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Entrapment and release of sodium polystyrene sulfonate (SPS) from calcium alginate gel beads

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Abstract

The release of sodium polystyrene sulfonate (SPS) from calcium alginate hydrogel beads has been studied. It has been shown that the structure of the cross-linked calcium alginate network is of primary importance in the retention and/or release of the SPS. This has been evidenced by studying the influence of Ca^{2+} concentration, molar masses (M_n) and the ratio of mannuronic acid/guluronic acid components. A minimum in the SPS release is observed in relation with the organization of the network structure. Conditions inducing the organization of a strong gel (e.g. high Ca^{2+} concentration for example) are not always related to a low release. A good control of release is found when a compromise between a well-structured hydrogel and sterical consideration of SPS is reached.

Keywords: Calcium alginate beads; M/G ratio; Entrapment; Network; Release; Sodium polystyrene sulfonate; SEC/MALLS

1. Introduction

Alginate is a naturally occurring biopolymer extracted primarily from brown algae. It has been used in the biotechnology industry as a thickening agent, a gelling agent and a colloidal stabilizer. Alginate also has a unique capacity to be used as a matrix for the entrapment and/or delivery of a variety of molecules or particles.

Alginate is a linear unbranched polysaccharide composed of two building blocks namely mannuronic acid (M) and guluronic acid (G) units (Fig. 1). These residues are linked in 1,4 and may vary widely in composition and sequence depending of the alga origin. In the presence of divalent cations, alginate shows gelling properties. Addition of calcium ions induces a cooperative effect between G-blocks until a 3D network is formed according to the well-known "egg-box" model (Fig. 2). Clark [1] had studied the influence of the divalent cations on the strength of the gel.

Ca alginate particles are generally prepared using two methods. The first method (called dripping), gives large particle (about 1 and 2 mm) (e.g. [2,3]). The second method (called emulsification) gives small but irregular particle with a high tendency to clumping (e.g. [3,4]). The large pore size of this particle (12–16 nm on the surface as reported by Klein [5] permits only to encapsulate and release macromolecules as protein (insulin [6], fibrinogen, gamma globulin [7] or to entrap cells [8]. Small molecules have high diffusion coefficient in Ca alginate beads and can be entrapmented and retained only

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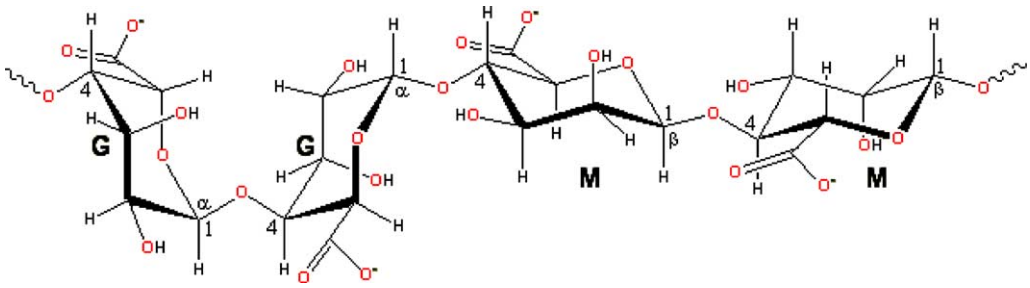

Fig. 1. Chemical structure of alginate. G is Guluronic acid group, M is Mannuronic acid group.

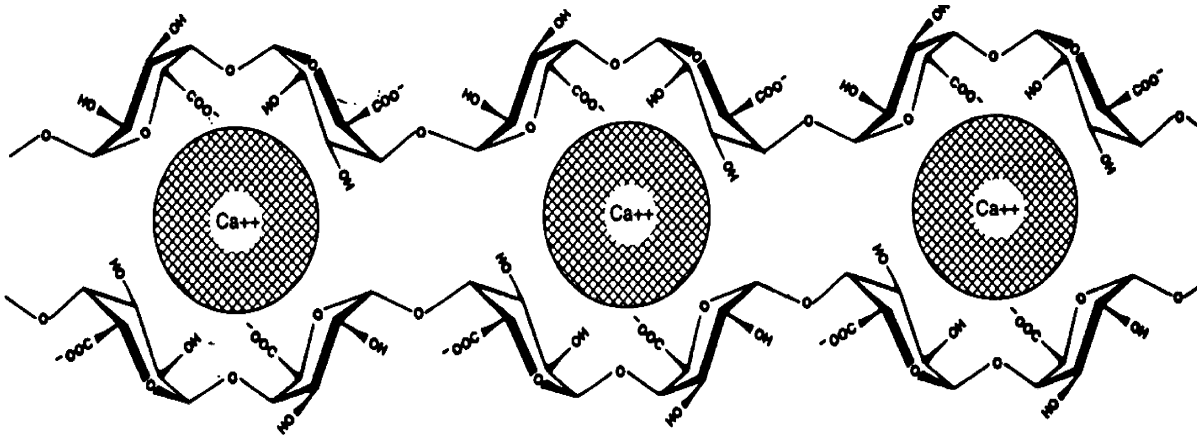

Fig. 2. The "Eggs-box" model for alginate gelation with calcium ions.

if beads are covered with a cationic polymer membrane (capsule) [9]. Data have been reported recently by Kikuchi [10] and Favre [11] about the release of polymer from such Ca-alginate hydrogel beads, however many questions still remain asked.

The aim of our work was to relate the macromolecular structure of alginate beads prepared using the dripping technique to study the entrapment properties and release capacities of a model polymer according to a specific application, a sodium polystyrene sulfonate (SPS) from various Ca alginate beads. The choice of this model polymer is attributed to the presence of sulfonate functions and a molecular mass of around 50 000 g/mol. The amount of calcium ions added, the molar weight and the G-blocks content of the alginate (M/G ratio) were particularly considered.

2. Materials and methods

2.1. Materials

Na-alginate samples with different M/G ratio and molecular weight were provided by SKW Biosystems (Baupte, France). Sodium polystyrene sulfonate (dry powder) and calcium chloride (anhydrous) were purchased respectively from Aldrich and Merck. For calcium chloride, the percentages of Mg^{2+} salts and $Ca(OH)_2$ are negligible (<0.005%). The water is treated with Milli Q system (Millipore, Massachusetts, USA).

2.2. Alginate characterization

2.2.1. Molecular weight

The absolute average molar weight and molar weight distribution was determined by coupling on-line a size exclusion chromatography (SEC), a multi-angle laser light scattering (MALLS) and a differential refractive index detector (DRI).

$LiNO_3$ 0.1 M used as carrier, was filtered through 0.1 μm filter unit (Millipore), carefully degassed (ERC-413 Erma CR Inc., Tokoy, Japan), eluted at 0.6 ml min^{-1} flow rate (Intelligent Pump 301, Flom, Tokyo, Japan), and clarified through a 0.45 μm filter upstream columns. The sample was injected through a 100 μl full loop.

The SEC line consisted of an OHPAK SB-G guard column as protection and two OHPAK SB 804 and

Table 1
Physicochemical characteristics of the studied alginates

Sample	M/G	Origin	M_n (g mol^{-1})	P_d	R_g (nm)	η (2%) (Pa s)
A	0.5	Lessonia	225 000	1.8	62	250
B	0.5		350 000	1.9	76	535
C	0.5		460 000	2.0	100	1000
D	1.4	Ascophyllum	380 000	1.5	73	1640

806 HQ columns (Shodex Showa Denko K.K., Tokyo, Japan) in series. The column packing is a polyhydroxymethylmetacrylate gel.

The MALLS photometer, a DAWN-EOS from Wyatt Technology Inc. (Santa Barbara, USA) is filled with a K5 cell and a He–Ne laser (λ = 633 nm). The collected data were analyzed using the Astra V-4.50 software package. The SEC-MALLS technique has been described elsewhere [12]. The concentration of each eluted fraction has been determined with the DRI (ERC 7515A Erma CR Inc.) according to the known values of dn/dC (0.171 ml/g and 0.133 ml/g for SPS and alginate respectively).

The on line quasi elastic light scattering (QELS) extent from Wyatt Technology was connected to the Down EOS (angle 13) in order to determine the SPS hydrodynamic radius (Rh).

2.2.2. Viscosity

The viscosity of 2% (w/w) Na alginate solutions was measured in water with a Carri-Med CSL 100 (TA Instruments, Delaware, USA) fitted with double-gap concentric cylinder geometry. The rheometer was equipped with a solvent trap to prevent any dehydration during measurement, and the temperature was controlled by circulation from an external water bath.

An Ubbelholde type viscometer (FICA, Sofica, Paris, France) was used for measuring relative viscosity of dilute SPS solutions.

$\eta_r = t/t_0$ where t_0 and t are the solvent and solution times of flow.

2.3. Preparation of Ca alginate particles

The Na alginate and the sodium polystyrene sulfonate (if necessary) are added slowly in water at a concentration of 2% and 4% respectively. The stirring is maintained gently during 24 h. Next the viscous solution is placed in a syringe equipped with a needle of 0.8 mm (Terumo 0.8 × 40 mm) and dropped into $CaCl_2$ solutions containing 10, 40 or 100 mM of Ca^{2+} respectively. The distance between the calcium chloride solution surface and the bottom end of the needle is fixed to 60 mm.

Ca alginate beads were slowly stirred for 15–60 min to allow alginate gel formation. The size of the beads is between 1 and 2 mm. The alginate solution viscosity (Table 1) had no effect on the size and the shape of the beads. This influences only the kinetics of the solution flow rate. The beads were then separated from the solution by Buechner funnel with sintered glass filter.

2.4. Loading of microparticles with SPS

A known quantity of Ca alginate beads is stirred during 24 h in a phosphate buffer (pH = 7.2). The gel particles are destructed and SPS is in solution that permits to determine the SPS concentration and consequently the loading efficiency (% of the actual content compared to the theoretical content (4%)).

But it is not possible to obtain the only SPS concentration with classical techniques as UV photometer or refractive index, which give a global response (SPS and Alginate).

Consequently, we used the SEC which permit to have the contribution of each species. Fig. 3 shows the good separation between the SPS and the alginate. The real quantity of SPS and alginate is evaluated from the refractometric signal of the SEC after calibration peak surface versus SPS and alginate quantity. Uncertainty is approximately 2%.

2.5. Release

The following procedure was used for measuring the release of SPS from particles. Successive aliquots (1 ml)

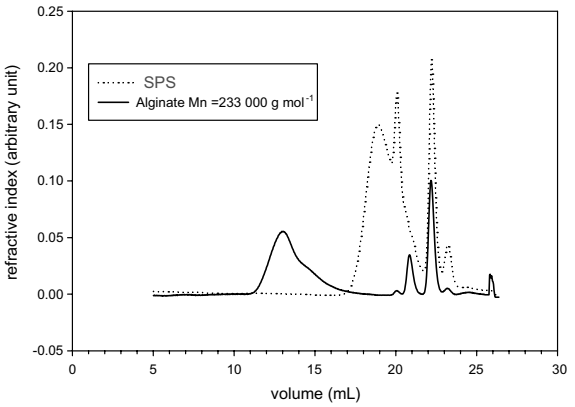

Fig. 3. Chromatographic profile of alginate (sample A) and SPS.

are drawn from a water solution (100 ml) containing 0.5 g (dry state) of slowly stirred Ca alginate beads. The quantities of SPS and alginate were calculated from SEC/RI analysis.

3. Results and discussion

3.1. Physicochemical characterization of alginates and SPS

The four alginate samples varying by their M/G ratio and molar masses have been analyzed by both SEC/MALLS/DRI and viscometry. The results are compiled in Table 1. As guluronic groups are involved in the gel formation with calcium ions, we have focused our attention on the lower ratio e.g. 0.5. Three samples of M/G = 0.5 have been studied differing by their number average molar masses (M_n) from 220 000 to 460 000 g/mol respectively from sample A to sample C. The fourth sample (D) has a M/G ratio value of 1.4 and its M_n is close to that of sample B, e.g. 350 000 g/mol. Both samples B and D were compared for studying the influence of M/G ratio.

As expected, it appears that for a constant M/G ratio, both radius of gyration and viscosity increase as M_n increases (sample A, B and C). The comparison between sample B and D shows the radii of gyration are similar indicating that the same conformation prevails. Concerning the viscosity at 2%, the results clearly indicate that the higher M/G ratio (1.4) gives higher viscosity as compared to the lower M/G (0.5). This can be attributed to specific interactions between mannuronic groups present in higher amount in sample D than in sample B. This result may be of importance considering the gel formation and moreover the release of SPS.

The sodium SPS has a M_n of about 50 000 g/mol together with a very low polydispersity index of about 1.1 and a hydrodynamic radius of 7 nm in $LiNO_3$ 0.1 mol/l.

3.2. SPS release from alginate-Ca beads

Most of the following results have been conducted with alginate B (M_n = 350 000 g/mol and M/G = 0.5) excepted when M/G or M_n has been taken as a specific studying parameter.

3.2.1. Influence of gel formation time

The time of gel formation is expected to be of primary importance as concerns the entrapment and release properties of the gel. The efficiency SPS entrapment as a function of hydrogel beads formation time (time of contact with the $CaCl_2$ solution) for two systems is reported in Fig. 4. It clearly appears that in the case when no SPS is present in the gel formation bath, the entrapment rate

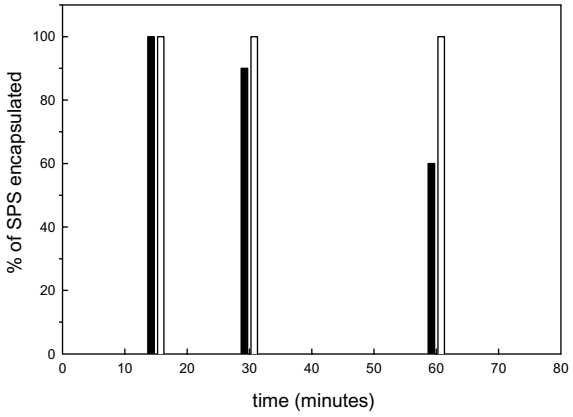

Fig. 4. Percentage of entrapment SPS as a function of beads formation time: in the absence of SPS in beads formation bath (black); in the presence of SPS (same concentration as inside beads) in beads formation bath (white).

of SPS decreases as the time of gel formation increases. This can be explained by the fact that the release of SPS starts to occur during the period (15 min) of the gel formation. To avoid this, the concentration of SPS inside and outside the beads must be the same during the gelation process as clearly evidenced by data of Fig. 4.

The time of gel formation should also influence the cohesion of the gel. If the structure of the gel is too loose, the polymer network is eroded. Consequently the SPS and also alginate release outside the beads. The release of alginate as a function of time for different times of gel formation is reported in Fig. 5. As expected, a certain amount of alginate is released from the particles. The released amount is more important when the time of gel formation is short suggesting that the structure of the gel is strongly dependent on the contact time between Ca^{2+} and alginate [13]. In our conditions the gel

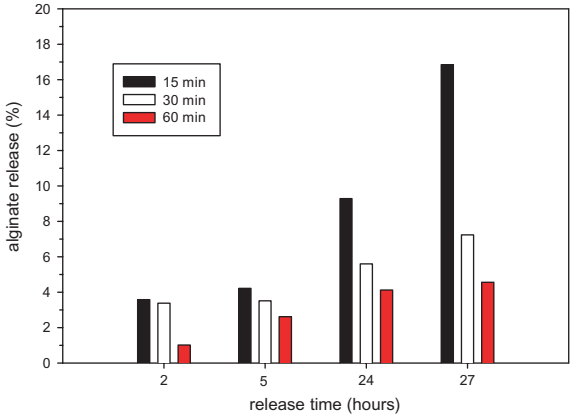

Fig. 5. Percentage of alginate released as a function of release time for different time of beads formation process.

formation cannot be considered as complete even after half an hour. In summary, the higher the gelation time, the stronger the gel and lower is the alginate release. Alginate release becomes really significant (more than 10%) only for equilibrium release time longer than 24 h. For this reason, in the following the experiment time of release will never be over 24 h and the gel formation time was fixed to 15 min.

3.2.2. Influence of Ca^{2+} concentration on gel and on SPS release

Before studying the influence of calcium ion concentration on the structure of the gel and on the release of SPS, it seemed of interest to focus on the SPS-Ca^{2+} system. Fig. 6 shows the evolution of the relative viscosity of SPS in dilute solution as a function of the ionic strength for both NaCl and $CaCl_2$. Obviously, as usual for a polyelectrolyte, the viscosity decreases as the ionic strength increases. Moreover the decrease of viscosity is more pronounced in the presence of divalent ions (Ca^{2+}) than in the presence of monovalent ions (Na^+). This is due to the existence of specific interactions between Ca^{2+} and SPS that induce a more compact conformation of SPS. This result has been confirmed by comparing at the same ionic strength (0.04 M) the intrinsic viscosity of SPS in NaCl ($[\eta] = 25$ ml/g) and $CaCl_2$ ($[\eta] = 18$ ml/g) solutions. In $CaCl_2$ the viscosity is 30% lower. QELS measurement has not permitted the determination of SPS hydrodynamic radius in $CaCl_2$. This point is of importance for the interpretation of the release process of SPS particularly when the concentration of Ca^{2+} will be changed in the gel.

The data reported in Fig. 7 show that the release of SPS is fast at first and reaches a plateau after about 500 min. Furthermore a certain amount of SPS (which is dependent of the Ca^{2+} concentration) remains entrapped in the beads. Two reasons can be suggested for explaining such a behavior:

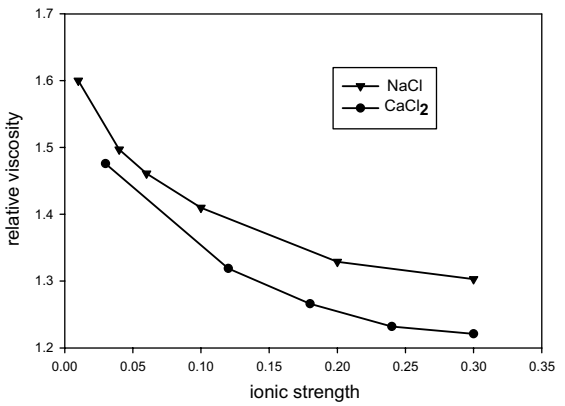

Fig. 6. Relative viscosity of SPS at 4% as a function of the ionic strength. NaCl (triangle), $CaCl_2$ (circle).

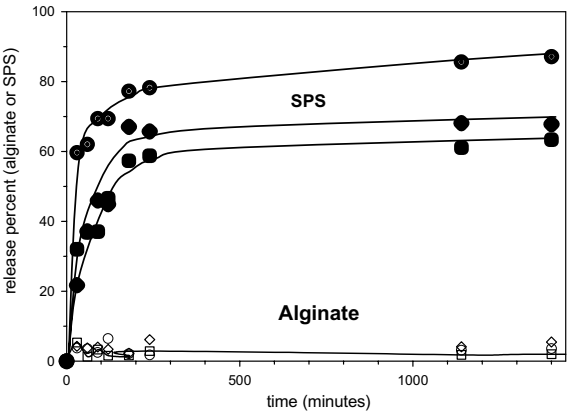

Fig. 7. Kinetics of alginate and SPS release as a function Ca^{2+} concentration. $[Ca^{2+}] = 10$ mM (losange); $[Ca^{2+}] = 40$ mM (square); $[Ca^{2+}] = 100$ mM (circle).

- A steric reason due to the existence of physical entanglements of cross-linked alginate-Ca^{2+} of lower dimensions than the hydrodynamic volume of SPS.
- Specific interactions could exist between SPS groups and Ca^{2+} ions, which are already involved in a complex with alginate within the gel.

The second mechanism is favored, as we have shown that specific interactions between Ca^{2+} and SPS exist.

It has been observed the SPS release presents a minimum for an intermediate concentration of 40 mM of Ca^{2+}, which can be explained by considering the structure of the gel beads. Low Ca^{2+} concentration leads probably to a loose gel. As a consequence, the SPS can be easily released from the beads, as the steric entanglements do not constitute a strong barrier. Further addition of Ca^{2+} increases gives a more structured gel and SPS is more retained inside the beads. For higher Ca^{2+} concentration the increase of SPS release can be explained by specific Ca^{2+}–SPS interactions, which can result in a very compact conformation of SPS and decrease the hydrodynamic radius of SPS. This finally facilitates its release due to sterical consideration. This tendency increases when Ca^{2+} concentration increases as shown in Fig. 6.

This result can be probably explained by both the structure of beads for low calcium contents and the SPS–calcium interactions for high calcium contents. The minimum in the plot corresponding to 40 mM in calcium ion could be a compromise of both tendencies.

3.2.3. Influence of M_n on release

The equilibrium release amount of both SPS and alginate has been measured for samples A, B and C varying by their M_n but with the same M/G (0.5) and the same Ca^{2+} concentration (40 mM). Results are presented in

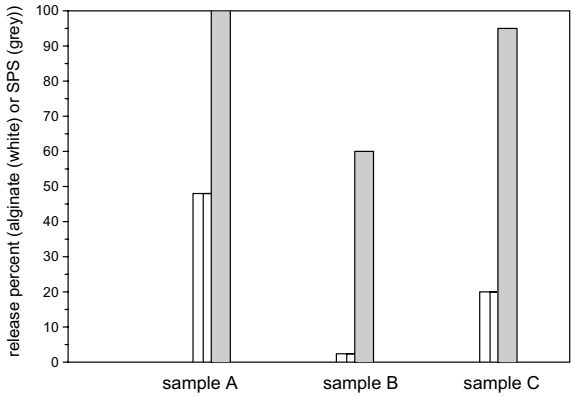

Fig. 8. Release of alginate (white) and SPS (grey) as a function of alginate number average molar mass (M_n) after 24 h.

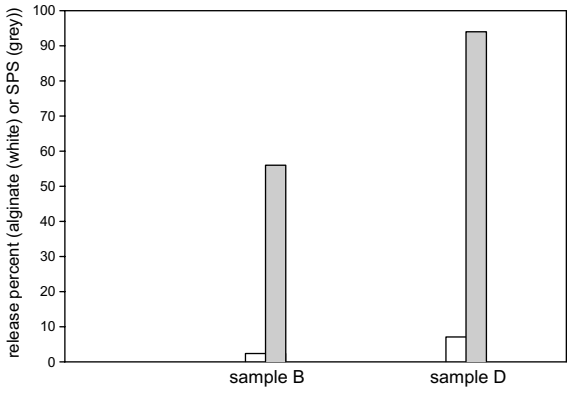

Fig. 9. Release of alginate (white) and SPS (grey) as a function of alginate M/G ratio after 24 h.

Fig. 8 and show that the amount of released SPS evidences a minimum for the M_n (350 000 g/mol). In the same time, a minimum has been also observed for alginate release. It seems that the molar mass also plays a great role in the structure of a such gel. In this case, no modification of specific interactions occurs. Only the influence of steric environment offered to the SPS can explain this result. The molar mass of alginate seems to modify the mesh size of the alginate-Ca cross-linked network which becomes more structured for the middle value of molar mass e.g. 350 000 g/mol. Below this value, the network appears to be not sufficiently structured according to the high amount of released alginate [14]. Above 350 000 g/mol, one can assume a heterogeneous network has been formed according to the higher viscosity of initial alginate solution inducing an increase of the matrix pore size.

3.2.4. Influence of M/G ratio on release

The influence of M/G ratio has been examined by studying the alginate and SPS release from two gel beads prepared with samples B and D which differ by the ratio M/G only (0.5 and 1.4 respectively). Results are compiled in Fig. 9. The sample with a lower M/G presents a better retention of the SPS than the higher M/G probably due to a better cross-linking with Ca^{2+} (according to the higher amount of guluronic groups). The diffusion of SPS is sterically slowed for the M/G value of 0.5 for which the number of cross-linking is more important and the mesh size is lower than for the M/G value of 1.4. This is confirmed by the lower amount of released alginate obtained in the case of the lower M/G value (0.5). This result does not agree with the model proposed by Draget [15], which suggests a larger pore size when the amount of G blocks is high. A different distribution of G groups on the macromolecular chain can explain the opposed result. Only blocks G are effective.

4. Conclusion

Entrapment and release of a low molar mass water-soluble polymer e.g. sodium polystyrene sulfonate (SPS) have been studied. The hydrogel matrix consisted of calcium alginate beads obtained by a dripping method. The ability of the system to encapsulate and control the SPS release has been investigated through the modification of the cross-linked calcium alginate network structure. Different alginates have been studied varying by their molar masses (M_n) and their mannuronic/guluronic acid (M/G) ratio. We have also focused our attention on the influence of calcium chloride ($CaCl_2$) concentration. The release of SPS shows a minimum as a function of the concentration of Ca^{2+}. This result may be explained either by a modification of the network mesh size, and/or by a decrease of hydrodynamic radius of SPS due to interactions with Ca^{2+} ions. A minimum in the SPS release has also been shown as a function of the alginate M_n. This result indicates that M_n plays a non-negligible role on the establishment and the structure of the cross-linked network. More obvious is the influence of M/G ratio, which shows a higher release amount of SPS for the higher M/G ratio. This can be explained by the formation of a network with a larger pore size when the amount of guluronic acid groups decreases. Consequently, the liberation of SPS is easier with a high alginate M/G ratio.

To complete this study, it will be of interest to investigate the entrapment and the release of water-soluble polymers of different molar masses but showing no interaction with Ca^{2+}.

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