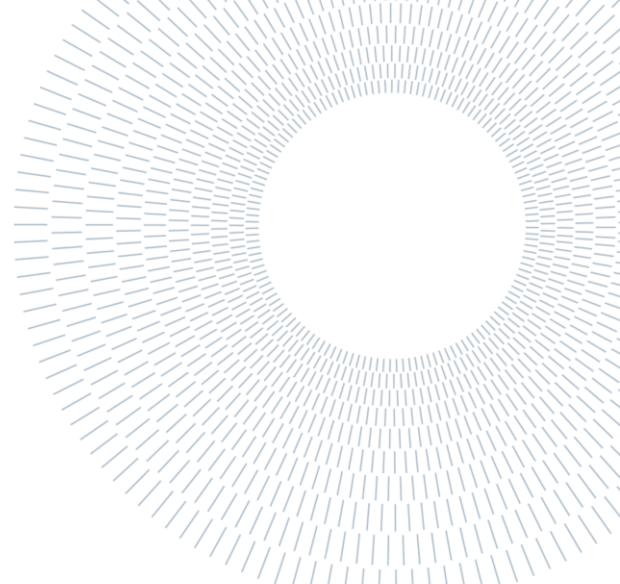




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EXECUTIVE SUMMARY OF THE THESIS

EFFECT OF THE SODIUM ALGINATE CONCENTRATION AND THE IONIC STRENGTH ON THE RHEOLOGICAL PROPERTIES OF SODIUM ALGINATE BASED HYDROGELS

MASTER DEGREE IN MATERIALS ENGINEERING and NANOTECHNOLOGY

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1. Introduction

Mucus is a biological hydrogel with important selective barrier properties for the human body. At the macroscopic level, it is a non-Newtonian, thixotropic hydrogel and its 3D structure, viscoelastic properties and compositional complexity define the relation between the human tissue and the external environment. The importance of developing in vitro models which can replicate both the microstructure and the chemical functionality of intestinal mucus is evident for drug delivery/screening, investigation of gut-brain-axis and research on regenerative medicine since in vivo and ex vivo models show some drawbacks such as low repeatability, high costs and the difficulty of obtaining large sample volumes. [1] Nevertheless, mucus constitutes a complex system when it comes to mimicking its structure and reproducing its properties by in vitro models, too. Its viscoelasticity and rheological properties mostly depend on its gel network which is influenced by some physicochemical parameters among which is ionic strength of the environment. Evolution towards a realistic in vitro mucus model

is possible with the use of sodium alginate(SA)-based hydrogels as the initial polymer structure. [2] SA-based hydrogels have proved to be efficient in mimicking the double layer structure of intestinal mucus through different gelation mechanisms.

In this thesis project, we investigate the effect of SA concentration and of the solvent NaCl concentration on the rheological properties of SA-based hydrogels which were prepared from SA solutions via the addition of calcium carbonate (CaCO_3) as the source of Ca^{2+} ions acting as crosslinker and Glucono delta-lactone (GDL) as the acidifier. Acidifier allows the slow dissociation of Ca^{2+} ions once the CaCO_3 is homogeneously dispersed in SA solutions, which leads to an internal homogeneous gelation resembling the uniform gel network of loose layer of intestinal mucus. This ionotropic gelation is described by “egg-box model” [3].

2. Materials & Methods

2.1. Sample Preparation

Sodium alginate (SA), calcium carbonate (CaCO_3), and glucono-delta-lactone (GDL) were purchased from Sigma Aldrich in the powder form. SA solutions were prepared by dissolution of SA powder in aqueous solutions of NaCl (NaCl 0, 0.057, 0.15, 0.7 and 1 (w/v)% in distilled water) upon magnetic stirring for 24 hours to ensure homogeneity. After the SA solutions were obtained, SA-based hydrogels were prepared with the addition of crosslinker CaCO_3 and the acidifier GDL by double-syringes procedure. Hydrogels were stored in refrigerator ($\sim 4^\circ\text{C}$) for 24 hours for the crosslinking process to be completed. As one would expect, SA concentration changes once CaCO_3 and GDL are added. Table 1 below shows the SA concentration (wt% = (w/v) %) of the solutions and of the hydrogels prepared from those.

SA wt% in solution	SA wt% in hydrogel	SA wt% in solution	SA wt% in hydrogel
0.1	0.07	0.8	0.57
0.2	0.14	1	0.7
0.3	0.21	1.5	1
0.4	0.28	2	1.4
0.6	0.42	3	2.1

Table 1: SA wt% in solutions and hydrogels.

2.2. Test Methods

An Anton Paar Modular Compact Rheometer (MCR 502) which operates in stress control was used for the rheological characterization. Steady state shear rheometry was applied to characterize the solutions whereas oscillatory rheometry was adopted to characterize the hydrogels. Parallel plate configuration in 50 mm and 25 mm were selected for solutions and the hydrogels, respectively. All tests were carried out at 25°C .

Via the flow tests on solutions, viscosity (η) dependence on shear rate ($\dot{\gamma}$) was investigated through a set of shear rate steps at shear rates increasing from 0.1 s^{-1} to 100 s^{-1} and then decreasing from 100 s^{-1} to 0.1 s^{-1} .

Via the frequency sweep tests on hydrogels, storage modulus (G') and loss modulus (G'') dependence on angular oscillation frequency (ω) was assessed. Angular frequency ranged from 0.1

to 20 Hz and the shear strain amplitude was chosen as 0.5% from the linear viscoelastic regime (LVER).

3. Results & Discussion

3.1. SA Solutions

Zero shear viscosities (η_0) were determined from the flow curve of the solutions and used to calculate the specific viscosity values (η_{sp}) from the Eq. 1 where η_s is the solvent viscosity. When η_{sp} is reported as a function of polymer concentration (c), a power-law relation (Eq. 2) is obtained wherein n is the scaling factor.

$$\eta_{sp} = \frac{\eta_0 - \eta_s}{\eta_s} \quad (3a)$$

$$\eta_{sp} = a * c^n \quad (3b)$$

When the relation Eq. 2 is plotted in a double logarithmic scale, the slope of the linear fit gives the scaling factor, n . Depending on the type of interaction between polymer-polymer and polymer-solvent interaction branches with different slopes are observed, corresponding to different regimes. The critical concentration corresponding to the transition from a regime to the subsequent can be distinguished from the points at which the slope changes.

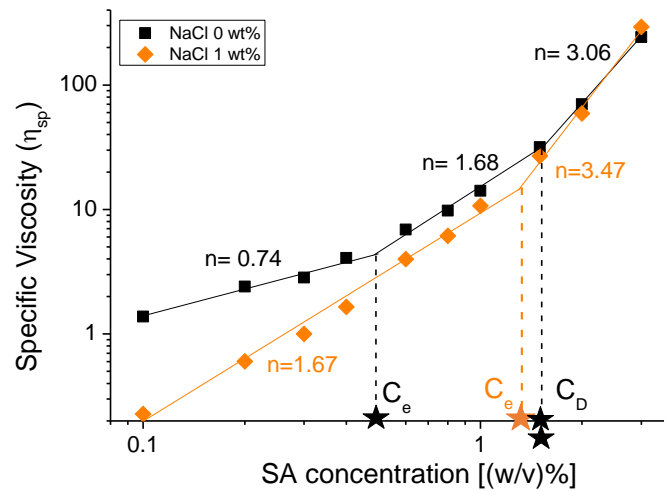


Figure 1: η_{sp} dependence on SA concentration (c). Black dashed lines separate the concentration regimes in salt-free case while the orange one does the same for NaCl 1 wt% solution. Black and orange stars on the x axis refer to the critical concentrations corresponding to concentration regimes for salt-free case and NaCl 1 wt% solution, respectively.

Figure 1 depicts the scaling behavior of η_{sp} with respect to polymer concentration c , for NaCl 0 wt% (black) and NaCl 1 wt% (orange). Scaling factors (n) are given for each concentration regime. Theoretical models [4] predict 4 concentration regimes for polyelectrolytes (such as SA) in salt-free case and 3 concentration regimes for neutral polymers. Both type of polymers shares the dilute regime which occurs at very low viscosity values out of the scope of this work. Instead, we observe semi-dilute unentangled and semi-dilute entangled regimes and the critical concentration for entanglement C_e (single stars on the x-axis) at which transition occurs indicated by dashed lines (black for NaCl 0 wt%; orange for NaCl 1 wt%). There is a concentrated regime beyond a further critical concentration (C_D) depicted with a double black star for salt-free case. The scaling factors for each regime and C_e values are reported for all SA solutions in Table 2. As expected from theoretical predictions [4] and other studies in literature [5], scaling factor increases along with the increase in NaCl concentration. Although the values found in our study do not coincide with the theoretical predictions, they are in accordance with previous studies. [5] The difference between experimental results and the theoretical predictions might be arising from SA polydispersity. These finding suggest the conformation change from polyelectrolyte in salt-free cases to neutral polymers via the addition of salt, as theory predicts.

NaCl wt%	Slopes			Entanglement concentration C_e
	Semidilute unentangled	Semidilute entangled	Concentrated	
Theoretical for polyelectrolyte	0.5	1.5	3.75	
0	0.74	1.68	3.06	0.47
0.057	1.41	2.59		1.01
0.15	1.51	3		1.24
0.7	1.55	3.46		1.23
1	1.67	3.47		1.26
Theoretical for neutral polymer	1.25	3.75		

Table 2: Scaling factors and C_e values reported with scaling factors from theoretical predictions.

The effect of ionic strength on the solution viscosity was investigated by plotting the η_0 values as a function of NaCl concentration of the solutions for each SA concentrations as in Figure 2. Figure 2 displays that two opposite effects can be observed:

- There is a remarkable decrease in η_0 with the increase of NaCl concentration when

the SA concentration is low (up to SA 0.8-1 wt%). This increase is probably due to the conformation change from rod-like polyelectrolytes in salt-free case to a more collapsed globular conformation of neutral polymers due to the screening of the electrostatic repulsion along the polyelectrolyte chain by the free ions introduced in the system. [4]

- Beyond SA 1 wt%, η_0 starts to increase as NaCl concentration increases above 0.7 wt%. This increase in η_0 becomes even more significant as SA concentration increases even further. For SA 2 wt% and 3 wt% solutions, the decrease in η_0 is observable only at the transition from salt-free case to NaCl 0.057 wt% solvent, then it increases as the ionic strength increases.

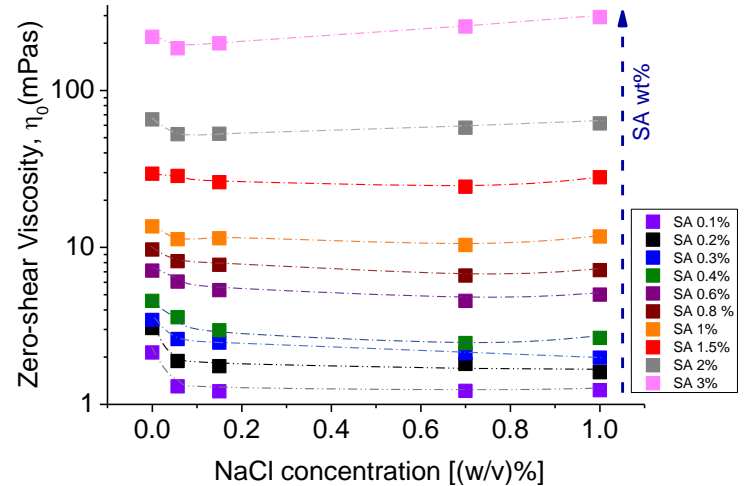


Figure 2: η_0 plotted with respect to salt concentration for all SA solutions. Legend shows the SA concentrations. The dashed arrow indicates the direction of SA wt% increase.

3.2. SA-based Hydrogels

Storage (G') and loss (G'') modulus dependence on angular frequency were revealed by frequency sweep tests. For all SA hydrogels we tested, G' is always higher than G'' , which confirms the solid-like (gel) state of the materials.

Given the limited effect of frequency (especially in the case of G'), from the experimental curves, the effect of SA and NaCl concentrations were investigated considering the response at a fixed frequency, which was arbitrarily set to 1 Hz.

G' increases with SA concentration for all hydrogels. The increase is more significant at low SA concentrations, and levels when moving

towards higher concentrations. An effect of NaCl concentration reflects as two separate regimes which can be distinguished, one for NaCl concentration lower than 0.7 wt%, and one for higher concentration.

The different increasing trends are represented by the blue and orange dashed lines in Figure 3 and Figure 4.

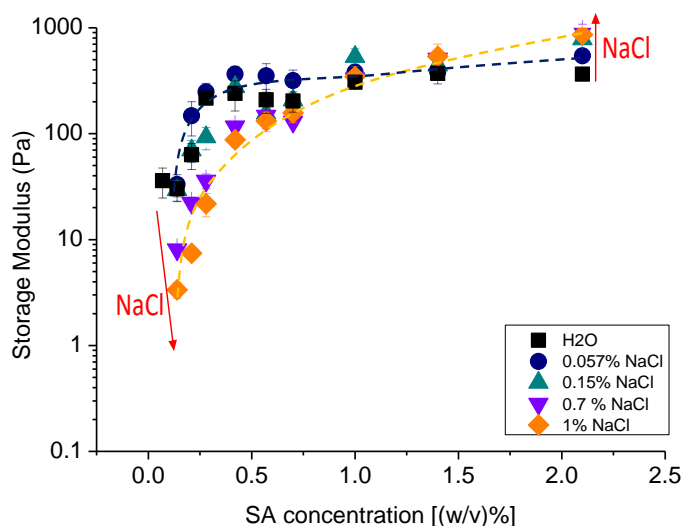


Figure 3: Storage modulus, G' dependence on SA concentration. Red arrows indicate the direction of increase in NaCl concentration.

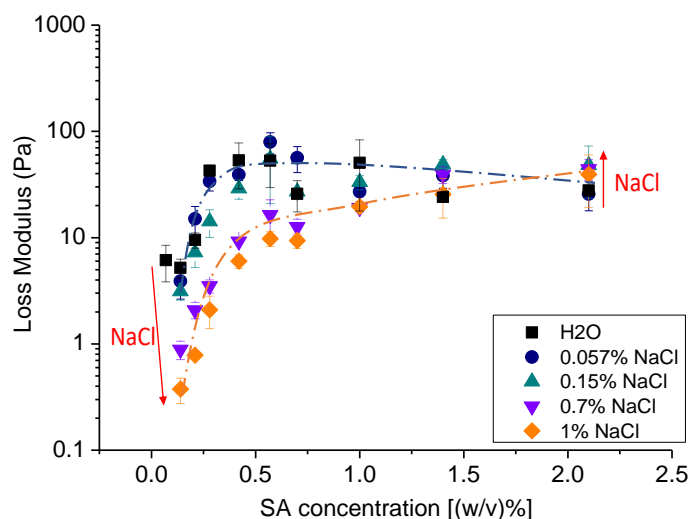


Figure 4: Loss modulus, G'' dependence on SA concentration. Red arrows indicate the direction of increase in NaCl concentration.

When the SA concentration is lower than ~1 wt%, SA hydrogels in distilled water, NaCl 0.057 wt% and 0.15 wt% seem to have storage moduli higher than those of SA hydrogels in NaCl 0.7 wt% and 1 wt%. When we go beyond SA 1 wt%, the situation

seems to be reversed and SA hydrogels in NaCl 0.7 wt% and 1 wt% have higher storage moduli than the others. Further in this second case, G' does not reach a plateau value. Similar observation can also be made for G'' .

These findings suggest that the effect of ionic strength on gels' dynamic mechanical response is therefore qualitatively similar to that observed in the case of SA solutions.

Considering the physical gelation mechanism of SA in the presence of divalent cations, introduction of Na^+ ions into the hydrogel leads to the partial replacement of Ca^{2+} ions (which are the crosslinkers between G blocks of adjacent SA chains) with Na^+ ions as represented in Figure 5. Hence, the addition of NaCl is expected to disrupt the crosslinking, therefore, to decrease the elasticity of the network which can explain the decrease in G' as the ionic strength increases.

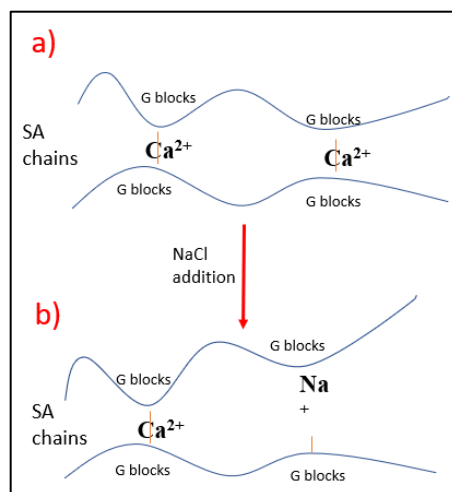


Figure 5: a) Ca^{2+} ions facilitate physical crosslinking between the G blocks of adjacent SA chains. b) Addition of Na^+ ions to the system leads to the partial substitution of Ca^{2+} with Na^+ and the consequent disruption of crosslinking.

The opposite situation in which the G' increases with increasing ionic strength above a certain SA concentration, requires more investigation to explain the mechanism behind it. Nevertheless, other studies have reported a similar interesting effect of ionic strength on the solutions' viscosity as well as the dynamic response.[5]

3.3. Relationship Between the SA Solutions' Viscosity and the Corresponding Hydrogels' Dynamic Mechanical Behavior

A correlation between the results of the rheological characterization of solutions and hydrogels obtained from the solutions was looked for, aiming at investigating if some link can be made with the structure of the solutions themselves, for example in terms of critical concentrations.

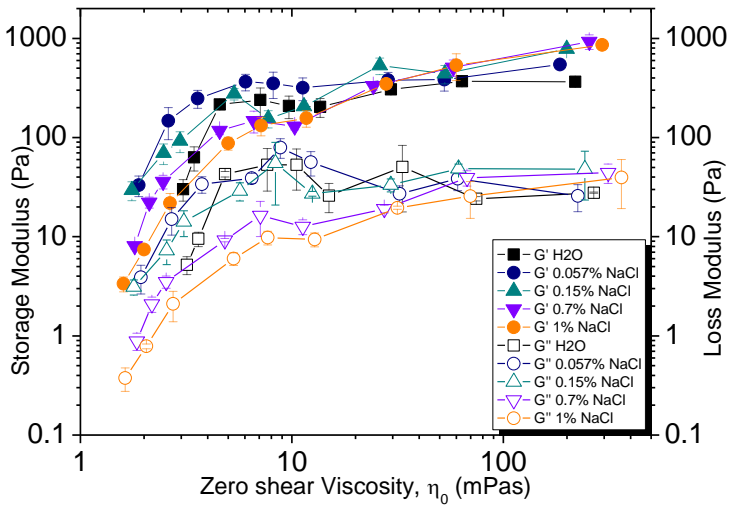


Figure 6: G' (solid symbols) and G'' (open symbols) values of SA hydrogels reported with the corresponding zero-shear viscosity values of the SA solutions from which SA hydrogels were prepared.

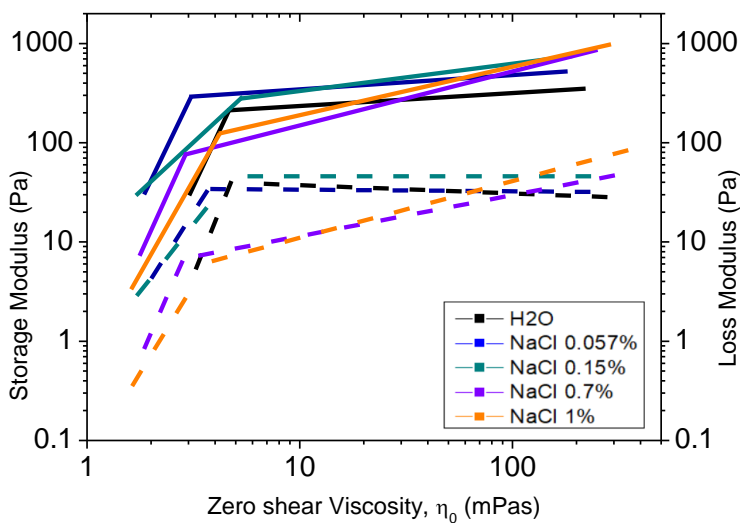


Figure 7: Trendlines of curves in Figure 6. Solid lines belong to the G' curves while dashed lines belong to the G'' curves.

For this investigation, G' and G'' of the hydrogels were plotted against the solutions' viscosity as in Figure 6. Each curve refers to a different NaCl concentration. Despite the curve-to-curve difference, a general trend can be observed, with a steep increase in G' and G'' at increasing solution zero shear viscosity in a low zero-shear viscosity range (up to about 10 mPa s), and then a further increase, which is however less steep. Given this trend, and in order to give an analytical description of the observation, the curves were fitted by a bilinear trend (in the log-log scale, Figure 7).

The zero-shear viscosity values at the intersection between the two lines were determined and the relevant specific viscosity values, which will be referred to as "critical", $\eta_{crit,sp}$ were calculated. Afterwards, $\eta_{crit,sp}$ values were compared with the specific viscosities at critical concentration for entanglement ($\eta_{sp,ent}$).

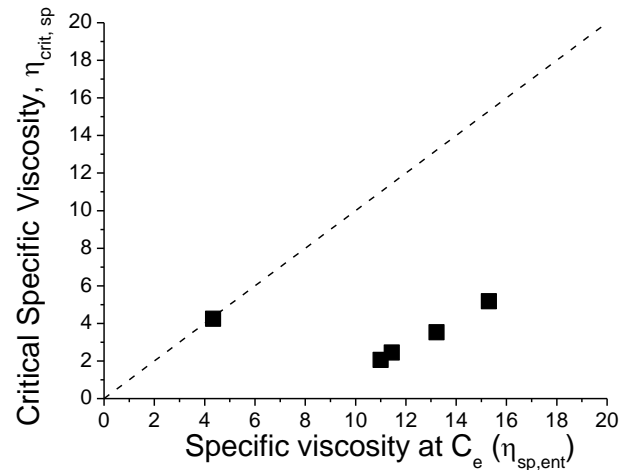


Figure 8: "Critical specific viscosity" $\eta_{crit,sp}$ plotted with respect to Specific viscosity at C_e , $\eta_{sp,ent}$. Dashed line is a linear 1:1 curve.

Figure 8 shows that, $\eta_{crit,sp}$ and $\eta_{sp,ent}$ interestingly coincide in the case of solution in distilled water, suggesting a structural cause for the change in G' and G'' dependence on solution viscosity; the dependence itself, requires further investigation. On the other hand, when NaCl is added to the solution, the matching of $\eta_{crit,sp}$ and $\eta_{sp,ent}$ values is lost, suggesting a complex combined effect of SA and NaCl concentration, which is still to be interpreted.

4. Conclusions

Steady state shear rheometry tests on sodium alginate (SA) aqueous solutions have shown that the viscosity of solutions strongly depends on the polymer concentration, and NaCl concentration in the solvent. The effect of NaCl was the expected neutralization of ionic sites on the macromolecule, so that even with the smallest amount of NaCl (0.057 wt%), rheological behavior of SA solution has turned to neutral polymer-like, showing only two inflection points in the concentration range investigated. Viscosity of the solutions in unentangled regime decreased with increasing ionic strength. However, an opposite effect has been observed at higher SA concentrations: an increase in viscosity was observable with ionic strength at concentrations beyond about SA 1 wt%.

Although the expected increase in scaling factor (for the specific viscosity dependence on SA concentration) was observable as the ionic strength increases, the values of the scaling exponents were not in complete accordance with the theoretical predictions, which might be due to the high polydispersity of SA deriving from its natural origin.

Frequency sweep confirmed the solid-like states of all SA-based hydrogels. Both properties increased with polymer concentration as expected. An interesting effect of ionic strength was observed: dynamic modulus of hydrogels with low SA concentration decreased in the presence of NaCl, which was probably due to the partial substitution of calcium crosslinkers with free sodium ions in the solvent. However, an opposite effect of ionic strength was observed at higher values of SA concentration (beyond 1 wt%), similar to what we observed in solution viscosity: dynamic moduli of the hydrogels increased with ionic strength. The inversion of the ionic strength effect occurred at higher SA concentration for G'' compared to G' .

Finally, a correlation between precursor solution viscosity and hydrogel dynamic moduli was looked for. G' and G'' increase with solution viscosity in a bilinear fashion in a double logarithmic plot, with larger slope in the low viscosity range and smaller slope at higher viscosities.

When the viscosity values at the inflection point of the bilinear curves were compared with the viscosity at critical concentration for entanglement;

they turned out to be very close in the case of solutions without NaCl. Absence of this coincidence in the presence of NaCl suggests a complex combined effect which requires further interpretation. Hence, the structural interpretation of the correlation between solution viscosity and hydrogel mechanical behavior is a point that remains open.

Nevertheless, this work allowed to build a map for the formulation of precursor solutions of SA in a physiological-like environment, which will lead to formation of hydrogels via crosslinking, with customized dynamic mechanical properties. Furthermore, the effect of Na^+ ions on both solutions and hydrogels has been assessed, and tentatively explained in terms of screening of anionic sites present on the Sodium Alginate macromolecules.

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