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Synthesis and characterization of ι-carrageenan hydrogel beads for BSA adsorption

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Abstract

Iota-Carrageenan (CAR) is a natural sulfated polysaccharide that can be extracted from the *Solieria filiform* seaweed and has the potential for application in the production of negatively charged adsorbents for this application. In this context, this study aims to synthesize a CAR hydrogel and evaluate its protein adsorption potential. Thus, CAR hydrogel was produced using $AlCl_3$ as a gelling agent, and its bovine serum albumin (BSA) adsorption potential was evaluated. The experiments were carried out in a batch system using 0.1M acetate buffer solution at different pH values (4.0, 4.8 and 5.2) at a concentration of 1mg protein/mL of buffer, at 25°C, for 60 minutes in triplicate. The adsorbent was also characterized using FTIR and NMR, and the total protein adsorption was determined using the Bradford method. Therefore, the experiments resulted in 95.8 ± 1.1 % adsorption of the BSA. This result contributes to future studies varying the buffer solution and protein concentration for future applications of this adsorbent in the pharmaceutical and biomedical industries.

Keywords: Carrageenan, Carrageenan-based hydrogels, protein adsorption, carrageenan-matrix hydrogel

1. Introduction

Adsorption is the phenomenon in which molecules from a fluid (gas, vapor, or liquid) spontaneously concentrate on a surface (usually solid) without undergoing a chemical reaction. This process results from interactions caused by unbalanced electrostatic forces on the surface of the adsorbent, which attract molecules present in a fluid such as proteins. This phenomenon is widely used in industrial applications for its potential in the purification, separation, and catalysis of various substances. For example, proteins can be adsorbed onto surfaces at specific pH levels due to their isoelectric potential, making adsorption a widely used methodology in the fields of medicine, fine chemistry, and pharmaceuticals. Protein adsorption on solid compounds may be utilized in biomedicine, chemistry, and pharmacy, where various positively or negatively charged materials can be employed as adsorbent materials. (NOGUEIRA et. al, 2016).

Previous studies have reported the use of these biopolymers as adsorbents for protein recovery, such as chitosan (LONG et al, 2015), agarose (DUMAN et al, 2020), and carrageenan (CHIBATA et al, 1981). In this context, carrageenan (CAR) stands out as a sulfated polysaccharide that can be extracted from the marine algae species *Solieria filiformis*. Sulfated polysaccharides are described as a class of bioactive macromolecules that are negatively charged, primarily due to the substitution of some hydroxyl groups in the carbohydrate residues with sulfate groups.

Carrageenan, which may occur in the form of its monomers, κ-carrageenan, ι-carrageenan, and λ-carrageenan, is used in the food industry to produce gelatin, cheese, canned goods, and sweets, functioning as a gelling, stabilizing, thickening, and emulsifying agent. In the pharmaceutical industry, it is utilized in the manufacture of laxatives, emulsifying agents, and stabilizers for medications (RINAUDO et al, 2008).

This polysaccharide can produce a biodegradable, renewable, biocompatible and non-toxic hydrogel adsorbent, containing one anionic sulfate group per disaccharide unit and produced

from the extraction of red seaweeds. This anionic structure can be used as adsorbent material. (DUMAN et al, 2020)

Bovine serum albumin (BSA) is a commonly studied protein for adsorption essays, due to its pharmaceutical applications, as it is also used to stabilize some enzymes during the digestion of DNA (deoxyribonucleic acid) and to prevent enzymes from adhering to reaction tubes, pipette tips, and other containers (NOGUEIRA et. al, 2016).

In this context, this work aims to study the potential of carrageenan extracted from *S. filiformis* to produce adsorbents for BSA adsorption. Thus, it presents new perspectives on the use of renewable adsorbents in pharmaceutical applications.

2. Material and methods

2.1 Materials

The algae of the species *Solieria filiformis* were gently provided by fishermen of the algae cultivation association of Flecheiras and Guajiru. Potassium hydroxide (KOH) was obtained from Dinâmica Química Contemporânea® in pellet form. Ethyl alcohol (C_2H_5OH) was obtained from Sigma (Brazil). Bovine serum albumin (BSA) was acquired from Inlab (Brazil).

2.2 Carrageenan extraction

The carrageenan was dried in an oven for 24 hours for the carrageenan extraction. Subsequently, 2.5 g was kept in a 1% KOH solution for 24 hours. After filtration, the carrageenan was extracted in distilled water at 80°C for 3 hours under constant. After filtration and centrifugation, the liquid portion was mixed with a 1:4 ethyl alcohol solution for 30 minutes and kept at 4 ºC for 24 hours. Then the precipitated carrageenan was recovered by centrifugation, followed by dialysis and lyophilization.

2.3 Production of carrageenan hydrogel

The hydrogel was produced by dissolving the lyophilized carrageenan in distilled water at a concentration of 1% at 60°C under agitation until complete solubilization. The dissolved solution is then slowly dripped into a 5% (w/v) $AlCl_3$ solution. The beads were kept in contact with the gelling solution for 2h and then rinsed with distilled water.

2.4 Protein Adsorption Assay

For adsorption assays, 1g of carrageenan hydrogel was added in a 50 mL Falcon tube containing 40 mL of a BSA solution (1 mg/mL) in sodium acetate buffer at pH 4.0, 4.8, and 5.2 was added to determine the most favorable pH for the adsorption assay. Subsequently, kinetic assays were conducted in triplicate, and samples were collected at intervals of 5, 10, 15, 30, 45, and 60 minutes.

2.5 Analytical methods

The Bradford method was used to obtain the total protein concentration (BRADFORD, 1976). The Fourier-transform infrared spectroscopy (FTIR) was performed in Cary 630 spectrophotometer (Agilent Technologies) in the range of 650-4000 cm^{-1}, with a resolution of 1 cm^{-1} and 32 scans. The ^{13}C NMR was obtained using Varian Inova 500 11.5 Tesla (300 MHz) at 25 ºC. Dimethyl sulfoxide was used as a solvent.

3. Results and discussion

Carrageenan is a negatively charged biopolymer, which may interact with proteins and present potential for pharmaceutical applications. The carrageenan beads presented high adsorbent potential, which may be observed in Figure 1, by the protein concentration reduction in the medium over the adsorption course.

Figure 1 presents the adsorption course over different pH. In all conditions, it was observed the adsorption of BSA. The equilibrium was achieved in approximately 30 minutes in all essays. However, pH 4.0 presented the higher protein adsorption, presenting 95.8 ± 1.1% of protein retention. This is probably due to the negative charges present in the carrageenan surface (Fig. 2), which may interact with positively charged molecules.

Fig.1: Kinetic adsorption of BSA on ι-carrageenan hydrogels at 25 ºC for 60 minutes in (■) pH 5.2, (●) pH 4.8 e (▲) pH 4.0.

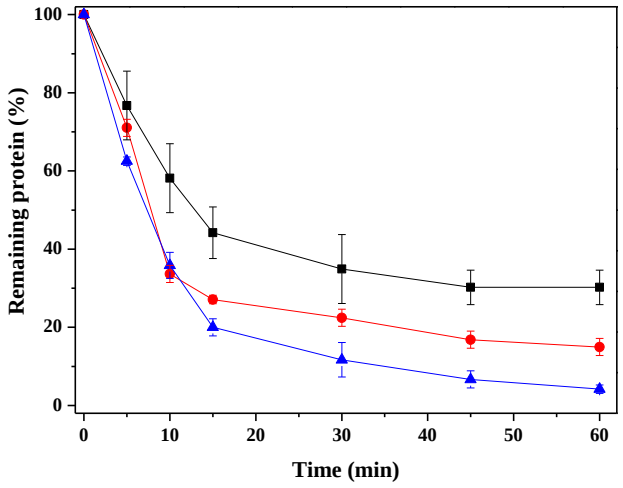
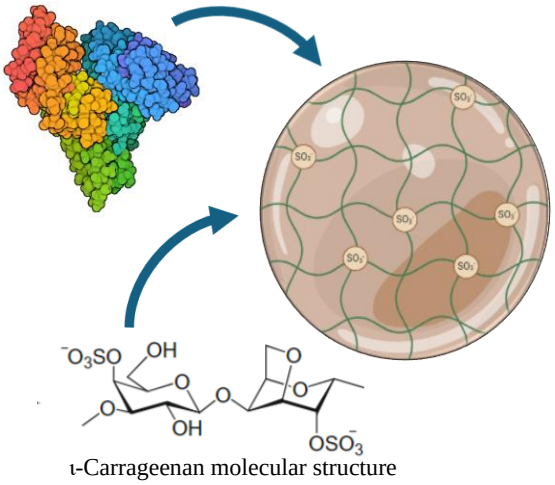


Fig. 2: Conceptual Diagram of Protein Adsorption (Bovine Serum Albumin) on Carrageenan

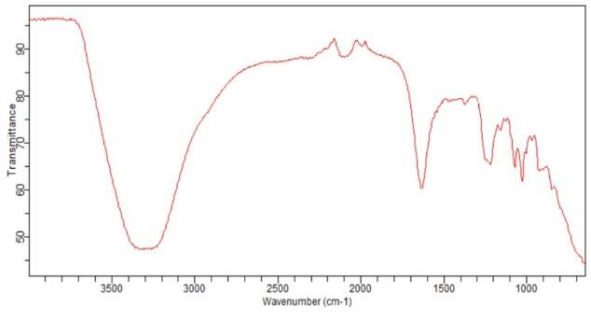


ι-Carrageenan molecular structure

The high adsorption of BSA is due to the interactions between the opposite charges of the protein on the hydrogel surface. The pI of BSA is 4.8. Below the pI the protein is positively charged and above the pI the protein is negatively charged. Thus, the observed result was expected.

This higher amount of adsorbed proteins may also be related to the carrageenan isomer present in the beads, as ι-Carrageenan presents two SO_3^- portions in its monomer, in comparison to κ-carrageenan, which presents only one. The FT-IR analysis presented bands usually related to ι-Carrageenan (Fig. 3)

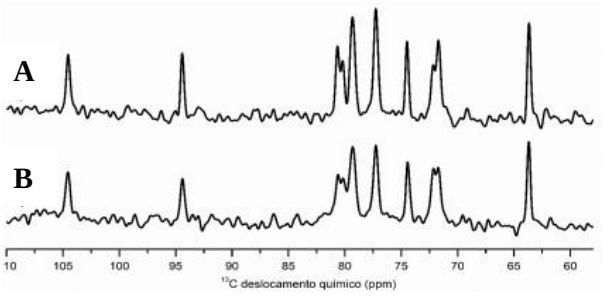
Fig. 3: Fourier Transform Infrared Spectroscopy (FTIR).



These bands, at 815 and 920 cm^{-1}, are associated with the second SO_3^- group in the carrageenan monomer. Also, ι-Carrageenans exhibit gelling potential at low concentrations of water due to the 1C4 conformation of the B unit (3,6-anhydrogalactose), which promotes the formation of a helical structure. Replacing the 3,6-anhydrogalactose with pyranosidic rings, as well as the number, type, and position of sulfate ester groups in the polymer chain, can have detrimental effects on the gelling properties of these hydrocolloids (VAN DE VELDE et al., 2002).

Additionally, the ^{13}C NMR analysis has presented similar peaks to the commercial ι-Carrageenan provided (Figure 4), which corroborates the presence of this isomer in the hydrogel. The presence of this monomer may result in a negatively charged hydrogel, suitable for positively charged protein adsorption.

Fig.4: ^{13}C NMR spectra performed with (A) extracted carrageenan and (B) standard ι-carrageenan.



Conclusion

This study has presented the synthesis and characterization of ι-carrageenan hydrogels for protein adsorption. The hydrogels exhibited high adsorption potential at pH 4.0, with protein retention of 95.8 ± 1.1%. This adsorption efficiency can be attributed to the structural characteristics and negative charges of ι-carrageenan, which favorably interact with positively charged proteins. FTIR and ^{13}C NMR analyses confirmed the presence of ι-carrageenan. These findings indicate that ι-carrageenan hydrogels are promising as sustainable and renewable adsorbent materials in the pharmaceutical and biomedical industries.

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References

[1] NOGUEIRA, K. A.B. Estudo da adsorção de biomoléculas (albumina de soro bovino e lisozima) em materiais mesoporosos. 2016. 65 p. Dissertação (Mestrado em Engenharia Química) – Universidade Federal do Ceará, Fortaleza, 2016.

[2] RINAUDO, M. Main properties and current applications of some polysaccharides as biomaterials. Polymer International, v. 57, p. 397-430, 2008.

[3] VAN DE VELDE, F. and de Ruiter, G.A. (2002) Carrageenan. In: Steinbuchel, A., DeBaets, S. and VanDamme, E.J., Eds., Biopolymers. Polysaccharides II: Polysaccharides from Eukaryotes, Wiley-VCH, Weinheim, Vol. 6, 245-274.

[4] BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, v. 72, p. 248-54, 1976.

[5] DUMAN O, Polat TG, Diker CÖ, Tunç S. Agar/κ-carrageenan composite hydrogel adsorbent for the removal of methylene blue from water. Int J Biol Macromol. 2020; 160:823–35.

[6] CHIBATA I, Tosa T and Sato T, J Chromatogr 215:93 (1981).

[7] LONG, J.; Yu, X.; Xu, E.; Wu, Z.; Xu, X.; Jin, Z.; Jiao, A. In situ synthesis of new magnetite chitosan/carrageenan nanocomposites by electrostatic interactions for protein delivery applications. Carbohydrate Polymers, 2015, 131, 98-10.