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EFFECT OF QUASI-CRYOGELATION ON ANTIMICROBIAL PROPERTIES OF CERIUM-DOPED BIOPOLYMER FILMS

Dikra Zeid AHMED

Master’s Thesis

Supervisor

Assoc. Prof. Dr. Hakan KAYGUSUZ

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The thesis titled EFFECT OF QUASI-CRYOGELATION ON ANTIMICROBIAL PROPERTIES OF CERIUM-DOPED BIOPOLYMER FILMS prepared by DIKRA ZEID AHMED and submitted on 02/07/2025 has been **accepted unanimously** for the degree of Master of Biomedical Science Program.

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I hereby declare that this thesis meets all format and submission requirements for a Biomedical Science Master's Thesis.

I hereby declare that all information/data presented in this graduation project has been obtained in full accordance with academic rules and ethical conduct. I also declare all unoriginal materials and conclusions have been cited in the text and all references mentioned in the Reference List have been cited in the text, and vice versa as required by the abovementioned rules and conduct.

Dikra Zeid AHMED

Signature

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ABSTRACT

EFFECT OF QUASI-CRYOGELATION ON ANTIMICROBIAL PROPERTIES OF CERIUM-DOPED BIOPOLYMER FILMS

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This study examines how cryogelation affects sodium alginate films crosslinked with cerium nitrate by comparing two types: $AlgCe^{3+}$ and Cryogelated $QuasiAlgCe^{3+}$. The films were prepared by dissolving sodium alginate in water, then crosslinking with cerium nitrate for 12 hours at room temperature, and $QuasiAlgCe^{3+}$, adding a freeze-thaw step at -21°C for 24 hours. FTIR spectroscopy, swelling behavior in different solutions, water vapor transmission rate (WVTR), and UV-Vis absorbance characteristics were used to examine the physical and chemical structure of the films. Swelling tests revealed $QuasiAlgCe^{3+}$ absorbed more water reaching 115% in isotonic sodium chloride solution and 98% in deionized water compared to $AlgCe^{3+}$, and its WVTR was higher at 2614.15 g/day·m² versus $AlgCe^{3+}$'s 1392.94 g/day·m², suggesting a more porous structure. However, when placed in PBS solution, they began to degrade and completely broke down in less than 15 minutes. UV-Vis results show $AlgCe^{3+}$ is clearer above 300 nm, making it better for transparent uses like bandages, while $QuasiAlgCe^{3+}$ blocks more UV light, which is better for protective coating applications. Overall, $QuasiAlgCe^{3+}$ is more suited for moisture-regulating and UV-protective applications, like wound dressing.

Keywords: Alginate, Cerium Nitrate, Wound Dressing, Quasi- cryogelation, WVTR.

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1. INTRODUCTION

The purpose of this chapter is to present this study. This section is divided into five sections, each of which will have extensive details related to the topic at hand. To make things clear, the review's basis is presented in section one, the issue clarification is presented in segment two, the analysis goals are presented in part three, and the review's significance is explained in segment four.

1.1 BACKGROUND

Biopolymers are widely used in a variety of applications due to their higher biodegradability, biomedical applications, and nontoxicity polymers. Ce^{3+}-containing antimicrobial Cryogelation fibers have been discovered. Cerium(III) ions stimulated fibroblast growth, while cerium-doped composites improved osteoblastic cell response in addition to its antibacterial activity. Elements from rare earths rarely have any harmful effects and cannot cross mammalian cell membranes. Wound dressings must have great mechanical qualities, good antimicrobial effects, and most importantly natural content ingredients, Chitosan and cerium ions are renowned for their antibacterial qualities, and alginate-based wound dressings are generally accessible. In the present research, the positive aspects of these materials were put together, and alginate films were subsequently crosslinked with cerium(III) solution and cerium(III) with chitosan incorporated [1].

Alginate films crosslinked with cerium ions and cerium ion-chitosan demonstrated antimicrobial activity toward both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) microbes. The films have been described using structural extending tests, vaporizing water transmittance tests, light transmittance, scanning electron microscopy (SEM), spectroscopy (FTIR), and swelling experiments. Biopolymer films are ideal for dressings for wounds because they are harmless to the body, disintegrate naturally, and can contain antibacterial substances. Seaweed alginate is used in filmmaking and can be combined with ions like cerium to form strong gels. Infections are a major problem with wound healing and medical equipment use, hence we must incorporate bacteria-killing chemicals in these films. Cerium, a rare metal, can destroy bacteria by converting them into two forms and producing deadly molecules that attack inside cells.

Quasi-Cryogelation is a process that could enhance these films' abilities to inhibit organisms [2].

By completely binding the biopolymer a process referred to as quasi-cryogelation fortifies the film and enhances its water absorption capacity. Research indicates that this approach is effective when alginate is mixed with other substances to increase its water-holding capacity. This might make it easier for cerium to come into touch with bacteria. Cerium-linked alginate films are advantageous for dressing wounds because they may inhibit the growth of bacteria This work investigates how quasi-cryogelation affects the antimicrobial properties of cerium-doped biopolymer films made from alginate that has been crosslinked with the element's cerium nitrate. Cerium nitrate, which holds alginate particles intact and promotes bacterial mortality, may work better with the holes created by quasi-cryogelation. By investigating the influence of the technique and cerium on bacteria, this study aims to improve biopolymer films for use in healthcare where infection prevention is vital [3].

Wound dressings speed up the rehabilitation cycle while shielding the injury from external factors and microbes. Work on the application of environmentally friendly substances that utilize biopolymers in wound dressing formulations is still in advancement, despite the abundance of lucrative options available on the market. Biopolymers more specifically, polysaccharides and polysaccharide derivatives are of special importance as they possess the potential for improved tissue adhesion due to hydrogen bonding between polysaccharides and protein structures. Those substances have high structural and water vapor transmission properties, but they can also develop antibacterial properties when mixed with various dopants [3].

Wound dressings must have strong mechanical properties, good antimicrobial properties, and above all natural material ingredients. Cerium ions and chitosan have been shown to have antibacterial qualities, and alginate-based dressings for wounds are commercially available. In this investigation, the advantages of both materials were integrated, and alginate cellulose sheets were combined using cerium(III) liquid and cerium(III) solution with chitosan applied. The polymers were evaluated utilizing expansion studies mechanical stretching tests, water vapor transmittance testing, light transmittance, scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR). The physical and antibacterial properties of the films were compared to those of conventional calcium alginate films. A substance crosslinker is used in their investigation to crosslink the biopolymers, necessitating an extra creation step. Another Alg/CMC-based superabsorbent has been described by El-Naggar. N, N-Methylene Bisacrylamide (MBAM) was employed as a crosslinker in the investigation, and γ illumination was used to start the crosslinking process [4].

Foam-based dressing for wound substances, made by scattering gas components in a polymeric substance, are soft, conform to the curves of the human body, and absorb wound exudate because of their porous nature. Many of these combinations are made of synthetic materials like polyurethane. Yet, biopolymers have appeared as a new player because of their biological compatibility and endurance. Biopolymers are increasingly being used in formulations in the field of science which involves bactericidal nanoparticles made of cerium oxide with a biopolymer composition [4].

Despite their many benefits, chitosan-based films are often fragile. To make films, chitosan must be combined with natural polymers that have strong film-forming properties. Because of their exceptional film-making capabilities, liquid cellulose replacements are ideal candidates for composite films. Using alternatives to cellulose improves the hydrophilia and elasticity of chitosan. Polyethylene glycol (PEG) is an additive substance that improves the properties of polymeric films, similar to cellulose counterparts. By reducing friction between polymer chains, PEG improves the mechanical and thermal endurance of polymer films. PEG and cellulose-derived compounds are both safe, agreeable, and renewable, much like chitosan [5]

Cerium ion-crosslinked alginate films have been shown to have antibacterial properties. Interest in this field of study has grown because of recent studies showing that nano cerium oxide particles possess antibacterial properties due to the reversible transition from the two valence states of Ce (III)/Ce (IV)]. Within an examination that looked at CeO_2 NPs' antibacterial approach against Escherichia coli (E. coli), the NPs were stronger than their majority forms [5].

Biopolymer films, which are generated using organic polymers like chitosan and alginate, have shown promise due in part to their inherent antibacterial properties, biocompatibility, and biodegradability. Cerium, a rare-earth component with antibacterial and anti-inflammatory properties, has been utilized for enhancing the antimicrobial performance of these bio-polymers matrices as the operational and structure. Characteristics of such films, such as porosity, durability, and ion release behaviour, can have an important influence on their efficacy towards infectious agents.

This process normally produces porosity designs because a solvent, commonly water, solidifies and then ice crystals are removed. In contrast to regular cryogelation, "quasi" suggests a variant or complete approach that would not exactly reflect established cryogenic temperatures or involve extra processes [6].

In this situation, biopolymer films are mainly comprised of native monomers like cellulose, alginate, or chitosan, which are commonly utilized in biomedical applications because of their degradation and biocompatibility. Utilizing activities like the development of reactive oxygen species (ROS) or direct disruption of bacterial barriers, and cerium supplementation inserts cerium ions (such as Ce^{3+} or Ce^{4+}) or cerium oxide nanostructures to the biopolymer structure, potentially increasing attributes like antibacterial activity. The antimicrobial capabilities of these materials are essential for applications such as wound dressings, surgical instruments, and even food containers in which they are necessary to prevent bacterial growth (e.g., against Staphylococcus aureus or Escherichia coli). The issue at hand is how quasi-cryogelation affects these characteristics in comparison to conventional film manufacturing techniques (such as casting or liquid dissolving [6].

The description aims to investigate quasi-cryogelation impacts on the antimicrobial abilities of cerium-doped biopolymer films, with a focus on the interplay among processing variables, cerium success, and microbial resistance. By carefully evaluating these factors, the research investigation seeks to provide insights into the development of advanced antimicrobial polymers with enhanced efficacy and usability. Wound dressings need to be antimicrobial, have durability, and contain organic substances. Cerium ions have known antimicrobial characteristics, and alginate-based wound dressings are marketed clinically. This study integrated the benefits of these materials by crosslinking alginate sheets with cerium(III) solvent and adding. Films were investigated using FTIR, light including transmittance, SEM, swelling, water vapor its transmittance and bending tests. The films' antibacterial and chemical compounds were evaluated against standard calcium alginate films. To improve the durability, adaptability, and permeability of biopolymer films, the process of cryogelation which involves reducing temperature and then thawing to generate porous gel frameworks has been thoroughly investigated. Cryogelation a modified technique that uses partial or controlled freezing conditions, provides a good alternative to standard cryogelation by allowing for increased control across the microscopic composition of the film and minimizing damage to dependent substances like cerium particles or nanoparticles. Despite these advancements, the

the precise impact of quasi-cryogelation on the antibacterial properties of cerium-doped biopolymer films remains unknown [7].

The antibacterial effectiveness of cerium-doped biopolymer films is mostly due to the generation of oxygen-releasing complexes (ROS) by Cerium's redox activity, which cycles between Ce^{3+} and Ce^{4+} states. These ROS can harm bacterial cell walls and halt the growth of microbes. However, the freezing and thawing cycles in quasi-cryogelation may alter the position, peace of mind, and release kinetics of cerium throughout the biopolymer matrix-like, which might influence the biopolymer matrix's interaction with microbial pathogens. Modifications in the film's porosity and surface shape caused by quasi-cryogelation may also influence the overall antibacterial efficacy. These modifications may also have a benefit on bacterial adhesion and cerium's permeability to target microorganisms [7].

There is evidence that cryogelation generally enhances the properties of biopolymer materials, yet "quasi-cryogelation" of cerium-doped biopolymer films. For instance, cryogels constructed from silk fibers or synthetic polymers have higher mechanical durability and permeation, which, if cerium has been added, could outcome in better antibacterial properties. The results of a "quasi" procedure may not be as evident as those obtained with full cryogelation, but they are still noteworthy when compared to films that were not managed. Biopolymer Abilities: One of the many biopolymers with intrinsic antibacterial characteristics is chitosan. By releasing additional functionality groups (such as the carboxy groups in chitosan) that engage with microbial cellular walls and lessen the impacts of cerium, quasi-cryogelation may be beneficial. By improving the morphology of the film in this way, the interaction between the biopolymer and cerium may increase [8].

2. LITERATURE REVIEW

2.1 GENERAL INFORMATION

Wound dressings require good antibacterial properties, physical durability, and, most significantly, organic ingredients. The work aims to demonstrate that an Alg/CMC superabsorbent can be created while using toxic substances for chemical alteration of the polymer chain. To do this, a green technology termed quasi-cryogelation was used. Quasi-cryogelation involves an entire ionic cross-link preceding freezing, like cryogelation, which happens under semi-frozen circumstances. This process involves the ionic (Ca^{2+}) cross-linking of alginate to form gels [9].

Crosslinker used for alginate precipitation. However, different metal ions give alginate polymers varied properties. For instance, they increase the gels' attraction to specific pollutants, enabling the development of novel adsorbents. Cerium(III) ions increased the growth of fibroblasts whilst cerium-enriched polymers improved osteoblastic cell response, additionally to their bactericidal capabilities. Because elements from rare earths cannot get through human cell membranes, adverse consequences are uncertain. To generate new, degradable films that can be used as dressings for wounds, this work aims to combine the water-friendly and flexible properties of alginate films with the antibacterial properties of Ce^{3+} ions and chitosan. The characteristics of alginate films cross-linked with chitosan and cerium ions have been related [10].

The antibacterial effectiveness of cerium-doped biopolymer films is mostly due to the antioxidant function of cerium (cycling between Ce^{3+} and Ce^{4+} states), which generates oxygen-producing substances (ROS) that will harm the cell walls of bacteria and stop microbial development. However, the repeated thawing and freezing cycles in quasi-cryogelation may alter the order, strength, and release kinetics of the element's cerium throughout the biopolymer matrix, which can affect the biopolymer matrix's interactions with microbial pathogens. Furthermore, modifications to the film's permeability and structure of the surface caused by quasi-cryogelation may have an impact on the overall antibacterial efficacy. These changes may also influence bacterial adhesion and cerium's availability to target bacteria [11].

Cerium is a chemical component. When confronted with air, this silvery-white, flexible, and supple metal harms. The second component in the lanthanide succession, cerium, has a stable +4 condition that does not oxidize water, even though it frequently exhibits the series' typical +3 oxidation state. It is regarded as a rare-earth element. Except for prolonged or severe exposure to sunlight, cerium is not highly poisonous and has no recognized biological function in humans. Furthermore, Cerium ions are known to have antibacterial properties, and alginate-based wound dressings are readily accessible in commerce. In this study, the advantages of both substances were integrated, and polyamide films were crosslinked using cerium(III) water and cerium(III) solution with chitosan applied. The films were characterized using mechanical stretching tests, water vapor absorbance examination, light transmittance, scan electron microscopy (SEM), infrared spectroscopy with the Fourier transform (FTIR), and swelling studies. The microbiological and physical properties of the films were compared to those of conventional calcium alginate films. The antibacterial qualities of cerium ion crosslinked, and cerium ion-chitosan-related alginate films were effective against bacteria of all types [12].

To improve the antibacterial, thermal, and mechanical properties of the composite material, nanosized cerium oxide was added to the polymer composite, which was established by the solvent-casting method. The films were described using thermogravimetric, examining electron microscopy with energy-dispersive X-ray spectroscopy, mechanical, ultraviolet-visible light transmittance, water solubility, pH, moisture content, water vapor transmission rate, swelling, and antibacterial tests. The films' physical and antibacterial qualities are encouraging for additional research as a possible wound-covering material. The polymer compound was generated with solvent casting. Water did not degrade any of the created films. The films were treated with micron-sized cerium oxide to increase their mechanical, thermal, and antibacterial abilities. The films were tested for mechanical, thermogravimetric, microscopy with scanning electron microscopy, energy-dispersive X-ray spectrum analysis, insoluble in water, pH, moisture content, water vapor transfer rate, swelling, and antibacterial qualities. The chemical and physical bactericidal features of the films seem promising for the future. As a result, swelling reduces along the polymer chain. Under contrary circumstances, -COOH groups dissociate and swelling rises due to negative charge aversion and hydrogel development. Crosslinked alginate chains transform into the compound sodium alginate through the process of ion exchange, which absorbs a large amount of water [12].

Metal oxide nanoparticles (NPs) can be added to polymer films to enhance their chemical composition and confer antibacterial properties, or they can significantly increase the biological activity of antibacterial films such as chitosan. Although studies on adding silver and zinc oxide NPs to chitosan-based films have recently increased, such experiments for cerium oxide NPs are rare. Ceramic nanoparticles have purportedly been incorporated into PVA films for optical applications. CeO_2^-NPs have been incorporated into chitosan-cellulose acetate films, chitosan-PVA films, and polycaprolactone (PCL)-gelatin nanofibers to produce wound dressing material [13].

It is widely acknowledged that for skin-regenerating and wound-healing dressings to be used in healthcare settings, some properties are required, such as biologic reliability, nontoxicity, porosity, and air and condensate flexibility. They also need to have an antibacterial effect, absorb waste products, provide humid conditions on the wound surface, and replicate the structure of the extracellular matrix. Hydrogels are substances and microfibers composed of synthetic and natural polymers modified by nanoparticles that exhibit these properties. Hydrogels create an atmosphere of moisture and have a cooling effect that makes sufferers feel less uncomfortable than gauze and roller dressings. A good substitute for antibiotic medications is microbial hydrogels, which are soft biomaterials that can eradicate germs without producing toxicity or other effects. The monomers, polymers, or biological components can be inherited for producing antifungal hydrogels. Including biopolymers in compositions has attracted scientific interest because of their biological properties and sustainability. Most of these compositions depend on artificial substances like polyurethane, but biopolymers have emerged as an emerging force when it comes partly because of their structure of pores that allows them to absorb wound liquid [13].

Metallic nanoparticles and oxide-based dressings show promise as wound-healing materials. The matrix for such dressings is composed of biocompatible and nontoxic hydrophilic polymers. In this review, we look at the antibacterial properties of nanoparticle-modified wound dressings versus a variety of infections, as well as their abilities to heal.

On the other hand, cerium enrichment can offer the biological polymer film antibacterial capabilities. When these ingredients combine, a biopolymer film with enhanced antibacterial activity may develop. Quasi-cryogelation is a method of freezing and then thawing the biopolymer liquid. This could lead to the formation of a structure that is porous with a bigger area of coverage. This greater surface area may boost the biopolymer film's capacity to interact with microbes, hence strengthening its ability to combat bacteria. Biopolymer-based wound dressings are appealing due to their sustainable characteristics and diverse possibilities for customization. The design of PVA-CMC-CeO_2 foam dressings results in a bactericidal and biodegradable compound that has excellent swelling and delivery of medications properties, making them acceptable for clinical use. The permeable composition of the foam gels was assessed using SEM. This innovative product can be used for a variety of applications, including direct drugs. Quasi-cryogelation can significantly enhance the antimicrobial activity of cerium-doped biopolymer films. The biopolymer film has a structure with pores due to quasi-cryogelation. This enhanced porosity also allows for greater absorption of cerium ions, which are known to have antibacterial characteristics. Improved Structural Strength: The biopolymer film is reinforced through quasi-cryogelation, which boosts its resilience to deterioration while also allowing for more effective cerium ion release and increased surface area. The porous nature of the quasi-cryogelation increases the area of the film's outer layer, allowing cerium ions to interact with bacteria more frequently. All of these variables have resulted in better antibacterial abilities for cerium-doped biopolymer films created using quasi-cryogelation. The films are suitable for use in food presentation, wound dressings, and several other medical applications since they significantly block the development of bacteria and fungi [14].

In contrast, the incorporation of bioactive nanoparticles into wound dressings has most recently given ensuring options in wound medication, with the benefit of considerably minimizing the quantity of antibiotics The element cerium oxide nanoparticles (CeO_2 NPs) are unique substances with powerful antioxidant and antimicrobial activity [10] the characteristics due to the permanent transformation between two valence states (Ce(III)/Ce(IV)). The redox activity of CeO_2 NPs due to oxygen vacancies by surface defects has given it numerous biological characteristics, such as anti-allergic and antioxidant. Biopolymeric hydrogels also exhibited wound-infection-desired biological reactions. The films are appropriate for use in packaging for food, wound dressings, and various other biomedical disciplines since they can successfully stop the growth of germs and fungi. Modifications in Structure and Mechanics Features: Quasi-cryogelation may alter the films' tensile strength or flexibility, which may have an indirect impact on their antibacterial efficacy. For example, a more resilient porous structure could remain intact under damp conditions (such as in wound dressings), guaranteeing continuous bacterial interaction and steady exposure to cerium [14].

The element cerium may be added to the biopolymer film to give it antibacterial properties. The form of biopolymer used, the elements cerium satisfied, and the quasi-cryogelation method parameters are only just some of the elements that will define the specific effect of cerium enrichment and quasi-cryogelation on the antimicrobial properties of the biologic film. The films are appropriate for use in food appearance, wound dressings, and various other uses in medicine since they greatly prevent the growth of microorganisms and viruses considering their advantageous qualities, including non-toxicity and biological compatibility, biopolymers are utilized in a wide range of biomedical fields. To date, it has proven especially appealing for applications involving wound healing. Materials with qualities appropriate for wound healing can be used to customize it. Multiple substances for wound dressings, including hydrogels, films, wafers, foams, nanofibers, and topical medications, have been made using alginate. Alginate-based wound dressings can reduce bacterial infections at the wound site, absorb excess wound fluid, and keep the surroundings physiologically moist. The proportion of other polymers combined with alginate, the type of cross-linkers applied, the crosslinking period, the type of additives used, the addition of nanoparticles, and antimicrobial substances all affect how effective these wound dressings are as treatments [15].

3. MATERIAL AND METHODOLOGY

3.1 MATERIAL

Alginic acid sodium salt (viscosity 2% solution), and cerium (III) nitrate hexahydrate were acquired from Sigma-Aldrich. Deionized water was used to prepare solutions from the Reptile-Direct Pure Up system.

3.2 METHODOLOGY

3.2.1 Preparation of Alginate Film

0.2 g of Alginate was dissolved in 20 mL of deionized water to give a 1% (w/v) solution under constant stirring at 40°C using a magnetic stirrer until a homogeneous solution is obtained. The mixture of 20 mL volume was poured into Petri dishes and dried at room temperature 25°C for 24 hours. After the drying step, transparent sodium alginate films were obtained.

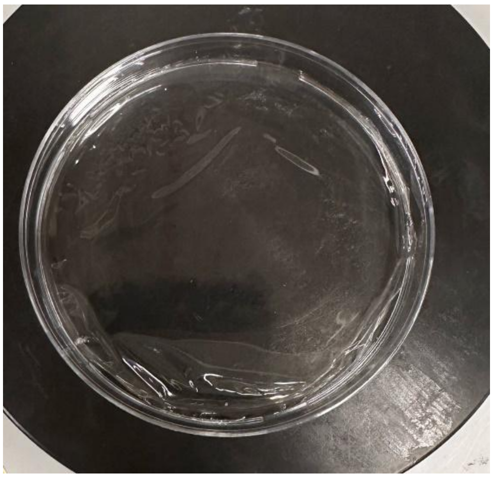


Figure 3.1: Alginate Film.

3.2.2 Preparation of Cerium Nitrate Crosslinking Solution

4.34 g of $Ce(NO_3)_3 \cdot 6H_2O$ was dissolved in 50 mL of deionized water and stirred until fully dissolved. 40 mL of mixture was poured into the alginate film for 12 hours at room temperature (25°C) to allow crosslinking. After 12 hours, the excess of cerium nitrate solution was removed and the film was rinsed gently with deionized water and air-dried for 24 hours. The film is named Alg Ce^{3+}.

Quasi-Cryogelated Films: Alginate film was prepared as shown above. After removing the excess cerium nitrate solution and rinsing the film. The film was kept in the freezer at -21 °C for 24 hours. Later the film was taken out of the freezer to thaw and dry at room temperature. These samples are named as QuasiAlg Ce^{3+}.

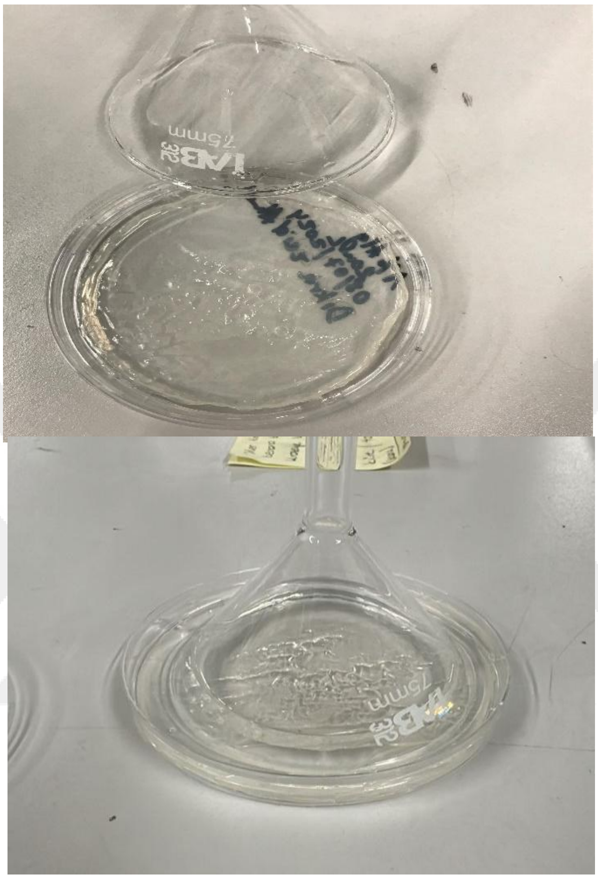


Figure 3.2: Cerium Nitrate Crosslinking Solution.

3.2.3 Characterization of The Films

The optical transmittance of the film was analyzed using a Shimadzu UV-1280 UV-VIS spectrometer (Kyoto, Japan). For the measurement, the film was cut into a rectangular piece and placed in the sample holder. Transmittance data were recorded over a wavelength range of 200–800 nm. Fourier Transform Infrared (FTIR) spectroscopy was performed on the dried film using a Shimadzu IRAffinity-1S infrared spectrometer (Kyoto, Japan) to analyze its molecular structure.

3.2.4 Swelling and Water Vapour Transmission Rates (WVTR) Tests

The swelling degree of the film was measured gravimetrically. The swelling test is completed in pure water, 0.9% of the isotonic solution, and citrate-phosphate buffer saline (Citrate-PBS) at pH 5.5.

$AlgCe^{3+}$ And $QuasiAlgCe^{3+}$ films were separately taken at 0.1 g sample of alginate film was submerged in swelling solutions and incubated in a Nüve ST-30 water-bath (Ankara, Turkey) at 37°C with 70 rpm shaking. At set intervals, the films were removed, filtered, and gently dried with filter paper to remove excess solution. The films were then weighed and returned to the solution. This process was repeated three times for each film type. The percentage of swelling was calculated using the provided

$$\text{Swelling (\%)} = \frac{Ws - Wd}{Wd} \times 100 \tag{3.1}$$

Where W_s and W_d define the Weight of the swollen film and the Initial dry weight of the film, respectively. The water permeability of the films was assessed using a modified form of the ASTM E96-96M "Desiccant Method." For the WVTR test, a vial filled with clean water was positioned so that the water level was 2 cm below the vial's neck. Films were placed over the vial opening and sealed securely. The test was conducted at a constant temperature of 37 °C and a qualified humidity. Over six hours, the mass of the vial was measured every hour. The loss in mass, attributed to water evaporation through the film, was recorded over time. The film area presented for vapor transmission was 0.000125 m^2. The water vapor transmission rate (WVTR) was then calculated in g/day·m^2 using the

$$WVTR = m/a \tag{3.2}$$

Where m is the slope of the weight change-time curve obtained by simple linear regression investigation and A is the superficial area of the film.

Figure 3.3: Swelling Tests.

Figure 3.4: Water Vapor Transmission Rate Test.

3.2.5 Antibacterial Activity

The antibacterial performance of the film samples was evaluated according to ISO 22196 by quantifying the number of viable bacteria after 2 hours of direct contact at 35 °C. A plain polypropylene film was used as the control. Prior to testing, the films (50 × 50 mm) were sterilized using ultraviolet irradiation and placed in sterile Petri dishes.

Bacterial suspensions of Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 6538) were prepared in 1/500 diluted nutrient broth (Liofilchem, Roseto degli Abruzzi, Italy) to obtain a final concentration of approximately 5×10^6 CFU/mL. A 200 µL aliquot of each suspension was pipetted onto the surface of the test films, which were then covered with sterilized plain polypropylene films of smaller dimensions (40 × 40 mm) to ensure uniform contact.

The samples were incubated at 35 °C under approximately 90% relative humidity. Following the incubation period, the number of surviving bacteria was determined using the plate count method. This involved serial dilution of the bacterial suspension recovered from the film surfaces, followed by plating onto tryptic soy agar (Liofilchem). The antibacterial activity was evaluated based on the bacterial count results, which were expressed as log CFU per sample.

4. RESULTS

4.1 CHARACTERIZATION OF THE FILMS

Differences in $AlgCe^{3+}$ and $QuasiAlgCe^{3+}$ films' FTIR spectra, shown in Figure 4.1 are observed over the range 600–3600 cm^{-1}. For $AlgCe^{3+}$, a broad peak at 3200–3400 cm^{-1} identifies O-H stretching from hydroxyl groups of alginates, and peaks at 1600 cm^{-1} and 1400 cm^{-1} indicate COO^- stretching confirming alginate's structure. Moreover, a sharp peak at 2100 cm^{-1} indicates some nitrate from crosslinking, which is most likely leftover after construction.

Its O-H peak at 3200 cm^{-1} was more pronounced in comparison to $QuasiAlgCe^{3+}$, presumably due to the freeze-thaw process affecting hydrogen bonds. COO^- peaks at 1600 cm^{-1} and 1400 cm^{-1} were stronger, suggesting better crosslinking after cryogelation. Possibly as a result of being trapped during freezing, the nitrate peak at 2100 cm^{-1} was more prominent as well.

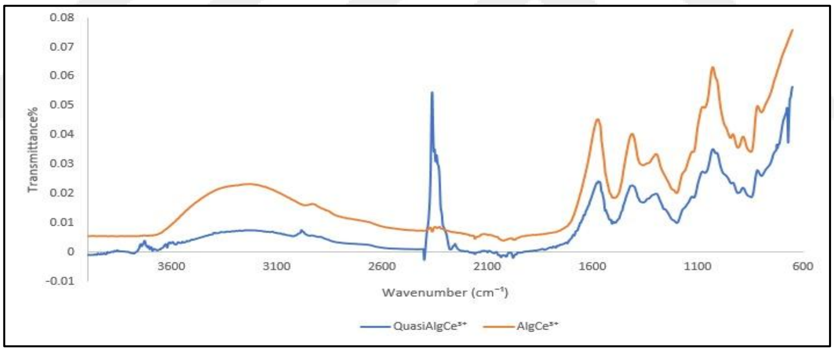


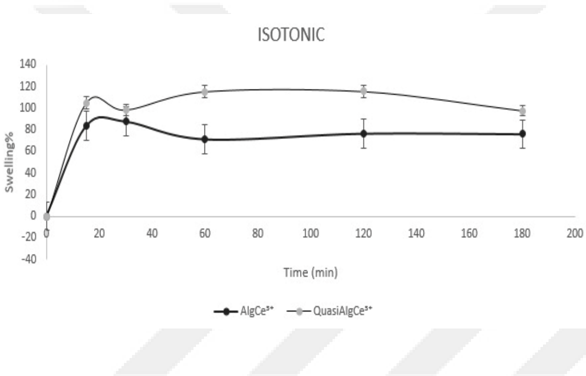
Figure 4.1: FTIR Spectra of the Sample.

4.2 SWELLING AND WATER VAPOR TRANSMISSION RATES (WVTR) RESULTS

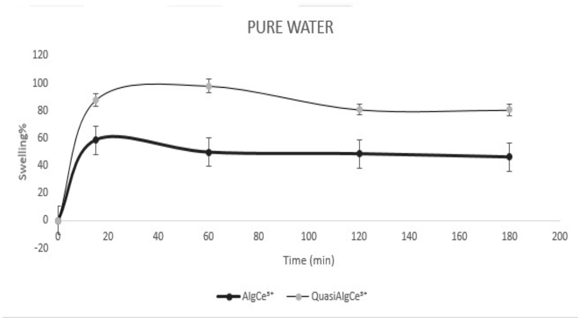
The films were immersed in deionized water and 0.9% isotonic solution for 4 hours in a shaking water bath at 37°C to reach their maximum swelling capacity. The $QuasiAlgCe^{3+}$ films reached their highest swelling—98% in pure water and 115% in an isotonic solution, as shown in Fig. However, when placed in PBS solution, they began to degrade and completely broke down in less than 15 minutes.

For the wound to heal naturally, the materials used for wound dressings must control the amount of moisture that escapes the wound. The water vapor transmission rate (WVTR) varies from 204 g/m²/day for skin that is healthy to 278 g/m² /Day for wounds or other harm and from 5138 g/m²/day for injuries that are separating. In 2000–2500 g/m² /day of water vapor transmission, there would be enough moisture present to avoid severe dehydration and encourage efficient wound healing [17]. QuasiAlgCe^{3+} films had a WVTR value of 2614,15 g/m²/day. Overall, the WVTR values in these samples are within the optimal range for keeping the wound moist.

(a)



(b)



(c)

Figure 4.2: Swelling Graphs of Films in (a) Isotonic (0.9%) Solution (b) Pure Water (c) Degradation of Swelling test Treatment in PBS Solution (Ph5.5).

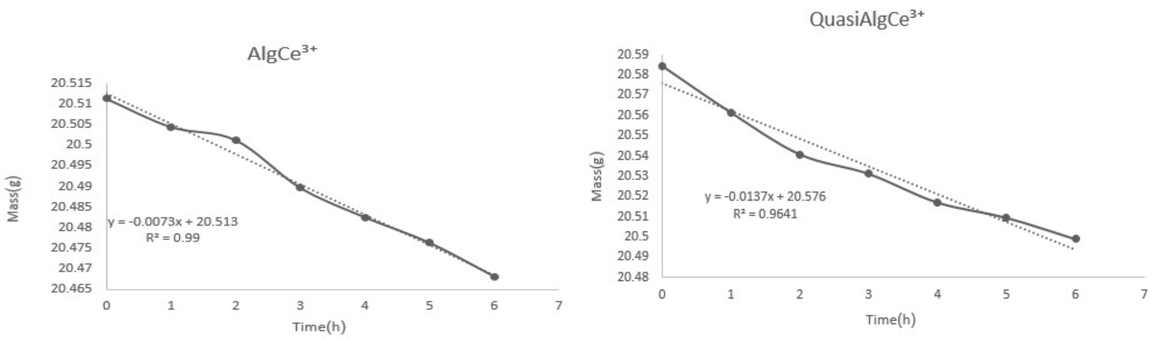


Figure 4.3: Graph of Water Vapor Transmission Ride.

4.3 UV-VIS TRANSMITTANCE OF THE FILMS

The UV-Vis spectra of QuasiAlgCe^{3+} and AlgCe^{3+} (Figure 4.4) demonstrate the amount of light absorbed by each film between 200 and 800 nm. With a large absorbance at 220 nm (about 1.6), AlgCe^{3+} blocks a significant amount of UV light there. However, it drops to nearly zero by 300 nm and remains low, allowing most light to pass through after that. With a tiny bump at 300 nm and a drop to almost zero by 400 nm, quasiAlgCe^{3+} likewise at 220 nm but much higher (around 4.0), demonstrating that it blocks even more UV light. Above 300 nm, AlgCe^{3+} is more transparent than QuasiAlgCe^{3+} because higher absorbance means less light passes through. The cryogelation process is probably what gives QuasiAlgCe^{3+} its stronger UV-blocking.

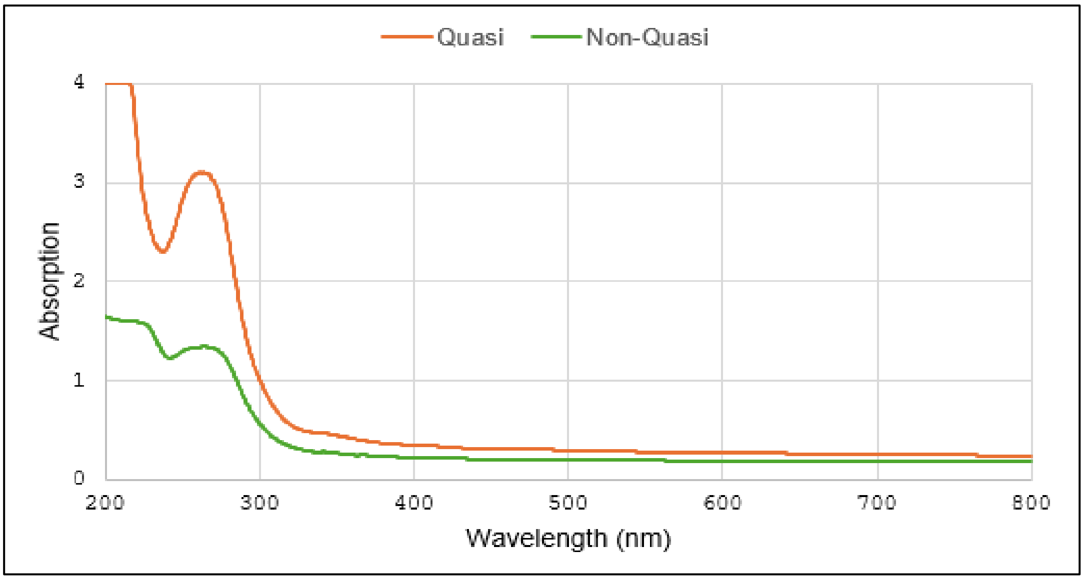
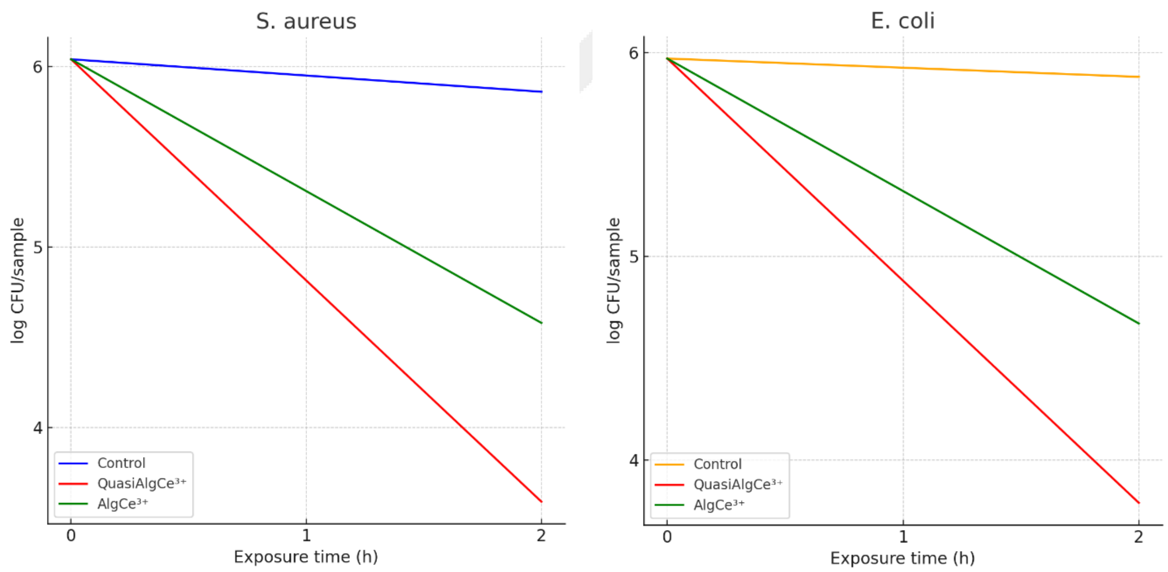


Figure 4.4: UV-VIS Transmittance of the Films.

4.4 ANTIBACTERIAL ACTIVITY

Figure 4.5 show log CFU/sample vs exposure duration (0 and 2 hours) for Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 6538) in Control, QuasiAlgCe^{3+}, and AlgCe^{3+} conditions. AlgCe^{3+} got log reductions of 1.30 for E. coli and 1.46 for S. aureus, whereas QuasiAlgCe^{3+} exhibited superior reductions of 2.19 for E. coli and 2.45 for S. aureus, compared to low Control reductions (0.09 for E. coli and 0.18 for S. aureus, respectively). QuasiAlgCe^{3+}'s increased efficacy shows powerful antibacterial components, with S. aureus being slightly more vulnerable. Cerium nitrate, on the other hand, has long been utilized in clinical settings to treat burns [18].

Cerium acts as a biological dressing, promoting wound healing. A recent study found that cerium ions boost the activity of human foreskin fibroblasts [19]. QuasiAlgCe^{3+} is presented as a promising wound dressing.



` **Figure 4.5:** Antibacterial Activity.

5. CONCLUSION

The tests demonstrate that QuasiAlgCe^{3+} differs significantly from AlgCe^{3+} due to cryogelation process, which might be due to the increased surface area and better mechanical properties as shown in previous studies. Because more cerium nitrate is trapped during the freeze-thaw process, QuasiAlgCe^{3+} exhibits stronger crosslinking, according to FTIR results. Compared to AlgCe^{3+}, QuasiAlgCe^{3+} swells more (115% in isotonic and 98% in pure water) and transmits more water vapor (2614.15 Vs. 1392,94g/day·m^2), making it more porous.

UV-Vis data show AlgCe^{3+} is clearer above 300 nm, making it better for transparent uses like bandages, while QuasiAlgCe^{3+} blocks more UV light, which is great for protective coatings. Overall, QuasiAlgCe^{3+} is more suited for moisture-regulating and UV-protective applications, like wound dressing.

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