International Journal of Biological Macromolecules
Volume 253, Part 8, 31 December 2023, 127494

Preparation and characterization of lysozyme loaded cryogel for heavy metal removal

Birnur Akkaya

Show more

Share Cite

https://doi.org/10.1016/j.ijbiomac.2023.127494
Get rights and content

Abstract

In the present study, monolithic poly(N-isopropylacrylamide-acrylamide)-acrilic acid (poly(npam-aam)-aac) cryogels were made. Swelling tests, SEM, XRD, and ATR-FTIR analyses revealed distinct cryogel and lysozyme-loaded cryogel properties. The equilibrium swelling degree was 6.2 g H_2O/g cryogel. The created poly(npam-aam)-aac with pores of 10–100 μm was obviously seen in SEM images. Lysozyme adsorption capacity on poly(npam-aam)-aac was found to be 260 mg/g at pH 7.4 and 40 °C. After that, we used lysozyme adsorbed cryogel for the removal of the model heavy metal ion (cadmium). A series of pH, duration, and ionic strengths were used to conduct Cd^{2+} adsorption experiments. The results showed that the new adsorbent had a considerable chemical affinity for Cd^{2+} ions in its ability to bind them under eye ocular conditions (pH 7.4, 32–36 °C, 0,15 M NaCl). The traditional Langmuir adsorption model was the most suitable, achieving maximum uptake of ~185 mg/g. Chemical adsorption was found to be the rate-controlling step, and the process was also compatible with the pseudo-second-order model. For the treatment of ocular pathologies, the most effective enzyme, lysozyme, must show its function. That is why there is a need for using lysozyme, and lysozyme is selected as a lignad to adsorb heavy metal ions because of its high heavy metal binding affinity. This material could be used for the treatment of ocular pathologies in the future.

Introduction

The knowledge of the etiological pathways underlying glaucoma is still insufficient, despite recent breakthroughs. Numerous ocular pathologies have been associated with heavy metal toxicity. Because glaucoma shares a pathophysiology, it is conceivable that heavy metal toxicity may contribute to the onset of glaucoma. A person may be exposed to heavy metals at work or through contaminated food or water. Due to the harmful consequences for human health, environmental contamination by heavy metals is a developing problem. Lead (Pb) and cadmium (Cd), the two most prevalent heavy metals, frequently coexist in a contaminated environment [1]. The multiple exposure pathways are another cause for concern regarding these dangerous substances. Contaminated water, lead paint, automobile emissions, industrial emissions, and mining extractions are the most typical sources of contamination. In scientific studies, it was reported that cadmium, arsenic, lead, zinc, and copper increased significantly, while manganese, vanadium, cobalt, nickel, and chromium increased moderately, especially in urban areas [[2], [3], [4], [5], [6], [7]]. It is also known from studies that cadmium ions have an effect on glaucoma [8,9]. Not only glaucoma but also dry eye disease and cataracts are affected by heavy metal toxicity [10,11]. Because the conjunctiva is not protected from the negative impact of microorganisms, eye infections result in a series of consequences that can occasionally result in blindness. This is because lacrimal fluid in ocular diseases contains little lysozyme, a hydrolytic enzyme that typically prevents eye infections [12]. Considering that eye infections and heavy metal accumulation in retina [13,14] toxicity are associated with the development of eye and eye-related diseases, it will be seen that new materials are needed to facilitate the removal of heavy metals from the eyes.

The human antibiotic is an enzyme called lysozyme, which is found in vertebrate cells and secretions. It is often encountered in 'egg white, sweat, tears, ruminant digestive tracts, papaya milk, horse, donkey, and camel milk, insect larvae', and other sources [15]. Because of its beneficial effects, lysozyme is widely employed in a variety of industrial sectors. Lysozyme, a type of mucopolysaccharide lyase, is a nonspecific immunity factor that is present in normal human body fluid and tissue. By breaking down the peptide glycans in the bacteria's cell wall through the use of acetylmuramic acid and *N*-acetyl ammonia in the base glucosamine-(isosorbide-5-Nitrae) glycosidic bond, it causes somatic cell wall destruction and cell rupture. Kill and dissolve the antibiotic. It has numerous pharmacological effects, including antiviral, antibacterial, and antitumor properties. It is also frequently used in pharmacology in anticancer medications, eye drops, and wound healing creams [16,17].

Synthetic materials have the potential to enhance the standard of living for dry eye sufferers, but further research is required to determine their optimal stability, pharmacodynamics, pharmacokinetics, long-term toxicity, and design. Traditional ophthalmic preparations have low medication adherence and a brief pre-corneal residence period. The ability to design intelligent and functionalized nanoscale systems, such as bio-responsive and thermo-responsive hydrogels, with improved corneal surface interaction leads to better mechanical characteristics and precorneal retention, which ultimately improves ocular comfort and compliance [18]. Among the synthetic materials, poly(Nisorpropylacrylamide) (PNiPAAm) as a thermo-responsive polymer and poly(acrylic acid) as a carboxyl group functional polymer have advantages for the corneal epithelium because of their hydroxyl and carboxyl functional groups. Supramolecular hydrogels were useful for ophthalmic applications [[19], [20], [21], [22]]. Due to their poor biodegradability, thermoresponsive polymer systems have a limited range of applications. To increase their application profile, they might be combined with a biodegradable polymer. One of the most prevalent proteins in human tears is lysozyme, and its immobilization will enhance the biodegradability of synthetic materials. In addition, it has a high affinity for heavy metal ions.

For the purification, separation, adsorption, or immobilization of proteins, one of the methods makes use of cryogel monolithic columns. Tris(hydroxymethyl)aminomethane served as the ligand group in the experiment by Verissimo et al. [23]. Erol et al. used poly(HEMA-GMA) and Cu (II) (polyethyleneimine-assisted two-step polymerization) to develop surface imprinted cryogels for lysozyme purification [24]; dye immobilized cryogels were used for lysozyme adsorption and purification [[25], [26], [27], [28]]; Bayramoglu and Arca used cation exchange-modified p(HEMA-GMA) cryogel [29]; Sun et al. prepared supermacroporous cryogel [30]; Liu et al. used tris(hydroxymethyl)aminomethane [31]. Avcbaşı et al. [32] employed hydrophobic albumin-capture cryogels. Cryogels based on polyacrylamide and containing Fe_3O_4 nanoparticles were used by Yao et al. [33] and Erzengin and Odabaşı [34] for the lysozyme adsorption process, respectively. Poly(N-isopropylacrylamide-*co*-acrylic acid) microgels that are responsive to pH and temperature were created by Huo et al. [35] for the purpose of binding albumin. Heavy metal removal is studied by lysozyme immobilized materials [[36], [37], [38]]. Among the materials cryogel will be investigated at the first time.

In this study, lysozyme on poly(npam-aam)-aac macroporous cryogel was investigated. In addition to being sensitive to temperature, pH, and ionic strength, cryogel also has a macroporous structure that has an effect on the adsorption procedure. Cryogel was examined using the swelling test, scanning electron microscopy (SEM), X-ray diffraction (XRD), and an attenuated total reflectance Fourier transform infrared (ATR-FTIR). At 40 °C and pH 7.4 (50 mM phosphate buffer), experiments on lysozyme adsorption were conducted. After that, we used lysozyme adsorbed (loaded) cryogel for heavy metal ion (cadmium) adsorption at the same experimental conditions as adsorbed lysozyme. It was the first time that a new material was used for heavy metal removal for eye treatment. The development of a new lysozyme loaded cryogel to extract heavy metal ions from the eye would therefore be greatly aided by the work done in this area.

Section snippets

Materials

All of the chemicals, including lysozyme (hen egg white lysozyme, lyophilized powder, protein ≥90%, ≥40,000 units/mg protein, Sigma), $CdCl_2$, and the necessary quantity of their salts (Merck, Darmstadt, Germany), were dissolved in water to create the standard solutions of Cd(II) at 1000 mg/L. By diluting the stock solution with water, working solutions were created and monomers, initiator, and TEMED were supplied from Sigma (St. Louis, MO, USA). All other chemicals were reagent-grade and used…

Characterization of cryogel

The prepared cryogel swells in an aqueous solution due to its cross-linked structure. The equilibrium swelling degree was 6.2 g H_2O/g cryogel. An increase in surface area and the presence of ionic functional groups like -COOH may have an influence on the matrix's swelling result.

Fig. 1 demonstrates how the cryogel's macropores range in size from 10 to 100 μm. The simple convective movement of the solution through the cryogel column is made possible by these massive, interconnected pores.…

Conclusion

This study created and characterized poly(npam-aam)-aac cryogels both with and without lysozyme. Lysozyme loaded poly(npam-aam) and poly(npam-aam)-aac cryogels were studied in an aqueous solution. The results showed that the adsorption capacity was improved by introducing acrylic acid into the structure, including the -COOH group. The adsorption procedure differs from the poly(npam-aam) cryogel in that it is influenced by pH, temperature, and ionic strength. In equilibrium, there was 6.2 g H_2…

CRediT authorship contribution statement

I am the single author that I did all of the research.…

Declaration of competing interest

There is not conflict of interest.…

Recommended articles

References (52)

C. Garudadri *et al.*
Prevalence and risk factors for primary glaucomas in adult urban and rural populations in the Andhra Pradesh Eye Disease Study
Ophthalmology (2010)

E. Apeagyei *et al.*
Distribution of heavy metals in road dust along an urban-rural gradient in Massachusetts
Atmos. Environ. (2011)

W.C. Prozialeck *et al.*
The vascular endothelium as a target of cadmium toxicity
Life Sci. (2006)

B. Bocca *et al.*
A pilot study to evaluate the levels of aqueous humor trace elements in open-angle glaucoma
J. Trace Elem. Med. Biol. (2020)

N.K. Wills *et al.*
Cadmium accumulation in the human retina: effects of age, gender, and cellular toxicity
Exp. Eye Res. (2008)

D.K. Sahu *et al.*
Recent trends in nanocarrier based approach in the management of dry eye disease
J. Drug Deliv. Sci. Techol. (2021)

L.J. Luo *et al.*
Long-acting mucoadhesive thermogels for improving topical treatments of dry eye disease
Mater. Sci. Eng. C Mater. Biol. Appl. (2020)

K. Erol *et al.*
Polyethyleneimine assisted-two-step polymerization to develop surface imprinted cryogels for lysozyme purification
Colloids Surf. B: Biointerfaces (2016)

S.S.-S. Wang *et al.*
Dye-affinity nanofibrous membrane for adsorption of lysozyme: preparation and performance evaluation
Food Technol. Biotechnol. (2018)

I.-S. Ng *et al.*
Purification of lysozyme from chicken egg white using nanofiber membrane immobilized with reactive Orange 4 dye

Int. J. Biol. Macromol. (2019)

View more references

Cited by (2)

Dna-Functionalized Cryogel Based Colorimetric Biosensor for Sensitive On-Site Detection of Aflatoxin B1 in Food Samples ↗

2024, SSRN

Preparation of Hydrophobic Cryogel Containing Hydroxyoxime Extractant and Its Extraction Properties of Cu(II) ↗

2024, Gels

View full text