total dissolution of the aggregates, respectively, observed at dilute concentration). The viscosity pattern of PQ-10/CAR ι could not be obtained at these experimental concentrations since this polyanion becomes gel by itself and increases markedly the dispersion viscosity. The patterns show a similar biphasic behavior for PQ-10/C-4S and PQ-10/ALG complexes, with an initial increase in viscosity at 1:0.5 molar ratio to reach values of 220 and 550 Pa s, followed by a drastic decrease in viscosity to values near 1 and 2 Pa s, respectively, when the concentration of polyanion increases to 1:2 molar ratio and remains low up to a 1:5.5 molar ratio when precipitate dissolves.

Fig. 8 shows the rheological behavior of PQ-10 alone and PQ-10 with C-4S, ALG and CAR at the PQ-10–Polyanion ratio where maximum viscosity was observed (i.e., 1:0.5) at 25 °C. The PQ-10 alone presents the typical behavior of

macromolecular homogeneous fluid dynamic modules that is viscoelastic behavior with a cross over point and zero shear viscosity. By contrast, the addition of C-4S, ALG or CAR induced a greater increment in G' than in G'' values. This observation confirms the existence of interactions between PQ-10 and each one of the polyanions studied. In addition, the values of both moduli were higher than for the dispersions with cationic cellulose alone and were almost independent of the angular frequency. G' is bigger than G'' from a very small angular frequency, which indicates that the systems are formed by cross-links of long lifetime. These results show that both, sulfate and carboxylate, are involved in the interaction. We also explored the viscoelastic behavior of PQ-10–C-4S complex at 37 °C (physiological conditions). The change in the temperature did not affect the hydrogel structure, but complex viscosity and modulus G' and G'' diminish as a consequence of higher fluidity of the polymeric chains compared to 25 °C. The results at different temperature are shown comparatively in the Supporting information.

These results show that the changes of the hydrogel depend on the total ratio of positive/negative charges. At a ratio lower than 1, the complex forms a strong hydrogel; however, the structure collapses and the complex precipitates. The structure conformation at this point conserves positive charges. Finally, an excess of polyanion produces an unfolding of the structure by charge repulsion and the aggregates are resuspended. This only occurs in the cases of C-4S and ALG. This phenomenon produces a highly negative hydrogel, with viscosity and module values below those found before precipitation conditions, but higher than those of PQ-10 alone. Fig. 5 shows a schematic phenomenon drawing.

We found that the behavior of PQ-10/C-4S and PQ-10/ALG complexes resembles that observed for other complexes formed by cationically modified hydroxyethyl cellulose and sodium dodecyl sulfate (SDS) [23–26] or drugs as ibuprofen [27]. These complexes also form hydrogels that increase modulus with higher proportion of negative charge; however, the precipitation occurs near the neutralization point. Then, an excess of surfactant renders a new negative coat charge, where free surfactant forms micelles able to solubilize the hydrophobic aggregates. In our case, the polymers cannot form micelles, therefore we suggest that the PQ-10/C-4S precipitate has remaining free positive charges, which are neutralized with an excess of negative charges that produces a strong electrostatic repulsion and generates the complex resuspension.

4. Conclusions

We evaluated the interaction and rheological properties of PQ-10/C-4S complex and found that the addition of low proportions of polyanion C-4S increases the entanglement of the system, which is still cationically charged, resulting in an increase in viscosity, G' and G''. Further addition of polyanion neutralizes the quaternary ammonium groups, reducing the effects of charge–charge repulsion which leads to aggregation. Finally, when C-4S or ALG concentration is above the concentrations required to neutralize

cationic groups, the repulsions of their anionic groups, and especially carboxylate groups, determine the rheological behavior of the system, and aggregates consequently redissolve. The addition of PQ-10 to the resolubilized PQ-10/C-4S complex produces a new cycle of precipitation and resuspension. In these experimental conditions, we were able to perform at least seven cycles of precipitation and resuspension of PQ-10/C-4S complex by addition of C-4S and PQ-10 respectively.

An attractive potential application of this type of complex is for osteoarticular problems, exploiting the rheological characteristics of PQ-10 combined with C-4S. The chondroitin sulfate, a sulfated glycosaminoglycan (GAG), is an important structural component of cartilage and has become a widely used biopolymer for treatment of osteoarthritis. The rationale for using chondroitin sulfate for the treatment of osteoarthritis was based on experimental data obtained in models in vitro and in vivo (animal and osteoarthritic patients) [28–30]. These studies showed that the sulfated glycosaminoglycans exogenous have an effect on the metabolism of chondrocytes which suggest a positive influence on the course of experimentally induced articular degenerative diseases.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurpolymj.2013.10.018.

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