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Surfactant and metal ion effects on the mechanical properties of alginate hydrogels

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ABSTRACT

This paper addresses the controlled variation of the mechanical properties of alginate gel beads by changing the alginate concentration or by adding different surfactants or cross-linking cations. Alginate beads containing nonionic Brij 35 or anionic sodium dodecyl sulfate (SDS) surfactants were prepared with two different types of cations (Ca^{2+}, Ba^{2+}) as crosslinkers. Compression measurements were performed to investigate the effect of the surfactant and cation types and their concentrations on the Young's modulus of alginate beads. The Young's modulus was determined by using Hertz theory. For all types of alginate gel beads the Young's modulus showed an increasing value for increasing alginate contents. Addition of the anionic surfactant SDS increases the Young's modulus of the alginate beads while the addition of non-ionic surfactant Brij 35 leads to a decrease in Young's modulus. This opposite behavior is related to the contrary effect of both surfactants on the charge of the alginate beads. When Ba^{2+} ions were used as crosslinker cation, the Young's modulus of the beads with the surfactant SDS was found to be approximately two times higher than the modulus of beads with the surfactant Brij 35. An ion specific effect was found for the crosslinking ability of divalent cations.

1. Introduction

Alginate is a material of interest due to its unique and useful properties. Being extracted from marine brown algae, alginates are non-toxic and edible polysaccharides. This polymer is a copolymer of 1–4 linked β-D-mannuronate (M) and α-L-guluronate (G) homopolymeric blocks. This polyelectrolyte forms crosslinked hydrogels with divalent cations, and this hydrogel structure is used in many applications. Applications of crosslinked alginates have a wide range, including controlled release, drug delivery formulations and waste removal agents [1–5].

Addition of various dopants to alginate formulations may increase the chemical and mechanical stability of the gels. One of the candidates for dopants is surfactants. Previously, the effect of cationic surfactant cetyltrimethylammonium bromide (CTAB) on viscosity and the effect of SDS on aggregation of alginate solution were studied by Yang et al. [6,7]. Rheological and turbidity measurements were carried out in aqueous mixtures of hydrophobically modified alginates with cationic, anionic and nonionic surfactants were also reported before[8].

The effect of different crosslinking cations on Young's modulus values of alginate beads [9], the effect of compression speed [10], the effects of M/G ratio of alginate and of the crosslinking cation type [11] on mechanical behavior have been reported before. Recently the effects of the crosslinking ion and polyamino acid coating on the mechanical properties of alginate beads were reported [12]. Besides beads, the effect of the crosslinking ion on the mechanical properties of disc-shaped alginates are also reported, such as the recent study by Kaklamani et al. [13].

The importance of the alginate materials in biomedical applications such as drug release studies and scaffolds for tissue engineering requires mechanical strength of these gels. Surfactants play an important role for the uptake and release of drugs. According to our knowledge, so far the effect of surfactant on the mechanical properties of alginate gel beads hasn't been studied. This paper reports for the first time, the effect of surfactant incor-

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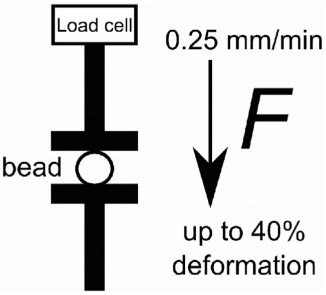

Fig. 1. Schematic representation of the unaxial compression measurement.

poration into alginate gels on the Young's modulus of alginate beads. Two different types of surfactants (nonionic: Brij 35 and anionic: sodium dodecyl sulfate) were used. Surfactant added alginates were crosslinked by calcium or barium ions. The effect of crosslinking ions on Young's modulus was also studied.

2. Materials and methods

Alginic acid sodium salt (viscosity of 2% solution ~250 cps) was from Sigma-Aldrich. This alginate is extracted from *Macrocystis pyrifera* and has a M/G ratio around 1.6 [14]. Calcium chloride dihydrate was purchased from J.T. Baker. Brij® 35, sodium dodecyl sulfate (SDS) and barium chloride dihydrate were obtained from Merck. All reagents were used without further purification. The critical micelle concentration (cmc) of Brij® 35 is about 0.09 mmol/L and about 8 mmol/L for SDS. In the present study both surfactants were used well above their respective critical micelle concentrations (cmc).

Accurately weighed alginate was dissolved in deionized water and necessarily amounts of surfactants were added into the alginate solutions. The solutions were stirred carefully in order to prevent bubble formation. The pH of the 1% alginate solution was around 6.7. Addition of SDS into alginate did not change the pH significantly. On the other hand, incorporation of Brij 35 into alginate solutions decreased the pH slightly to around 6.45. After complete dissolution, the mixture of alginate and surfactant were added dropwise to the gelling solution (e.g. $CaCl_2$ or $BaCl_2$) using a syringe of 0.8 cm inner diameter. The concentration of the crosslinking solutions was selected as 3% of $CaCl_2$ or $BaCl_2$ (w/v) in the experiments dealing with the effect of surfactants. For the effect of crosslinker ion experiments, the concentrations were 2, 3 and 5% of $CaCl_2$ or $BaCl_2$ (w/v). Formed beads were transferred into storage vessels and kept in the gelling media for 12 h at room temperature in order to complete gelation.

Compression measurements were carried out using an Instron 3345 testing machine attached with a 10 N force transducer. The diameter of each bead was measured using a digital caliper and all of the measurements were conducted at least in triplicate. A single bead was placed onto a platform, as shown in Fig. 1. A probe with a flat end was used to compress the bead. Compression measurements were performed at a speed of 0.5 mm/min and up to 40% deformation ratio at 25 °C.

In order to clarify the statistical significance of the results, single factor analysis of variance (ANOVA) tests were conducted for each data set. The level of statistical significance was assumed as 0.05 and statistical calculations was done using R statistical software v. 3.02 [15].

3. Results

The effect of crosslinker cation, alginate and surfactant concentrations on bead size is shown on Table 1. Barium alginate beads have bigger diameter than calcium alginate beads. The sizes increased with increasing alginate concentrations for all formulations. Incorporating both types of surfactants into formulations decreases the sizes of the beads initially, then the sizes increase with increasing surfactant concentrations. For 333 cmc of Brij 35 (i.e. the molar concentration of Brij 35 has 333 times of cmc of Brij 35) and 50 cmc of SDS, the sizes became almost equal to formulations without any surfactant for each crosslinker concentration.

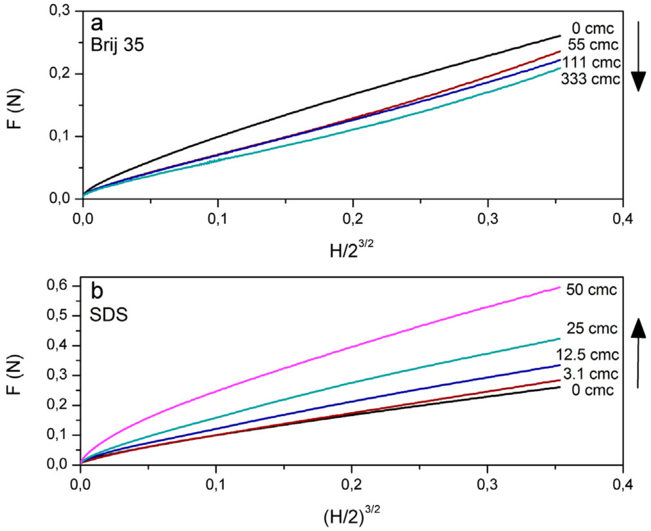

Fig. 2. The Force F (N) and $(H/2)^{3/2}$ curves of 4% (w/v) alginate beads crosslinked with 3% (w/v) $BaCl_2$ containing (a) 0, 5, 111 and 333 times cmc Brij 35 and (b), 0, 3.1, 12.5, 25 and 50 times cmc SDS. The arrows indicate the direction of increasing surfactant concentration.

The force (F) versus displacement (H) data was generated from the compression measurements. Hertz Theory [16] was used to determine the Young's modulus, as shown below:

$$F = \frac{4R^{0.5}}{3}\frac{E}{1-\upsilon^2}\left(\frac{H}{2}\right)^{3/2} \qquad (1)$$

where R is the radius of a bead, E is the Young's modulus, H is the displacement, and υ is the Poisson's ratio. First, the force (F) was plotted against the displacement $(H)^{3/2}$. The Poisson ratio was taken as 0.5 for 0.5 mm/min compression speed applied. This value is compatible with literature values [10,17]. In the literature, for the compression speed range between 0.075 mm/min [17] and 60 mm/min, [10] the Poisson ratio was selected as 0.5. The Young's modulus was then determined from the slope of linear region using the least square regression of the plot of F versus $(H/2)^{3/2}$.

Two examples of force versus $(H)^{3/2}$ curves for 4% (w/v) alginate beads crosslinked with 3% (w/v) $BaCl_2$ are shown in Fig. 2(a) and (b). Fig. 2(a) corresponds to the 4% (w/v) alginate beads containing 0, 55, 111 and 333 times cmc of Brij 35. Fig. 2(b) shows the curves of 4% (w/v) alginate beads containing 0, 3.1, 12.5, 25 and 50 times cmc of SDS. From comparing Fig. 2(a) with Fig. 2(b), the larger concentrations of SDS surfactant required larger force values to produce a given degree of deformation. On the other hand, the nonionic surfactant Brij 35 shows the opposite effect. The same trend was observed for calcium alginate beads, with smaller slope values. For each formulation, the Young's modulus of at least three different beads were calculated from the linear region of the force versus $(H)^{3/2}$ curves.

Statistical significance of Young's modulus values among changing surfactant concentrations at each alginate concentration was investigated using one way ANOVA tests at $p = 0.05$. Except the 1% and 2% (w/v) alginate beads crosslinked with barium, all series resulted in $p < 0.05$. Thus, incorporation of Brij 35 shows no effect

Table 1
Effect of crosslinker cation, alginate and surfactant concentrations on bead size.

	Ca^{2+} beads Bead size (cm)				Ba^{2+} beads Bead size (cm)			
	1% Alg	2% Alg	3% Alg	4% Alg	1% Alg	2% Alg	3% Alg	4% Alg
0 cmc	3.1 ± 0.1	3.3 ± 0.2	3.4 ± 0.2	3.8 ± 0.1	3.0 ± 0.1	3.5 ± 0.1	3.8 ± 0.1	3.9 ± 0.1
Brij 35								
55 cmc	2.8 ± 0.5	3.2 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	2.6 ± 0.1	3.0 ± 0.1	3.5 ± 0.1	3.8 ± 0.1
111 cmc	2.7 ± 0.2	2.7 ± 0.2	3.2 ± 0.2	3.0 ± 0.1	2.7 ± 0.1	3.3 ± 0.1	3.5 ± 0.1	4.0 ± 0.1
333 cmc	2.7 ± 0.2	3.6 ± 0.2	3.2 ± 0.2	3.3 ± 0.1	2.8 ± 0.1	3.3 ± 0.1	3.7 ± 0.1	4.2 ± 0.2
SDS								
3.1 cmc	2.6 ± 0.3	2.7 ± 0.1	3.1 ± 0.1	3.2 ± 0.6		3.1 ± 0.2	3.3 ± 0.1	3.2 ± 0.2
12.5 cmc	3.0 ± 0.2	3.2 ± 0.1	3.1 ± 0.1	3.2 ± 0.1		3.1 ± 0.2	3.4 ± 0.1	3.8 ± 0.2
25 cmc	3.1 ± 0.5	3.4 ± 0.1	3.2 ± 0.1	3.8 ± 0.3		3.3 ± 0.1	3.7 ± 0.2	3.6 ± 0.1
50 cmc	3.2 ± 0.2	3.5 ± 0.2	3.2 ± 0.1	4.0 ± 0.2		3.4 ± 0.3	3.8 ± 0.1	4.1 ± 0.5

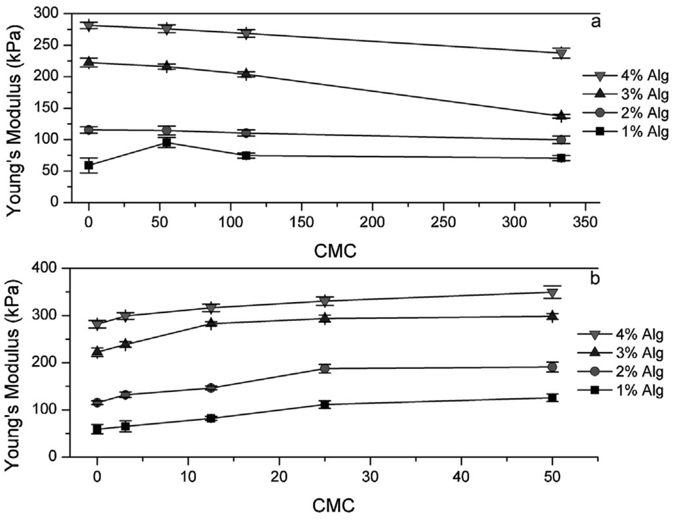

Fig. 3. Effect of (a) Brij 35 and (b) SDS concentrations on the Young's modulus of the alginate beads with various alginate concentrations. Crosslinking solution: 3% (w/v) $CaCl_2$. Error bars indicate standard errors of the mean.

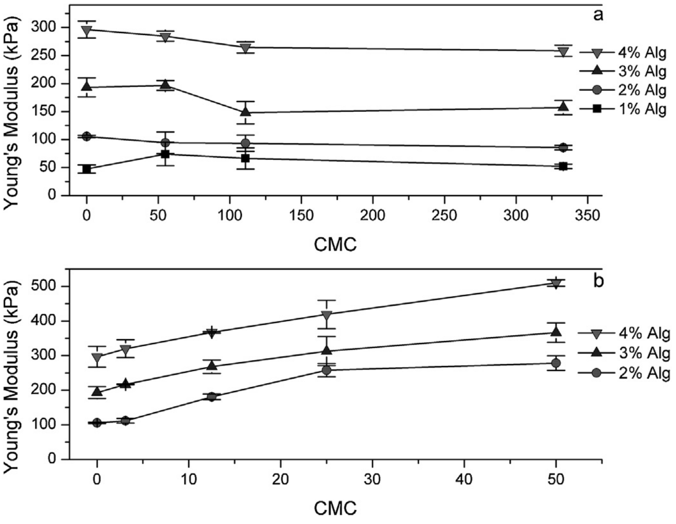

Fig. 4. Effect of (a) Brij 35 and (b) SDS concentrations on the Young's modulus of the alginate beads with various alginate concentrations. Crosslinking ion: 3% (w/v) $BaCl_2$. Error bars indicate standard errors of the mean.

on barium alginate beads at an alginate concentration up to 2% and other results are statistically significant.

Fig. 3(a) and (b) show the effect of surfactant type and surfactant concentration on the Young's modulus of the beads with varying concentrations of alginate solutions by crosslinking with 3% $CaCl_2$ ions. The most pronounced effect on the Young's modulus is given by the alginate concentration. By increasing the alginate concentration from 1 to 4% (w/v) the Young's modulus increase by factor 5–6 from about 50 kPa to almost 300 kPa. As seen from Fig. 3(a), the Young's modulus of alginate beads decreases with Brij 35 concentration. This decrease is more apparent for higher alginate concentrations. On the other hand, the value of the Young's modulus of alginate beads increases with SDS concentrations for beads containing different alginate concentrations. It is important to note that the Young's modulus for calcium crosslinked pure alginate beads are in the range of 60–300 kPa, which are comparable with the results by Kaklamani et al. reported for 2.5–5% alginate and 1–5 M Ca^{2+} although they manufactured disc-shaped pure alginate hydrogels [13].

The effect of Brij 35 and SDS concentrations on the Young's modulus of alginate beads crosslinked with 3% (w/v) $BaCl_2$ ions is shown in Fig. 4. While the Young's moduli of the beads slightly decrease with Brij 35 concentration, a considerable increase in the Young modulus is observed with increasing SDS concentration. From comparing Fig. 3(b) and Fig. 4(b), the SDS effect on the elastic modules of alginate beads is stronger for Ba-Alginate beads compared to Ca-Alginate beads. The Young's modulus of barium alginate beads containing 50 cmc SDS was approximately two times higher when compared with the modulus of pure beads. It should be noted that there was no spherical barium bead formation for the formulations containing SDS and 1% (w/v) $BaCl_2$. The formed gels were flat and therefore are not included in the measurements.

In order to understand the effect of cation concentration on the beads containing the anionic surfactant SDS, the alginate and SDS concentrations were kept constant (2% and 50 cmc, respectively), whereas the concentrations of both $CaCl_2$ and $BaCl_2$ were varied from 1 to 5% (w/v). The cation effect on Young's modulus of the beads is given in Fig. 5. As seen from Fig. 5, the Young's modulus of alginate beads crosslinked with Ca^{2+} ions decreases slightly with increasing calcium ion concentration, whereas the Young modulus of barium alginate beads increases dramatically with increasing barium ion concentration. Barium alginate gels have larger Young's modulus than calcium alginate ones and this order is the same for formulations containing SDS.

4. Discussion

4.1. Effect of alginate concentration

The strongest effect on the Young's modulus is caused by the change in alginate concentration. The increase in alginate from 1 to 4% leads to an increase in Young's modulus by a factor of about 5–6. The increase in alginate leads to a densification of the beads' mate-

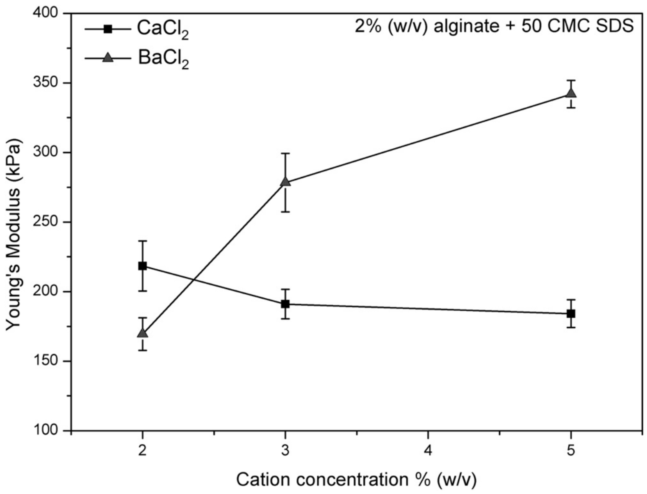

Fig. 5. Effect of cation crosslinker concentration on the Young's modulus of 2% (w/v) alginate beads containing 50 cmc SDS. Error bars indicate standard errors of the mean.

rial. One has to keep in mind that the alginate chains themselves form aggregates via hydrogen bonding.

4.2. Effect of surfactant

The constituents of the alginate copolymer, mannuronic acid and guluronic acid, are acidic monomers having pK_a values 3.2 and 3.6, respectively [18]. However, there is not any literature report about the pK_a value of the copolymer. According to our previous electrophoretic study with alginate polymer, alginate does not gain a noticeable electrophoretic mobility between pH = 3.5 and 8.5. However, when SDS added to the medium above its cmc, alginate gains an electrophoretic mobility like a negatively charged molecule. This behavior showed us an interaction between alginate biopolymer and SDS micelles [19]. Since the interaction is not electrostatic, it can be theoretically expected that the SDS carbon chain and the alginate copolymer chain show this hydrophobic–hydrophobic interaction. In the present study, since the used SDS concentration is well above the cmc, it can be assumed that half micelles are formed along the alginate chains which increase the negative charge density of the alginate/SDS associate. This additional charge offers more adsorption site for the divalent cations and therefore a higher amount of cross-linkers than in the SDS-free gel bead. This increasing amount of cross-linker points increases the stiffness and the Young's modulus. Since the SDS concentrations are well above the cmc, a salt effect of Alginate on the formation of micelles can be neglected.

Brij 35 can interact with the alginate via hydrogen bonding mediated by the head group or via association by the aliphatic chain. This could lead to a reduction in charge of the alginate chain, since potential charges of the alginate chain are covered by nonionic Brij 35 half micelles. The association with Brij 35 would reduce the density of adsorption sites for the divalent cations and therefore the density of cross-linked alginate and the Young's modulus decrease. On the other hand the association via the head group could also hydrophobize the alginate chains, which would lead to an association between hydrophobic domains related to an increase in Young's modulus. Obviously, this influence is minor since the Young's modulus decreases with increasing Brij 35 concentration. Fig. 6 schematizes this discussion.

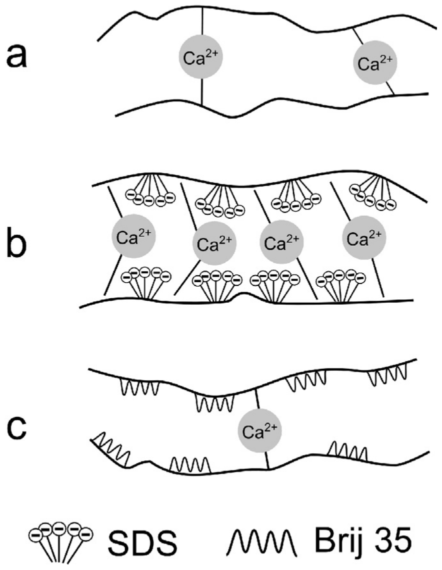

Fig. 6. Scheme of surfactant effect. Calcium ions binds to negative centers on alginate chain (a) Addition of SDS increases the negative charge density and therefore a higher amount of crosslinkers (calcium or barium ions) localizes around the alginate chain. This increased amount of crosslinkers causes an increase in the Young modulus and stiffness (b) Nonionic Brij 35 covers some of negative centers of alginate chains, leading to a decrease in crosslinking ions and consequently gelation. Thus the resistance to being deformed elastically decreases (c).

4.3. Ion specific effects in cross-linking

Since the ionic radius of barium (135 pm) is larger than calcium (99 pm), the hydration shell around Ba^{2+} is smaller and less ordered than for Ca^{2+}.This makes it easier for the negative groups of alginate to interact with Ba^{2+} than to interact with Ca^{2+}. This leads to a stronger cross-linking and therefore a higher Young's modulus in presence of Ba^{2+} than of Ca^{2+}. Of course an increase in cross-linker (Ba^{2+}) increases the density of cross-linking points which increases the Young's modulus of alginate particles. It is also known from literature that the affinity of alginate towards barium is greater than towards calcium [20,21]. This is supposed to result in stronger gel formation in the presence of barium ions. The same order was also observed for barium and calcium alginate microbeads. [20]. It is not really clear why the Young's modulus decreases with increasing Ca^{2+} concentration. Here, we can only speculate. Perhaps the Ca^{2+} acts rather like a salt in the system than as a cross-linker. This means that the Ca^{2+} ions screen the charges in the system, which might reduce the Young's modulus of the particles.

5. Conclusion

The present study shows that the Young's modulus can be easily varied by more order of magnitude by changing the alginate concentration or the type or concentration of the surfactant or the cross-linking divalent cation. The effect of surfactant on mechanical properties of calcium and barium alginate beads was investigated by compression measurements combined with the Hertz Theory. The Young's modulus of alginate beads changes with the type of used surfactant and metal ions. Brij 35 addition decreases the Young's modulus while SDS addition increases the Young's modulus of both calcium and barium alginate beads. This indicates a different type of association of SDS and Brij 35 to the alginate beads due to different charges and the different ability to form hydrogen bonding. In case of SDS only a hydrophobic interaction at low alginate charge can take place, while Brij 35 can associate with the alginate chain via it head group or via the aliphatic chain. The increase in Young's modulus with increasing SDS concentration is considerably more pronounced for barium alginate beads than for calcium alginate beads. Since alginate beads are widely used as drug release agents, more rigid beads can be obtained by the addition of SDS to beads for this purpose. It is assumed that the beads are not in thermodynamic equilibrium and that the mechanical properties depend on the order of the addition of chemical compounds during formulation. It would be interesting in future investigations to study the effect of order on the Young's modulus.

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