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Synthesis of i-carrageenan hydrogels beads with different gelling agents for protein adsorption.

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Abstract

Iota-Carrageenan (i-CAR) hydrogel beads were synthesized to adsorb standard protein bovine serum albumin (BSA). For this purpose, i-CAR was extracted from *Solieria filiformis*, a macroalga abundant on the northeastern coast of Brazil. To do this, i-CAR was dissolved in water at a ratio of 1% (w/v), then the solubilized i-CAR was added to solutions of different gelling agents (Na^+, K^+, Cu^{2+}, NH_4^{2+}, Mg^{2+}, Ca^{2+}, Fe^{3+}e Al^{3+}) at 5% (w/v) to assess the formation of the hydrogel beads. The adsorption experiments were conducted in a batch system using a 0.1 M acetate buffer solution at pH 4.8 and a protein concentration of 1 mg per 0.5 g of adsorbent at 25 °C, for 15, 30, and 60 minutes, in triplicate. Thus, the experiments showed that the most stable and consistent hydrogel beads are those produced from gelling solutions of salts with trivalent cations ($FeCl_3$ and $AlCl_3$), probably due to the greater interaction with i-CAR isomers. In the adsorption experiments, the beads formed with Al^{3+} achieved 80.7% protein adsorption, maintaining consistent performance at all time points analyzed, in addition to maintaining the shape and diameter of the hydrogel unchanged. This stability establishes $AlCl_3$ as the ideal gelling agent for the i-CAR hydrogel beads.

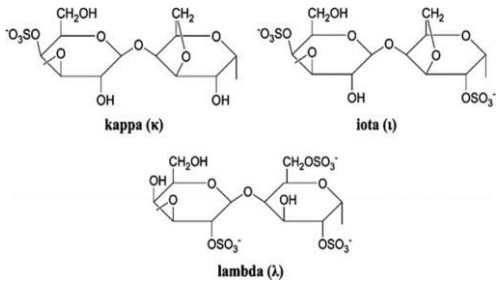
Keywords: Carrageenan, carrageenan hydrogel, protein adsorption;

1. Introduction

Carrageenan (CAR) is a sulfated polysaccharide that can be extracted from red algae and is widely used in the food and pharmaceutical industries due to its thickening, stabilizing and gelling properties. Hydrogels, with their high-water retention capacity and three-dimensional structure, are ideal for the controlled delivery of proteins, essential in pharmaceutical therapies and tissue regeneration. So, the interaction between hydrogels and proteins has been studied in medical treatments, tissue engineering and wound healing [8].

CAR has a variety of isomers, such as kappa (κ), iota (ι) and lambda (λ) carrageenan, which differ from each other in the number of sulphated groups (Fig. 1). These isomers form gels of different consistencies and porosities according to the gelling agents used, which can be cations of metallic origin, originating from chloride and sulfate salts [1]. In this context, this study seeks to synthesize a i-CAR hydrogel and identify the best gelling agent to obtain the hydrogel with the greatest protein adsorption capacity.

Fig.1. Molecular structure of the main types of carrageenan [4].



2. Materials and methods

2.1 Materials

The standard protein used was Bovine Serum Albumin (BSA) was purchased from Inlab (Brazil). The macroalgae *Solieria filiformis* was supplied by the Association of Seaweed Producers of Flecheiras and Guajiru, cultivated on the beaches of Trairi, CE - Brazil. The salts of NaCl, $(NH_4)_2SO_4$, $CuSO_4$, $CaCl_2$, $MgCl_2$ and $FeCl_3$ were obtained from Dinâmica Química Contemporânea® (Brazil), KCl from Mallinckrodt Chemicals® and AlCl from Fluka Analytical® (Brazil).

2.2 Extraction of i-carrageenan

Initially, 2.5 g of dried macroalgae were crushed and stirred in a 0.1 M KOH solution 1:100 (m/v) for 24 h. Then, the polymer was extracted in 100 mL of water at 80°C for 3 h. The resulting supernatant was centrifuged (2400 rpm for 30 min) and precipitated with ethanol in a ratio of 1:4 (v/v). The carrageenan obtained was kept at 4 °C for 24 h, dialyzed for 24 h and subsequently lyophilized.

2.3 Hydrogel production

The hydrogel beads were prepared by dropping a 2% (w/v) solution of i-carrageenan into different 5% (w/v) gelling solutions, following the methodology adapted from Wahba et al. (2021).

2.4 Adsorption experiments

The BSA adsorption experiments were carried out using the methodology adapted from De Oliveira *et al*. (2020). Thus, 0.5 g of hydrogel beads formed from each gelling agent were added to 50 mL Falcon tubes containing 20 mL of a 1 mg/mL BSA solution in acetate buffer (pH 4.8) and placed on a rotary shaker for agitation (model TECNAl-165, Brazil) for 60 minutes, in duplicate. During this time interval, aliquots were removed to evaluate the percentage of adsorbed protein.

The following salts were used as possible gelling agents in order to assess the gelling effect of monovalent, bivalent and trivalent cations: NaCl, KCl, $CuSO_4$, $(NH_4)_2SO_4$, $MgCl_2$, $CaCl_2$, $FeCl_3$ and $AlCl_3$.

2.5 Analytical method

The total amount of protein adsorbed by the hydrogel beads was determined by calculating the difference between the initial protein mass and the residual mass after the contact period at each time. The Bradford method (1976) was used, comparing the results with a standard curve of bovine serum albumin (BSA) to measure the concentration of protein in the medium during the adsorption test.

3. Results and discussion

3.1 Production of hydrogel beads in different gelling agents

The use of different cations as gelling agents interferes with the interactions and crosslinks formed between the carrageenan monomers, thus forming gels with different strengths and porosities [6].

The solutions were separated according to the valence of their cations: Na^+ and K^+, monovalent; Cu^{2+}, NH_4^{2+}, Mg^{2+} and Ca^{2+}, bivalent; Fe^{3+} and Al^{3+}, trivalent. Figure 2 shows the appearance of the beads produced with each gelling agent. The hydrogels formed from the salts of bivalent alkaline earth metals (Mg^{2+} and Ca^{2+}) and trivalent cations (Fe^{3+} and Al^{3+}) were uniform beads with a characteristic shape (Table 1). The beads formed with trivalent cations had a larger average diameter than those produced with trivalent cations, which may be a result of the interactions caused by these atoms, since trivalent cations can promote greater interaction in certain carrageenan monomers with a greater number of SO_3^- groups, such as λ- and ι-carrageenan [3].

On the other hand, those beads obtained from solutions of sulphate salts (Cu^{2+} and NH_4^{2+}) and monovalent cations (Na^+ and K^+) did not have the desired spherical shape, consistency or stability, and disintegrated after stirring in the protein adsorption experiments. Thus, these gels were unviable for protein adsorption applications. Therefore, the cations Cu^{2+}, NH_4^{2+}, Na^+ and K^+ were discarded in subsequent analyses.

The potential of gel-forming agents can also vary between carrageenan monomers. Previous reports have shown the gelling potential of kappa-carrageenan in the presence of monovalent cations [9]. In this work, the biopolymer used was previously identified as ι-carrageenan, a monomer with a greater negative charge than kappa-carrageenan due to the presence of a second SO_3^- group, which probably interferes with the interactions between the monomers and the gelling

agents, which may favor the formation of gels from trivalent cations.

Fig. 2. i-CAR hydrogel spheres from solutions of different gelling agents.

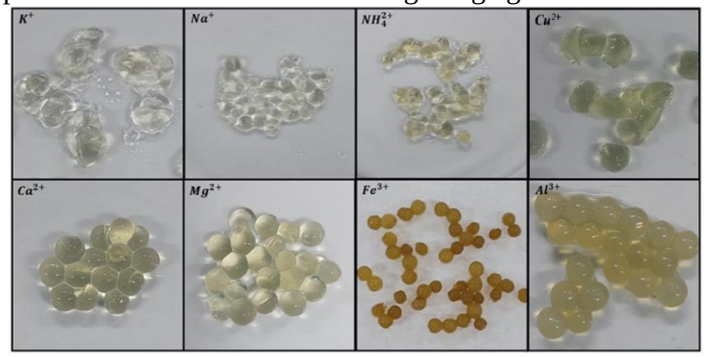


Table. 1. Average diameter of i-CAR spheres for each gelling agent.

Cation	K^+	Na^+	NH_4^{2+}	Cu^{2+}	Mg^{2+}	Ca^{2+}	Fe^{3+}	Al^{3+}
Standard diameter (*mm*)	6.94 ± 0.55	6.05 ± 0.43	3.82 ± 0.10	4.08 ± 0.28	4.87 ± 0.07	4.70 ± 0.05	1.98 ± 0.13	2.78 ± 0.08

3.2 Adsorption experiments of BSA

After selecting the most promising cations, the protein adsorption analysis was carried out. After 60 min of agitation, the adsorbents produced were separated and washed with distilled water and their final appearance is shown in Fig. 3.

All the beads increased in size compared to their initial diameters (Table 3). Those made from Al^{3+} proved to be the most stable with little variation in diameter (8%), while those made from Mg^{2+} and Fe^{3+} had a greater increase in final diameter, of 58% and 113%, respectively, compared to their initial measurements. Regarding the spherical shape, the Mg^{2+} and Ca^{2+} spheres showed greater fragility after agitation, with the Mg^{2+} sphere showing ruptures and breaks after the tests, while the others remained without significant changes in sphericity and resistance.

In the adsorption experiments, the aliquots removed had their absorbance (ABS) and BSA concentration quantified at intervals of 15, 30 and 60 minutes (Figure 4). The total amount of protein absorbed by the hydrogel was obtained by expressing the Bradford standard curve. The adsorbent potential of carrageenan at the evaluated pH (4.8) is due to the isoelectric point of BSA (pI = 4.8), at which the surface charges of the protein are balanced, allowing the interaction between the protein, positively charged, and the biopolymer, negatively charged.

Fig. 3. Adsorbents after 60 minutes of agitation at 25°C in a BSA adsorption test.

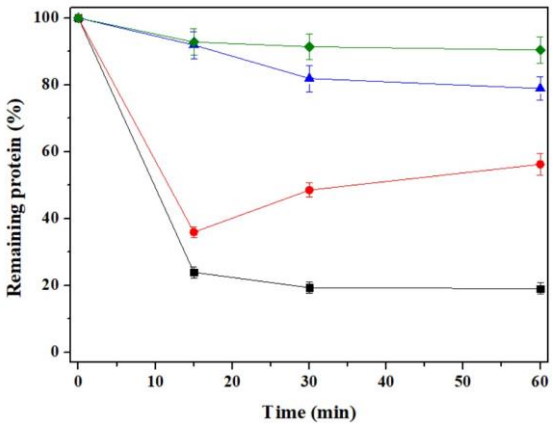
From these results, it can be inferred that the CAR hydrogel, produced from divalent salts, had low protein adsorption, with the Mg^{2+} hydrogel requiring a longer stirring time to reach equilibrium, while the Ca^{2+} hydrogel presented its maximum point and adsorption equilibrium, not exceeding 10% adsorption. These results point to a lower efficiency of divalent cations, which is in line with

reports in the literature for the formation of kappa-carrageenan hydrogels, probably due to the greater number of SO_3^- ions present in i-carrageenan [9], which may have benefited the formation of resistant gels using trivalent cations.

Table. 3. Diameter of hydrogels after 60 min of agitation.

Cation	Average diameter after test (*mm*)	Swelling post adsorption (*mm*)	Expansion (*%*)
Mg^{2+}	7.41	2.71	58%
Ca^{2+}	6.05	1.18	24%
Fe^{3+}	4.23	2.25	114%
Al^{3+}	3.01	0.23	8%

Fig. 4. Adsorbed amount of BSA by each CAR/Cation hydrogel in the 60-minute test at 25°C: (■) i-CAR/Al^{3+}, (●) i-CAR/Fe^{3+}, (▲) i-CAR/Mg^{2+} e i-CAR/Ca^{2+} (♦).



In contrast, the hydrogels originated from trivalent salts showed significant BSA adsorption in the first 15 minutes, Al^{3+} with 80.1% and Fe^{3+} with 64.1%. However, in the subsequent periods (30 and 60 minutes), the i-CAR/Fe^{3+} hydrogel probably led to the desorption of the previously adsorbed protein, resulting in a decrease in its adsorption percentage. In contrast, in the i-CAR/Al^{3+} hydrogel, the adsorption remained stable, having reached equilibrium and high adsorbent potential (80.7%).

4. Conclusion

This study showed the potential of iota-carrageenan extracted from algo *Solieria filiformis* as a hydrogel for the adsorption of BSA. It was concluded that the most suitable gelling agent for the formation of the gel sphere is Al^{3+} (from $AlCl_3$), since, due to its trivalence, it forms more resistant gels with a BSA adsorption capacity of 80%, similar to those observed in the literature for i-carrageenan.

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