Research Article

The Size Stability of Alginate Beads by Different Ionic Crosslinkers

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Few studies have discussed the stability of gelled alginate bead size. Therefore, the present study investigated the dynamic shrinkage of gelled alginate beads affected by two common ionic crosslinkers at different concentrations and temperatures. The results indicate that the gelled alginate beads gradually shrank with longer gelling times. The beads incubated in a Ca\(^{2+}\) solution shrank more dramatically than those incubated in a Ba\(^{2+}\) solution. Those incubated at room temperature exhibited greater shrinkage than those incubated at a low temperature. A 25% size reduction occurred in the 1% Ca\(^{2+}\) solution at room temperature after 300 minutes of gelling time. The alginate beads gelled took at least 120 minutes to become stable after the ionic gelation process.

1. Introduction

Because of easy availability, cost effectiveness, biodegradability, and biocompatibility [1], natural polymers have been used extensively over the past few decades [2–4]. Among them, alginate, a type of polysaccharides composed of 1,4\(^-\) \(\beta\)-d-mannuronic acid (M-block) and \(\alpha\)-l-guluronic acid (G-block), is plentiful in our environment [5, 6]. Alginate was discovered as a structural component of brown marine algae [7]. Until now, alginate has been processed extensively as capsules, beads, and fibers for various application fields, especially in drug delivery [8]. It is suitable for encapsulating targeted drugs because of favorable properties such as water solubility [9], nontoxicity [10], and biodegradability [11].

Alginate particle size is a crucial factor for many applications [12–15]. The bead size is determined by the manufacturing process and alginate properties [16, 17]. Among these properties, alginate's affinity with various divalent ions has a crucial influence on the particle size [18]. Divalent cations such as Ca\(^{2+}\) and Ba\(^{2+}\) are common crosslinkers for producing alginate particles [19]. A previous study indicated that alginate beads gelled in Ba\(^{2+}\) solutions were larger than those gelled in Ca\(^{2+}\) solutions [20]. Another study found that alginate beads incubated in a 10 or 20 mM Ba\(^{2+}\) solution were larger than those incubated in a 50 mM Ca\(^{2+}\) solution [18]. These results show that alginate bead size is easily influenced by these two crosslinkers. However, few studies have compared the dynamic size changes of alginate beads gelled by these two crosslinkers in a single experiment. One study demonstrated that alginate bead sizes incubated in Ca\(^{2+}\) solution decreased continuously over a 30-minute gelling period [21]. Another study reported that a prolonged gelling time did not affect bead size in Ba\(^{2+}\) solution after 2 or 20 minutes [22].

In addition to detecting size changes due to crosslinkers, one study has indicated that the size of the beads diminishes by 8% after being gelled at a high temperature (90°C) [23]. However, another paper claimed that incubation at temperatures between 5°C and 40°C had no significant effect on the bead size [13]. Accordingly, this paper discusses whether bead size is affected by temperature changes.

According to the aforementioned studies, alginate bead size is changed by crosslinkers or temperature. However, until now, the effects of Ca\(^{2+}\) and Ba\(^{2+}\) and temperature on dynamic alginate size have seldom been reported. The present study observed the dynamic shrinkage of alginate beads due to gelling by Ca\(^{2+}\) and Ba\(^{2+}\) at various temperatures.
2. Materials and Methods

2.1. Materials. Sodium alginate powder (A0682, M/G = 69/31, MW = 12,000–80,000) was purchased from Sigma Aldrich Chemical Co., Ltd. (St. Louis, USA), and Coomassie Brilliant Blue G-250 was obtained from One Star Biotechnology Co., Ltd. (Taipei, Taiwan). Barium chloride dihydrate and anhydrous calcium chloride were supplied by Eco Chemical Co., Ltd. (Taichung, Taiwan).

2.2. Preparation of Alginate Beads. Sodium alginate solution was prepared in distilled water at a concentration of 2% (w/v), and Coomassie Brilliant Blue G-250 was added at a concentration of 0.05% (w/v) for observation. For simple and straight comparison, CaCl\(_2\) and BaCl\(_2\) were also prepared by 10% w/v and diluted to 1% w/v, respectively. A disposable Terumo\textregistered syringe (3 mL) was filled with homogenized alginate solution, which was subsequently extruded using a KDS230 syringe pump (KD Scientific Inc., Holliston, USA). Alginate dispersion was then added dropwise into the cuvettes filled with 3 mL of Ca\(^{2+}\) solution and Ba\(^{2+}\) solution at a constant injection rate, enabling calcium and barium ion-crosslinked alginate and the gelled beads to be created uniformly [24] (Figure 1). We examined the beads at various predetermined times (5, 20, 30, 60, 120, 180, 240, and 300 minutes) after initializing the gelling process until reaching the equilibrium size state. This experiment was controlled around the set room temperature (25°C) and low temperature (8°C) and repeated at least three times. Student t-test was used for the statistical analysis.

2.3. Characterization. A digital camera (DP70, Olympus, Taiwan) was employed for imaging to estimate the bead morphology. At a predetermined time, the alginate beads were removed from the crosslinking solution for imaging. To ensure statistical representativeness, three alginate beads were analyzed for each condition. The bead sizes were obtained from recorded photographs and are expressed as the mean ± standard deviation.

3. Results and Discussion

Table 1 shows a series of photographs for four alginate beads gelled by two concentrations (1% w/v and 10% w/v) of two crosslinkers (Ca\(^{2+}\) and Ba\(^{2+}\)) at different gelling times (5, 20, 30, 60, 120, 180, 240, and 300 minutes) at room temperature (25°C). The results indicate that all the alginate beads shrunk with longer gelling times. Furthermore, the bead sizes in the Ca\(^{2+}\) solution were initially larger than those in the Ba\(^{2+}\) solution because the beads incubated in the Ca\(^{2+}\) solution did not form sufficiently tightly and had a lower affinity than the beads incubated in the Ba\(^{2+}\) solution [25, 26]. At 300 minutes, the beads in high concentrations of crosslinkers were larger than those in low concentrations of crosslinkers. By analyzing the statistical results in Table 1, we plotted Figures 2 and 3 for the dynamic size shrinkage of alginate at room temperature. Figures 2 and 3 show the original bead size and normalized size by the initial bead size, respectively.

According to Figure 2, all alginate beads shrunk with longer gelling times, and the beads in the Ca\(^{2+}\) solution were...
Table I: Photographs of alginate beads incubated in different concentrations of Ca\(^{2+}\) and Ba\(^{2+}\) at room temperature (25°C) for various gelling times.

| Time (minutes) | 1% Ca\(^{2+}\) | 10% Ca\(^{2+}\) | 1% Ba\(^{2+}\) | 10% Ba\(^{2+}\) |
|---------------|----------------|----------------|--------------|---------------|
| 5             | ![Image](1.png) | ![Image](2.png) | ![Image](3.png) | ![Image](4.png) |
| 20            | ![Image](5.png) | ![Image](6.png) | ![Image](7.png) | ![Image](8.png) |
| 30            | ![Image](9.png) | ![Image](10.png) | ![Image](11.png) | ![Image](12.png) |
| 60            | ![Image](13.png) | ![Image](14.png) | ![Image](15.png) | ![Image](16.png) |
| 120           | ![Image](17.png) | ![Image](18.png) | ![Image](19.png) | ![Image](20.png) |
| 180           | ![Image](21.png) | ![Image](22.png) | ![Image](23.png) | ![Image](24.png) |
| 240           | ![Image](25.png) | ![Image](26.png) | ![Image](27.png) | ![Image](28.png) |
| 300           | ![Image](29.png) | ![Image](30.png) | ![Image](31.png) | ![Image](32.png) |

To conform to a standard to easily observe the shrinking rates, Figure 3 shows the normalized sizes of the beads obtained at room temperature, thereby demonstrating that the beads incubated in the Ba\(^{2+}\) solution did not shrink more notably than those in the Ca\(^{2+}\) solution. After 60 minutes, the shrinking curves had become smooth, but 1% of the Ca\(^{2+}\) curve required 120 minutes to become smooth. Compared with different concentrations of crosslinkers, the beads in high concentrations of crosslinkers barely shrank.

The final gelled bead sizes in high concentrations were between 0.9 (10% Ca\(^{2+}\)) and 0.93 (10% Ba\(^{2+}\)), and those in low concentrations were between 0.75 (1% Ca\(^{2+}\)) and 0.85 (1% Ba\(^{2+}\)). Therefore, the effect of the Ca\(^{2+}\) concentration on the gelation-shrinkage process was more pronounced than that of the Ba\(^{2+}\) concentration. The gel-forming ability of alginites depends on the G-blocks binding divalent cations [27]. Sufficient divalent cations are present in high concentrations of crosslinkers, leading to the tight formation of gel and low structural rearrangement to reduce the size. A previous
study [18] demonstrated that alginate's affinity toward various divalent ions can decrease in the order of \(\text{Ba}^{2+} > \text{Ca}^{2+}\). Hence, the beads incubated in the \(\text{Ba}^{2+}\) solution were able to form tightly, resulting in minimal shrinkage. The outcome is in accordance with Figure 2.

With the same gelling conditions as those in Table 1, Table 2 shows alginate beads at a low temperature \(8^\circ\text{C}\). The outcomes were similar to those when the experiment was conducted at room temperature; the bead sizes in the \(\text{Ca}^{2+}\) solution were initially larger than those in the \(\text{Ba}^{2+}\) solution. The images in Table 2 were also subjected to a statistical analysis. Figures 4 and 5 show the original and normalized bead sizes at a low temperature, respectively.

Figure 4 demonstrates the same trend as Figure 2. All the curves for the low temperature were much smoother than those for room temperature, indicating that the beads incubated at a low temperature did not shrink substantially, even with a low concentration of \(\text{Ca}^{2+}\). This is because the gelled alginate structure has low rearrangement mobility at low temperatures, resulting in a minor size change.

Figure 5 shows the normalized size shrinkage in \(8^\circ\text{C}\). The results indicate that the final gelled bead sizes were between 0.85 (1% \(\text{Ca}^{2+}\)) and 0.95 (10% \(\text{Ba}^{2+}\)). By contrast, Figure 3 shows that the gelled bead sizes were between 0.75 (1% \(\text{Ca}^{2+}\)) and 0.93 (10% \(\text{Ba}^{2+}\)). It reveals that four groups were significantly different from each other with \(p\) value less than 0.05 in Figure 5. Except groups between 10% \(\text{Ca}^{2+}\) and 1% \(\text{Ba}^{2+}\), the other pairs in Figure 3 also showed significant difference with \(p\) value less than 0.05. The beads incubated...
Table 2: Photographs of alginate beads incubated in different concentrations of Ca\(^{2+}\) and Ba\(^{2+}\) at a low temperature (8°C) for various gelling times.

| Time (minutes) | 1% Ca\(^{2+}\) | 10% Ca\(^{2+}\) | 1% Ba\(^{2+}\) | 10% Ba\(^{2+}\) |
|---------------|----------------|----------------|---------------|----------------|
| 5             | ![Image]       | ![Image]       | ![Image]      | ![Image]       |
| 20            | ![Image]       | ![Image]       | ![Image]      | ![Image]       |
| 30            | ![Image]       | ![Image]       | ![Image]      | ![Image]       |
| 60            | ![Image]       | ![Image]       | ![Image]      | ![Image]       |
| 120           | ![Image]       | ![Image]       | ![Image]      | ![Image]       |
| 180           | ![Image]       | ![Image]       | ![Image]      | ![Image]       |
| 240           | ![Image]       | ![Image]       | ![Image]      | ![Image]       |
| 300           | ![Image]       | ![Image]       | ![Image]      | ![Image]       |

in 1% Ca\(^{2+}\) solution exhibited considerably more evident size changes than those in the other three conditions, because a temperature increase activates motion in water molecules and a higher temperature raises the shrinkage rate of alginate beads. Shrinking is governed by cooperative diffusion of the gel network. The final gelled alginate beads in low crosslinker concentrations had minor mobility restrictions, resulting in major shrinkage compared with those in high crosslinker concentrations.

The shrinkage, tightness, release behaviors, and swelling properties of alginate beads are all relating to each other. Kaygusuz et al. reported metal ion and surfactant effects on the mechanical strength of alginate beads, and they concluded similar trends in bead sizes of pure Ca\(^{2+}\) or Ba\(^{2+}\) alginate by changing alginate concentration [28]. Harper et al. reported effects of various cations on the physical properties of alginate films and found Ba\(^{2+}\) ions produced strong alginate films [29]. Kaygusuz et al. reported cation effects on the slow release from alginate beads [30]. Encapsulation efficiency of model dye in Ba-alginate beads was much higher than that of Ca-alginate and dye release from Ca-alginate beads was much faster. Darrabie et al. reported the effect of...
gelling cation on microbead swelling and concluded that Ca-alginate microbeads were more prone to swelling than the corresponding Ba-alginate beads [31].

4. Conclusion

All alginate beads in this study shrank with longer gelling times. However, the beads incubated in the Ca\(^{2+}\) solution shrank more substantially than those in the Ba\(^{2+}\) solution. The beads incubated at a low temperature exhibited minor size changes compared with those incubated at room temperature, which exhibited dramatic shrinkage such as the 25% size reduction in 1% Ca\(^{2+}\) solution. The results show that the alginate beads gelled stably at least 120 minutes later, thereby providing a guideline for utilizing alginate properties in different fields.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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