
T.C.

ALTINBAŞ UNIVERSITY

Institute of Graduate Studies

Mechanical Engineering

# Synthesis and Characterization of Copper Alginate Quasi-Cryogel Beads

Ali Taissir BELHI

Master of Science

Supervisor

Asst. Prof. Dr. Hakan KAYGUSUZ

Istanbul, 2021

# Synthesis and Characterization of Copper Alginate Quasi-Cryogel Beads

by

**Ali Taissir BELHI**

Mechanical Engineering

Submitted to the Institute of Graduate Studies

in partial fulfillment of the requirements for the degree of

Master of Science

ALTINBAŞ UNIVERSITY

2021

The thesis titled “Synthesis and Characterization of Copper Alginate Quasi-Cryogel Beads”, prepared and presented by “Ali Taissir BELHI”, was accepted as a Master of Science Thesis in Mechanical Engineering.

Asst. Prof. Dr. Hakan KAYGUSUZ

Supervisor

Thesis Defense Jury Members:

| | | |
|---|---|---|
| Asst. Prof. Dr. Hakan KAYGUSUZ | School of Engineering and Natural Sciences, Altınbaş University | |
| Asst. Prof. Dr. Süleyman BAŞTÜRK | School of Engineering and Natural Sciences, Altınbaş University | |
| Assoc. Prof. Dr. Bengü ÖZUĞUR UYSAL | School of Engineering and Natural Sciences, Kadir Has University | |

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Approval Date of Institute of Graduate Studies:
____/____/____

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Ali Taissir BELHI

Signature

# DEDICATION

This dissertation is dedicated to all the professors that helped me in my learning journey. Even though they were my inspiration to pursue my master's degree, they were unable to see my graduation.

This is for them.

# ACKNOWLEDGEMENTS

I want to thank my mother, who is also my biology teacher, and the one who inspired my pursuit of material science and chemistry and my father who helped me in all things, great and small.

I want to thank my academic advisor Dr Hakan Kaygusuz who guided me in this process, and his research assistant Burcu Orhan who kept me on track and taught me so much in a very few time.

# ABSTRACT

## Synthesis and Characterization of Copper Alginate Quasi-Cryogel Beads

Belhi, Ali Taissir,

M.Sc. Mechanical Engineering, Altınbaş University,

Supervisor: Asst. Prof. Dr. Hakan KAYGUSUZ

Date: 10 June 2021

Pages: 50

Alginate biopolymer has several applications-especially in biological fields such as drug release, tissue engineering, and wound dressing, due to its biodegradable, biocompatible, and non-toxic properties. Copper ion has been used in previous wound dressing studies for its antimicrobial property. In this thesis study, firstly, copper crosslinked alginate beads (Alg) were prepared using the quasi-cryogelation method. Their swelling profile was investigated since alginate forms hydrogel in the presence of divalent cations. Swelling experiments were performed in two solutions, pure water and 0,9% (w/v) isotonic. Swelling ratios were found as 29% in purified water and 24% in an isotonic solution. Secondly, Alginate beads were modified by the quasi-cryogelation method. In this way, it was aimed to improve the swelling degree with the increasing porosity of beads. The swelling degree of Quasi-Alg beads was found as 295% in pure water and 260% in an isotonic solution. This study exhibits a method to increase the swelling ratio of hydrogels. Since the swelling ratio is essential for drug release to effectively release the drug or for wound dressing films to supply enough moisture to wound, Quasi-Alg beads may comprise a basis for such applications.

**Keywords:** Antimicrobial, Copper, Alginate, Quasi-Cryogelation.

# TABLE OF CONTENTS

**Pages**

# LIST OF TABLES

**Pages**

# LIST OF FIGURES

**Pages**

# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| AFBI | : | Agri-Food and Biosciences Institute |
| ATSDR | : | Agency for Toxic Substances and Disease Registry |
| PEG | : | Polyethylene glycol |
| PAA | : | Polyacrylic Acid |
| SPH | : | Super Porous Hydrogels |
| SAP | : | Superabsorbent Polymers |

# LIST OF SYMBOLS

Alg : Alginate

Cu : Copper

GPC : Gel Permeation Chromatography

pH : Acidity-Basicity scale

# SUMMARY

Hydrogels have been used for a wide range of uses in everyday life, including as superabsorbent and drug delivery. Hydrogels with functional properties that enable the insertion of extra functions to have a considerable amount of potential for future expansion, by including reactivity to external stimuli, the characteristics of these hydrogels might be varied. The crosslinked polymers respond to temperature, pH, pressure, chemical and electric stimuli, irradiation, and magnetic fields. Possible applications arise due to this responsive nature in many areas, such as implementation as actuators and drug delivery, purification, tissue engineering, and medical coatings or biosensors. Hydrogels have been introduced as innovative technologies for several uses over the past decades, for example, biomedical engineering or sanitary products and agriculture[1]. They have been utilized effectively as superabsorbent materials and in drug delivery, cell encapsulation, and tissue healing due to their high-water content and associated biocompatibility.

Given that hydrogel's high-water retention offers a practical pathway for drug diffusion, several hydrogel networks have been developed and manufactured as intelligent drug carriers. The most significant parameters that regulate the solvent and drug release patterns from all of these polymeric networks are the rate and degree of hydrogel swelling. Therefore, the main goals of several experiments were the detailed description of hydrogel behavior and the statistical explanation of equilibrium swelling, structural changes as a result of solvent absorption, desorption, and release of drug profiles[2].

Copper (Cu) is an essential trace element present in both organs and cells; Its oxidation-reduction chemistry makes copper highly desirable for biological processes such as cellular respiration, iron transfer, oxidative stress defense, development of peptide hormones, neurotransmission, connective tissue biosynthesis, and many others as a catalytic cofactor of many enzymes[3]. For its antimicrobial activities, copper is frequently used, for example, in cotton and polyester fabric impregnation or coating and dental infection prevention applications. In pharmaceutical science, through the effect of physical (electrostatic) forces between polyelectrolytes and current polyvalent ions, polyvalent ions such as $Cu^{2+}$ can be used as a practical approach to formulating polyelectrolytes with negatively charged groups (alginates, carmellose, pectins, etc.) into gel systems[4].

In the study, spherical beads have been prepared by crosslinking sodium alginate with copper(II) solution, then undergone the quasi-cryogelation step at -21$^{o}$C, and their swelling behavior has been studied; some experiments revealed that prepared copper-alginate beads might be employed successfully for drug delivery, and even a coat weight of 20% water retention was enough to give a robust and resistant quality to the beads for effective drug release at higher pH values. The goal of this experimental research was to prepare new drug-free particles with potential antimicrobial, antifungal, and antiviral properties, based on the excellent mucoadhesive polymer sodium alginate crosslinked by $Cu^{2+}$ ions[5], [6]. Using the Quasi-Cryogelation method, beads were prepared, and their effects were measured.

# 1. INTRODUCTION

Today, in our everyday lives and technology, medicine, and engineering, polymers are widespread; we utilize melamine plates, polyacrylate superabsorbent diapers, and electrical cords free of polyvinyl chloride. Polymers are applied to measure concrete hardening, while aromatic polyamides are employed in safety equipment, Polyethylene glycol (PEG) is engaged in dentistry, medication delivery, and tissue engineering, and polystyrene is utilized to fill GPC columns. Hydrogels, which are typically swollen yet insoluble, have grown in popularity for a variety of applications.[7], [8]. Over the last decades, most of the pharmaceutical development efforts have concentrated on discovering or synthesizing novels that have been used as drug containers or release control barriers; hydrogels have acquired significant attention and have been reviewed from many points of view. Hydrogels are a form of the macromolecular network which can retain a large amount of aqueous solvent inside its structure[9]. They are especially suitable for biomedical and tissue engineering applications due to their ability to mimic biological tissues and expanded applications in bioseparation, agriculture, and enhanced oil recovery.

The abuse of antimicrobial agents is a significant challenge to future pharmacotherapy, and the answer can be obtained in the most straightforward polymeric structures with intrinsic antibacterial characteristics without the requirement for additional antibacterial agents or adding medications, e.g. alginate beads crosslinked with copper ions[5]. The suggested study's key goal was to increase the swelling ability of crosslinked copper alginate beads.

Copper-Alginate beads have been made using the extrusion dripping method by crosslinking sodium alginate with copper ions[10]–[12]. Morphological characteristics, swelling ability, ion release were evaluated, antimicrobial resistance is a significant problem for the current and future

pharmacotherapy of infectious diseases. The dilemma could be partly solved by using basic polymeric structures having antibacterial characteristics intrinsic without the need for natural drugs to be integrated. Several innovative concepts have recently been introduced. One such solution may be the use of crosslinked alginate particles with copper ions. Alginates are frequently usedin the medical and pharmaceutical industries[13]. They have a wide variety of beneficial properties, such as biocompatibility, biodegradability, bioadhesivity, and structural resemblance to the extracellular matrix, making them a biomaterial of choice in the fields of wound dressing, tissue engineering, and regeneration. The development of controlled release dosage forms and the possibility of protein drugs, probiotics, and other live-cell encapsulation are different areas of their numerous applications. Of the high number of varying dosage formulations based on regularly published alginates, crosslinked alginate particles have a particulate character that enables fast dosage dispersal at the administration site, eliminates local adverse effects, making it possible to use dispensing tools when delivered to body cavities, and is quickly customizable for controlled release through pharmaceutical technology[14]. These advantages are strengthened by alginate itself because of its Previously reported mucoadhesive effect, increasing contact time and hence lowering dose frequency. While the most physiological ion for external ionic gelation is commonly agreed as the calcium (II) ion, quite a few papers have been published dealing with crosslinking via copper ion mixture, although some advantages may be offered, including the reduction of single cation vulnerabilities concerning both technical and biological aspects.

Some of the polyvalent ions in the affinity sequence, such as $Cu^{2+}$, have their own pharmacological effect, which provides the possibility of formulation of the dosage type without the need for any other drug encapsulation[8]. While human tissue sensitivity to copper is low, the sensitivity of

many microorganisms is extreme, including Escherichia coli, Staphylococcus epidermidis, or even viruses such as bacteriophages, herpes simplex virus, poliovirus, hepatitis A virus, and HIV.

The goal of this thesis was to prepare alginate-based mucoadhesive copper crosslinked alginate beads for use against the most common bacterial infections. The current study's goal is to look at the swelling properties of the copper-alginate hydrogel; copper alginate beads have been prepared by the extrusion dripping method; the purpose of this experimental research was to design new drug-free particles with desired antimicrobial, antifungal, and antiviral properties.

# 2. COPPER

Copper is a naturally occurring element and metal; people have used it for thousands of years, and copper, along with copper alloys and compounds, may now be found in everything from jewellery and coins to construction material and technologies., in the wiring in our phones and motors, the pipes in our walls. We also need it for car batteries and solar panels, and wind turbines, to name some application, It is what makes our cities glow, our factories hum, and our telecommunications buzz, with the urgency of a telegram and the force of an electric shock copper, helped catapult the world into the modern era[15]–[17].

Copper is a chemical element that is gentle, composable, flexible, and ductile metal that is also highly thermal and electrical, the symbol of copper is Cu, and its atomic number is 29; The newly revealed pure copper surface is pinkish orange in tone. Copper serves as both a thermal and an electrical conductor[18], as a building material, and also as a component of a variety of metal alloys, including stainless steel used in watches, cupronickel, which is used in the manufacture of marine gear and coinage, also used in pressure gauges and thermocouples for temperature measurement.

**Figure 2.1:** Some water contamination is very colorful and visible such as Acid Mine Drainage seen here.

## 2.1 PRODUCTION

The majority of copper is mined or produced as copper sulfides from huge mining sites in porphyry copper deposits with 0.4% to 1%copper content. Copper may also be extracted by an in-situ leaching operation. The quantity in use is increasing, and the amount available is insufficient to allow all countries to meet the level of usage in the developing world[19].

## 2.2 COPPER ABSORPTION

Copper is absorbed in the stomach and transported to the liver in the form of albumin. Copper is transferred to other tissues in the second stage after treatment in the liver. This contains the ceruloplasmin protein, which is responsible for the bulk of copper in the blood. Ceruloplasmin also contains copper that is secreted in milk and is an excellent copper source. Copper typically circulates in the bloodstream via enterohepatic circulation (about 5 mg per day vs approximately 1 mg per day consumed in the diet and excreted from the body) and, if necessary, the body is able to eliminate any excess copper via bile, which contains some copper from the liver but is not absorbed by the intes[20].

## 2.3 BIOCHEMISTRY

Copper proteins have several roles in the transfer of biological electrons and oxygen, processes that rely on the easy interconversion of Cu(I) and Cu(II). Copper is required for aerobic respiration in all eukaryotes. It is present in mitochondrial cytochrome c oxidase, which is the last protein in oxidative phosphorylation. Cytochrome c oxidase is an enzyme that bonds O2 between copper and iron and transfers 8 electrons to the $O_2$ molecule, reducing it to two molecules of water[21]. Copper is also found in several superoxide dismutase proteins, which catalyze the breakdown of superoxide via disproportionation to oxygen and hydrogen peroxide.

## 2.4 COPPER TOXICITY

Copper toxicity is a type of metal poisoning caused by an excess of copper in the body. Copperiedus can be caused by eating acidic meals cooked in non-coated copper cookware or by being close to waste copper in drinking water and other environmental sources. Copper in the blood and bloodstream exists in two forms: the one that is bound to ceruloplasmin and that which

is secure, partially attached to albumin, and tiny molecules. Organic and inorganic copper are distinguished nutritionally by whether the copper ion is bonded to an organic ligand[22][20].

## 2.5 COPPER IN HEALTH

Copper is a trace element that is essential to the health of all living beings (humans, plants, animals, and microorganisms). Copper is required for the correct functioning of organs and metabolic processes in humans. The human body contains complex homeostatic systems that seek to keep a stable supply of valuable copper while eliminating excess copper whenever feasible; however, like with all critical elements and minerals, too much or too little copper consumption will result in a copper surplus or shortage in the body, each of which has its own set of negative health repercussions. Several health organizations throughout the globe have created standard copper dietary guidelines.[3], [4]. Standards adopted by individual nations prescribe various standards of copper consumption for men, pregnant women, infants, and children, matching the varying requirement for copper during multiple stages of life [23].

**Figure 2.2:** Copper deficiency is uncommon, but certain medical disorders and other circumstances might raise the risk.

## 2.6 ANTIMICROBIAL PROPERTIES OF COPPER

Copper and copper alloys have antibacterial properties of natural origin resources. Ancient cultures used the antimicrobial qualities of copper well before the idea of bacteria was recognized in the nineteenth century [14].

In addition to many coppers therapeutic preparations, it was often observed centuries earlier that the water stored in copper vessels or transported in copper conveying systems was of higher consistency (i.e. no or little apparent sludge or biofouling formation) than the water contained or transported in other products. The antimicrobial effects of copper are also under study [40]. Intensive research has been performed on the molecular processes responsible for the antibacterial activity of copper. Scientists are now successfully demonstrating the inherent effectiveness of copper alloy "touch surfaces" to kill a wide variety of microorganisms that risk public health.

# 3. BIOPOLYMERS AND HYDROGELS

## 3.1 BIOPOLYMERS

Biopolymers are a special kind of polymers that are derived from biological systems that are environmentally friendly and biodegradable. However, polymers can also be identified as biopolymers on the condition that they have been chemically synthesized by humans from organic sources, including vegetable oils, fats, resins, sugars, proteins, and amino acids. They have monomers bound with larger complexes in a covalent manner; They are inexpensive, recyclable, and indeed low resistance and manufacture biopolymers; no dangerous toxins are used[11].

**Figure 3.1:** Biodegradable polymers are becoming increasingly popular as an alternative material for plastic packaging.

Biopolymers are structurally more complicated and have specifically defined 3D forms compared to synthetic polymers. Biopolymers are categorized into two primary groups; microorganisms, plants, and animals form the first one. The second is produced synthetically but extracted by biological starting materials such as amino acids, carbohydrates, fats, or oils.

Biodegradable biopolymers are created through renewable resources, allowing them to combine or replace fossil fuels because they are biocompatible and biodegradable; biopolymers generated in biological systems are sustainable materials[24].

## 3.2 HYDROGELS

A hydrogel is a three-dimensional (3D) system that may expand in water and retain considerable amounts of water while retaining its structure owing to the chemical or physical crosslinking of polymer chains. The hydrophilicity of the network is dependent on the presence of Hydrophilic functional groups.

**Figure 3.2:** 3D printed bioprinted scaffold in the form of a human hand.

Hydrogels are generated through Cross-linking of copolymers and homopolymers via physical or chemical and effectively give 3D structures have unique chemical and mechanical properties. The crosslink can be produced by covalent or noncovalent interactions; Chemical gels are often called covalently crosslinked hydrogels, although noncovalent gels are labelled physical gels. Hydrogels could potentially be biochemical, mechanical, or physical. In reaction as a result of a change in ambient circumstances such as temperature, ionic concentration, pH, etc., or other conditions such as combining two elements, Physical gels may undergo a liquid-to-gel transition. Chemical gels, as opposed to other fragile materials, utilize covalent bonding, which adds mechanical stability and resistance to deterioration. In biochemical hydrogels, biological agents such as enzymes or amino acids participate in the gelation process. Hydrogel swelling is influenced by a few parameters, such as pH, temperature, ionic force, and electromagnetic radiation[25]. This enables them to be used for the pulp and paper industry and the manufacture of contact lenses, cellulosic membranes, and biomedical applications.

### 3.3 SUPERABSORBENT HYDROGELS:

Around three decades ago, superabsorbent polymers (SAPs) in Agriculture industries were introduced, their uses were then extended to other sectors where excellent water holdings were critical. Super porous hydrogels (SPHs) have just been introduced as a distinct form of the water-absorbent polymer system. Hydrophilic polymers joined together by crosslinking of covalent polymer bonds or ionic and secondary forces in the form of hydrogen bonds or hydrophobic interactions generate three-dimensional networks of superabsorbent hydrogels capable of absorbing and holding vast quantities of water while preserving the physical dimensional structure[26], [27]. Many researchers have recently focused their efforts on superabsorbent hydrogels for the production of new technologies, such as material processing, detectors, and releasing materials are all examples of biomaterials. Their affinity for water makes them essential, particularly for industrial absorbents, agriculture, hygiene supplies, cosmetics, and healthcare. Water-absorbing and retention capabilities can be controlled by the kind of hydrophilic monomer

or polymer utilized in their synthesis, the degree of crosslinking, and the nature of the swelling medium; SAPs can be classified in a variety of ways. They may be divided into particle, powder, filament, membrane, among others, based on their morphology. SAP's morphology is intended to adapt to the various application needs, a powder product, for example, can be installed on a multilayer sheet to make wet wipes and diapers, the spherical product can be used as a deodorant, fiber product can be used as an antistatic electrical fiber, a membrane item can be used as an anti-frost piece of paper, and an emulsion product can be used in soaking and painting. SAP can also be divided into natural macromolecules, semi-synthesized polymers, and synthetic polymers in terms of material resources. From the standpoint of preparation technique, it may be divided into graft polymerization, crosslinking polymerization, water-soluble polymer network growth, and radiation crosslinking, among others. In today's market, there are several varieties of SAPs.

### 3.3.1 Stimuli-Responsive Hydrogels:

Significant attention has been drawn over the last two decades to so-called smart hydrogels, intelligent hydrogels, or stimulus-responsive hydrogels that, in response to numerous external physicochemical influences, can undertake a treatable and discontinuous volume phase change, primarily because of their unique properties but also because of their potential for severe technical amplification.

The relevance of these materials resides not only in the rapid macroscopic transformations in their composition but also in their reversibility.

The table below summarizes the environmental conditions that influence the swelling or shrinkage of hydrogels and their uses:

**Table 1:** Environmental conditions that induce hydrogels to respond sharply.

| Factor | Applications |
|---|---|
| Temperature | Drug delivery<br>Separation<br>Bioreaction<br>Shape memory<br>Artificial muscle<br>Enzyme immobilization |
| pH | Drug delivery |
| Electric field | Drug delivery<br>Artificial muscle |
| Pressure specific molecules | Drug delivery |
| Ions | / |
| Solvent's light | / |

### 3.3.2 pH-Responsive Hydrogels:

Polymeric Backbones are used to create the pH-responsive hydrogels of groups of ions; the most widely studied pH-responsive ionic polymers are polyacrylamide (PAAm), polyacrylic acid (PAA), polymethacrylic acid (PMAA), poly diethylamino ethyl methacrylate (PDEAEMA), and poly dimethylamino ethyl methacrylate (PDMAEMA). Several synthetic or natural polymers have been employed up to this point. As pH-sensitive controlled release methods, acid or simple pendant groups have been developed. Natural polymers with properties such as non-toxicity, non-immunogenicity, and biodegradability have been widely employed in recent years. In aqueous conditions with adequate pH and ionic strength, the groups ionize and form fixed charges on the polymer network, resulting in electrostatic repulsive forces that cause pH-dependent swelling or deswelling of the hydrogel, thus regulating the release of the drug[5]. Minor pH variations will lead to drastic changes in terms of mesh size of the polymer networks. Anionic hydrogel pendant groups are un-ionized below and ionized far above the polymer network pKa, allowing the hydrogel to expand at pH above the polymer pKa due to a significant osmotic swelling force generated by the presence of ions. Cationic hydrogels, on the other hand, swell at lower pH. The graphic depicts the differential swelling of ionic hydrogels in acidic and alkaline buffers.

## 3.4 ALGINATE

### 3.4.1 General Information

Alginates are brown seaweed-derived hydrocolloids and water-soluble biopolymers. They are found naturally in seaweed (algae), primarily as sodium, magnesium, or calcium salts.

Alginates are polymers of carbohydrates (e.g., Polysaccharides) consisting of two uronate sugars, Guluronic acid salts, and mannuronic acid salts[28]. The uronic acids are neutralized and transformed into their salt forms, mannuronate/guluronate. The physical and chemical properties of alginate blocks are determined by their length, relative amount, and dispersion.

Alginate is one of the world's most commonly used marine biopolymers. Alginate is found in the cell walls and the intracellular regions of brown algae[2]. For the extraction of alginate, the milled algal tissue is obtained with mineral acid for the Transferring ions for protons., Algal tissue is extracted with a mineral acid to excrete proton ions.

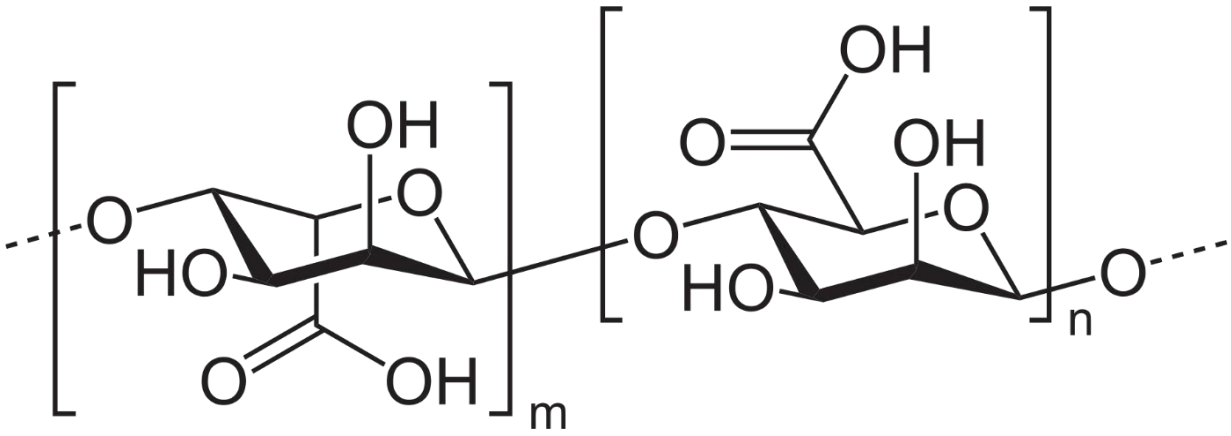


**Figure 3.3:** Structure of Alginic acid.

Then the method of neutralization is used for sodium carbonate or hydroxide in alginic acid. Then Sifting, floating, centrifugation, and filtration techniques are applied following this method to isolate algal particles. In the end, precipitation and drying techniques are used, respectively. There is a relation between the properties of alginate and its industrial application.

Alginates have unique features in water, becoming gel and remaining stable properties. They are amazing, soft, and have a transition to the sol-gel of multivalent cations like calcium and barium[29]. So, alginate is cell-living compliant. It is used in pharmaceutical and medical applications.

### 3.4.2 Properties of Alginates

#### 3.4.2.1 Solubility

The solubility of alginate is actively determined by the state of the carboxylic groups' backbone. There are typically three water solubility guidelines for alginates. Firstly, due to the electrostatic charge present on a substratum of uronic acid, it is the medium pH that determines the solubility of the alginates[30]. There is also the ionic strength of the environment from salting the impact of non-gelling cations. A switch in the ionic strength of alginates influences the actions of alginates, such as their viscosity, polymer conformation, chain length, and ultimately solubility. Finally, the presence of gelling particles in the medium lowers the solubility of alginates and causes gels to form instead. Alginic acid does not dissolve itself in a solution, but hydrocolloids are sodium alginates in the presence of water; they include a sticky solution or gel. The sodium alginates in organic media are not fully soluble[31].

#### 3.4.2.2 Stability

Sodium alginate's pure, dried, and pulled form will last for several months, as long as it stays in a cold, dark, and dry place. The sodium alginate can also be retained for several years under such conditions, and no substantial decrease in molecular weight can still be observed[32].

#### 3.4.2.3 Viscosity

When immersed in water, the alginate molecules get hydrated, and the solution becomes viscose; because of the blocked rotation and the glycosidic connections of the G-block sections, the dissolved molecules are not entirely flexible, resulting in a very viscous chain stiffening[33]. The length of the alginate molecules or the total molecular weight and concentration of the alginate influence the viscosity of the alginate in the liquid. As a result, with the same concentration and increased chain length, viscosity rises[8].

#### 3.4.2.4 Capabilities of Alginates

Alginates have a wide range of uses due to their qualities in the foodservice industry, medical companies, chemical, fabric, and other fields:

*Formation of Gel*

Alginates are capable of forming gels when there are sufficient quantities of guluronate monomers in the block to cause them to respond to divalent cations. Thus the average level of the GG portions determines the reaction with the calcium and the subsequent gel-forming[34].

*Film-Forming Capability*

Alginates, like other biopolymers, can form films; the films created are following flexible plasticizers, solid, transparent, and impermeable to oxygen. The film may be either soluble or insoluble[35].

### 3.4.3 Application of Alginates

Alginates have different uses in many industries due to their flexible properties. They are currently used in the food, nutrition, fiber, and paper industries as coatings, pharmaceuticals, and source electrodes.

#### 3.4.3.1 Food

Alginate is often used as a stabilizer, additive, and thickener in many food products to make the substance fine, smooth and tasty.

#### 3.4.3.2 Textile printing

Alginate is of great importance in the textile industry. A small amount of alginate is enough to substitute a significant amount of starch in the yam spinning phase. Alginate is also used as an intermediate in the dyeing and printing industry and as a thickening agent for dyes and inks[36].

#### 3.4.3.3 Pharmaceutical and medical uses

Alginates are also used in pharmaceutical and medical uses due to their extreme purity and compatibility with the body; sodium alginate, for example, is used for the encapsulation of proteins, enzymes, and live cells[8].

## 3.5 MECHANICAL PROPERTIES OF ALGINATES

Mechanical properties are features that signify the elastic or inelastic behavior of the material under force, stress, and strain. The mechanical strength rises with the G-content in beads. It is observed by chemical structure and composition when the molecular weight reaches a certain amount. It should, therefore, be remembered that the gel formulation capacity reduces under a particular molecular weight[37].

# 4. MATERIALS AND METHODS

## 4.1 MATERIALS

Alginic sodium salt with high viscosity, sodium chloride, and Montmorillonite K10 is supplied from Sigma-Aldrich (USA). Copper sulfate pentahydrate is supplied from Edukim (Turkey). Deionized water was used in the experiments, and all chemicals were used without further purification.

## 4.2 INSTRUMENTS

Shimadzu UV-1280 (Japan) Spectrophotometer was used in the spectrometric experiments. Nüve ST30 (Turkey) shaking water bath was employed for repetitive and reproducible shaking experiments. A laboratory heater with a magnetic stirrer and precision balance was used for the preparation of the solutions and weighing steps, respectively.

## 4.3 PREPARATION OF COPPER ALGINATE BEADS

### 4.3.1 Copper(II) Alginate Beads (Quasi-Cryogelation):

A homogenous solution of sodium alginate in water was prepared by dissolving 0.4 g in 20 mL of deionized water, covering it with parafilm plastic to protect the water from evaporating and risking any change in concentration in the solution, and then the mixture is placed on a stirrer and stirred until a homogenous solution (2% w/v) was obtained.

Crosslinker stock solution was prepared by dissolving 6.755 g of $CuSO_4$ 100 mL of deionized water; then, the solid crystals were dissolved in 50 mL of pure water and finally diluted to 100 mL.

In crosslinking step, 50 mL of $CuSO_4$ solution was taken, placed over a stirrer, and sodium alginate solution was added dropwise using a 0.8 mm diameter syringe, above 20 cm from the solution to create uniformly spherical beads. After the addition of sodium alginate, the following reaction is expected to occur:

$$Cu^{2+}(aq) + Na^{+}Alg^{-}(aq) \rightarrow \quad Cu^{2+}Alg^{-}(gel) + Na^{+}(aq)$$

**Equation 1 Chemical Reaction between to form copper-alginate beads**

In Eq.1, copper ions in copper sulfate solution react with alginate in the sodium alginate solution to form copper alginate hydrogels aimed to be obtained in this study, where $Cu^{2+}Alg^{-}$ represents the copper alginate gels. Then the beads were stirred for 20 minutes for complete gelation, and then the beads were filtered. At this step, beads contain entrapped water in hydrogel form; then, the beads were carefully placed in a laboratory refrigerator at - the degree of -21 $^{o}$C for quasi-cryogelation.

Cryogelation is based on performing gelation reactions in a frozen reaction environment; as water freezes, monomers are expelled from the ice concentration in the unfrozen domains, resulting in an extremely concentrated solution, cryogels can be obtained even at low concentrations.

Quasi-cryogelation is a modified freezing step reported for the first time by Uyar et al. [41] and Balkız et al. [42], which is helpful for obtaining hydrogels with high surface area, better adsorption properties, and other characteristics with the use of a facile method. Since the method resembles the cryogelation, the name quasi-cryogelation is used since the freezing occurs after the gelation step.

Finally, after keeping the beads at -21 $^{o}$C for 24 h, the beads were taken out and left to melt at room conditions. After that, they were collected, washed three times with deionized water, and left to dry at room temperature. Dry beads lost the spherical appearance of wet beads and emerged as pellets.

### 4.3.2 Copper(II) Alginate Beads (Normal Beads) :

A homogenous solution of sodium alginate in water was prepared by dissolving 0.4 g in 20 mL of deionized water, covering it with parafilm plastic to protect the water from evaporating and risking

any change in concentration in the solution, and then placing it over a stirrer and waiting for it to dissolve, until finally obtaining the 2% w/v concentration wanted.

Later 50 ml of Copper(II) sulfate solution was prepared by dissolving 6.755 g of $CuSO_4$ in 100 mL of deionized water; firstly, by pouring the dissolved 6.755 g in 50 mL of $CuSO_4$ into a flask, thenceforth completing it with deionized water till it reaches 100 mL.

After that, 50 ml of CuSO4 solution was placed over a stirrer, and sodium alginate solution was dropped using a 0.8mm diameter syringe from a steady height of 20 cm in order to create uniformly spherical beads.

After this step, the created beads were stirred for 20 min for coagulation, cleaned three times with deionized water, and left directly to dry at room temperature; dry beads lost the spherical appearance of wet beads and emerged as pellets.

## 4.4 SWELLING TEST

The swelling test is a test to measure the swelling ratio that is defined as the percentage weight increase of the hydrogel as a result of water absorption.

$$Swelling\ Ratio = \frac{Weight\ of\ swelled\ sample}{weight\ of\ dry\ sample} = \frac{W_2 - W_1}{W_1}$$

**Equation 2.1 The general formula for swelling ratio**

In Eq.1.1 The general formula used to calculate the swelling ratio for polymers, further calculation and results in this study were obtained using this formula.

### 4.4.1 SWELLING TESTS IN PURE WATER:

For determination of swelling in pure water, 0.1 g of beads (normal and quasi) were placed in 30 mL of distilled water separately and then placed in a water bath at a temperature of 37$^{o}$C and shaking at pre-determined time intervals for intervals of [15 min - 240 min].

### 4.4.2 SWELLING TESTS FOR ISOTONIC SODIUM CHLORIDE:

For determination of swelling in isotonic sodium chloride, 0.1 g of beads (normal and quasi) were placed in 30 mL of isotonic sodium chloride separately and placed in a water bath at a temperature of 37oC and shaking for intervals at pre-determined time intervals of [15 min- 240 min].

# 5. RESULTS AND DISCUSSION

## 5.1 SWELLING TESTS IN PURE WATER

Normal beads Samples are Labeled as A1, A2, A3

Quasi Beads Samples are Labeled as C1, C2, C3

The results are shown in the graph and table below:

| Time (min) | A1 | A2 | A3 | C1 | C2 | C3 |
|---|---|---|---|---|---|---|
| **0** | 0.1026 | 0.1013 | 0.1019 | 0.1012 | 0.1023 | 0.1002 |
| **15** | 0.1423 | 0.1433 | 0.143 | 0.3231 | 0.2986 | 0.2945 |
| **30** | 0.132 | 0.1352 | 0.134 | 0.33 | 0.355 | 0.3256 |
| **45** | 0.134 | 0.1366 | 0.1386 | 0.3256 | 0.3638 | 0.355 |
| **60** | 0.1277 | 0.1283 | 0.1325 | 0.3245 | 0.3318 | 0.3423 |
| **90** | 0.1276 | 0.13 | 0.1315 | 0.3116 | 0.3444 | 0.322 |
| **120** | 0.129 | 0.1435 | 0.1443 | 0.3631 | 0.3879 | 0.3613 |
| **180** | 0.1298 | 0.127 | 0.1392 | 0.3448 | 0.3723 | 0.3763 |
| **240** | 0.1539 | 0.1377 | 0.1345 | 0.4183 | 0.3918 | 0.3912 |

**Table 2:** Swelling Results (SR) and standard deviations for the measurements of two sorts of beads in pure water.

The beads are swelling remarkably in pure water, and the swelling ratio was found as 29% in pure water when Alginate beads were modified by the quasi-cryogelation method, it was aimed to improve swelling degree with the increasing porosity of the beads, the swelling ratio of Quasi-Alginate beads was found as 295% in pure water.

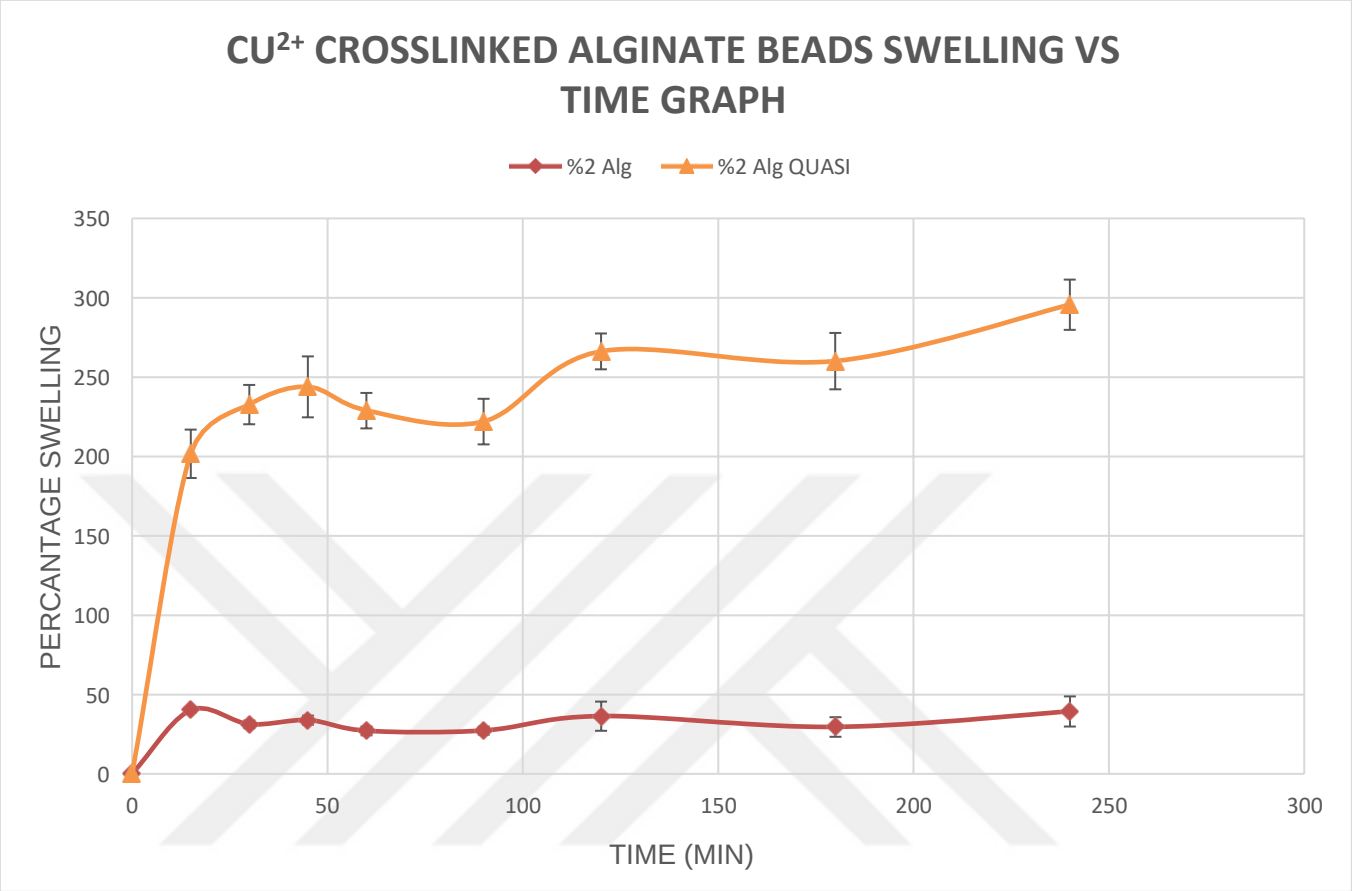


**Figure 5.1:** $Cu^{2+}$ Crosslinked Alginate Beads Swelling vs Time Graph in pure water.

## 5.2 SWELLING TESTS FOR ISOTONIC SODIUM CHLORIDE 0.9

Normal beads Samples are Labeled as A1, A2, A3

Quasi Beads Samples are Labeled as C1, C2, C3

The results are shown in the graph and table below:

| Time (min) | A1 | A2 | A3 | C1 | C2 | C3 |
|---|---|---|---|---|---|---|
| **0** | 0.1025 | 0.1001 | 0.1023 | 0.1012 | 0.102 | 0.1015 |
| **15** | 0.1412 | 0.1389 | 0.1455 | 0.272 | 0.334 | 0.274 |
| **30** | 0.133 | 0.1275 | 0.14 | 0.3524 | 0.3445 | 0.3092 |
| **45** | 0.128 | 0.1298 | 0.1308 | 0.386 | 0.3778 | 0.37 |
| **60** | 0.131 | 0.1288 | 0.1278 | 0.356 | 0.312 | 0.307 |
| **90** | 0.125 | 0.1232 | 0.125 | 0.3295 | 0.3835 | 0.3143 |
| **120** | 0.1224 | 0.1232 | 0.1307 | 0.3364 | 0.3442 | 0.326 |
| **180** | 0.1243 | 0.1245 | 0.1324 | 0.3454 | 0.3826 | 0.322 |
| **240** | 0.1257 | 0.1271 | 0.1263 | 0.3667 | 0.3508 | 0.3797 |

**Table 3:** Swelling Results (SR) and standard deviations for the measurements of two sorts of beads in isotonic sodium chloride.

The beads are swelling in isotonic sodium chloride solution; the swelling ratio was found as 24% in isotonic sodium chloride solution. When Alginate beads were modified by the quasi-cryogelation method, it was aimed to improve swelling degree with the increasing porosity of beads.

The swelling degree of Quasi-Alginate beads was found as 260% in isotonic sodium chloride solution.

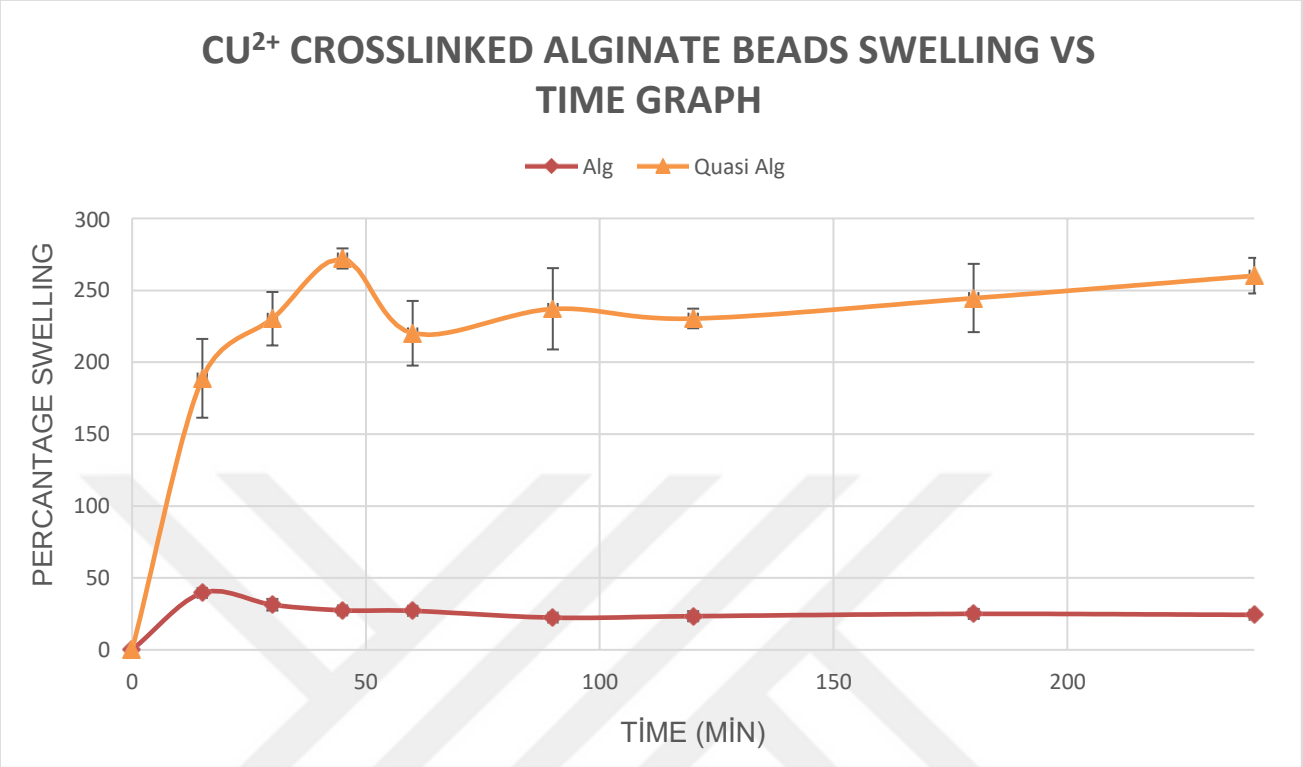


**Figure 5.2:** $Cu^{2+}$ Crosslinked Alginate Beads Swelling Vs Time Graph in Isotonic Sodium Chloride.

## 5.3 DISCUSSION

The beads are swelling more in pure water than in isotonic sodium chloride solution; among the two formulations, swelling ratios were found as 29% in purified water and 24% in an isotonic solution.

Adjusting the beads via the Quasi-cryogelation method led to a significant improvement in the swelling ratio; the aim was to boost swelling degree as the porosity of the beads increased.

The swelling degree of Quasi-Alginate beads was found as 295% in pure water and 260% in an isotonic solution.

# 6. CONCLUSIONS

In this research, using the antimicrobial properties of both copper and alginate as advantages, novel alginate biopolymer beads were prepared. Alginate-Copper quasi-cryogels were prepared, and their swelling potential was studied by placing the beads in different aqueous solutions and shaken them at a water bath for different time intervals. Quasi-cryoglation and the integration of copper have significantly improved swelling efficiency. Freezing the beads led to a considerable improvement in swelling activity through growing the adsorbent surface area without harming the integrity of the bead. Swelling experiments were performed in two solutions, pure water and 0.9% (w/v) isotonic. Swelling ratios were found as 29% in purified water and 24% in an isotonic solution.

Alginate beads were modified by the quasi-cryogelation method. In this way, it was aimed to improve the swelling degree with the increasing porosity of beads. The swelling degree of Quasi-Alginate beads was found as 295% in pure water and 260% in an isotonic solution. This study exhibits a method to increase the swelling ratio of hydrogels. Since the swelling ratio is essential for drug release to effectively release the drug or for wound dressing films to supply enough moisture to wound, quasi-alginate beads may comprise a basis for such applications.

Where most experiments involved the addition of another adsorbent and/or surface adjustment by chemicals, the advantage of this novel approach is its simplicity. Quasi-cryogel beads are shown to have a higher capacity for swelling when compared with normal beads. Copper alginate beads have been found to have the highest antibacterial properties. In addition, future research may show the feasibility of crosslinked alginate with copper or other metal ions as effective drug delivery materials. Present results indicate that alginate-copper quasi-cryogels can be used as a good

biopolymer for drug delivery. Future studies might reveal the water purification behavior of this material for other types of solutions.

# 7. BIBLIOGRAPHY


[1] L. Jin *et al.*, "Monitoring of swelling and degrading behavior of alginate beads using optical tweezers," *Biochip J.*, vol. 3, no. 3, pp. 213–218, 2009.

[2] A. M. Morales-Burgos *et al.*, *Polysaccharides in Alternative Methods for Insulin Delivery*. Elsevier Inc., 2018.

[3] L. Landner and R. Reuther, *Metals in Society and in the Environment. A critical review of current knowledge on fluxes, speciation, bioavailability and risk for adverse effects of Copper, Chromium, Nickel and Zinc.* 2005.

[4] F. Solomon, "Impacts of copper on aquatic ecosystems and human health," *Mining.com Mag.*, no. January, pp. 25–28, 2009, [Online]. Available: https://yukonwaterboard.ca/registers/quartz/qz08-084/Volumes 9-11/5.0/5.2.1.pdf.

[5] M. Pavelková *et al.*, "Biological Effects of Drug-Free Alginate Beads Crosslinked by Copper Ions Prepared Using External Ionotropic Gelation," *AAPS PharmSciTech*, vol. 18, no. 4, pp. 1343–1354, 2017, doi: 10.1208/s12249-016-0601-4.

[6] J. Rui Rodrigues and R. Lagoa, "Copper ions binding in Cu-alginate gelation," *J. Carbohydr. Chem.*, vol. 25, no. 2–3, pp. 219–232, 2006, doi: 10.1080/07328300600732956.

[7] M. Punter, *The mechanics of soft porous solids : from hydrogel dynamics to fibrin compression Melle Punter*. 2020.

*[8]* K. Y. Lee and D. J. Mooney, "Alginate: Properties and biomedical applications," *Prog. Polym. Sci.*, vol. 37, no. 1, pp. 106–126, 2012, doi: 10.1016/j.progpolymsci.2011.06.003.

[9] J. D. Bronzino and D. R. Peterson, “Biofunctional Hydrogels,” *Biomed. Eng. Fundam.*, pp. 681–694, 2020, doi: 10.1201/b15482-40.

[10] M. Chi, C. Liu, J. Shen, Z. Dong, Z. Yang, and L. Wang, “Antibacterial superabsorbent polymers from tara gum grafted poly(acrylic acid) embedded silver particles,” *Polymers (Basel).*, vol. 10, no. 9, 2018, doi: 10.3390/polym10090945.

[11] H. Kaygusuz geboren in Fatih, F. Bedia Erim Berker, R. von Klitzing, M. Gradzielski, and A. Nevin Öztekin, “Layered and Composite Biopolymers: Mechanical, Physical and Antimicrobial Properties vorgelegt von,” 2017.

[12] Z. Kalaycıoğlu *et al.*, “Antibacterial nano cerium oxide/chitosan/cellulose acetate composite films as potential wound dressing,” *Eur. Polym. J.*, vol. 133, no. May, pp. 1–7, 2020, doi: 10.1016/j.eurpolymj.2020.109777.

[13] D. Thomas, M. S. Latha, and K. Kurien Thomas, “Evaluation of the antibacterial activity of calcium alginate beads modified with ethanolic extract of adhatoda vasica leaf extract on Staphylococcus Aureus and Escherichia coli,” *Asian J. Pharm. Clin. Res.*, vol. 11, no. 3, pp. 68–70, 2018, doi: 10.22159/ajpcr.2018.v11i3.23367.

[14] L. P. Arendsen, R. Thakar, and A. H. Sultan, “The use of copper as an antimicrobial agent in health care, including obstetrics and gynecology,” *Clin. Microbiol. Rev.*, vol. 32, no. 4, pp. 1–28, 2019, doi: 10.1128/CMR.00125-18.

[15] U. W. Engineering, *Coagulation Process Characteristics and Pollutant Removal from Urban Runoff Fredrik Nyström*. 2019.

[16] Z. Aksu, Y. Sag, and T. Kutsal, “The biosorption of copperod by c. Vulgaris and z.

ramigera," *Environ. Technol. (United Kingdom)*, vol. 13, no. 6, pp. 579–586, 1992, doi: 10.1080/09593339209385186.

[17] C. A. Woody and S. L. O. Neal, "Effects of Copper on Fish and Aquatic Resources By," no. March 2012, 2012.

[18] M. Florio, "The economic rate of return of infrastructures and regional policy in the European Union," *Ann. Public Coop. Econ.*, vol. 68, no. 1, pp. 39–64, 1997, doi: 10.1111/1467-8292.00035.

[19] S. I. Report, "Porphyry Copper Deposit Model Scientific Investigations Report 2010 – 5070 – B," 2010.

[20] L. Troccoli, W. Senior, R. De, and L. Cruz, "Copper: Essential and Noxious to Aquatic Organisms 105 5 Copper Essential and Noxious to Aquatic Organisms 5 Copper Essential and Noxious to Aquatic Organisms," no. October, 2019, [Online]. Available: https://www.researchgate.net/publication/336899879.

[21] I. Villaescusa, N. Fiol, M. Martínez, N. Miralles, J. Poch, and J. Serarols, "Removal of copper and nickel ions from aqueous solutions by grape stalks wastes," *Water Res.*, vol. 38, no. 4, pp. 992–1002, 2004, doi: 10.1016/j.watres.2003.10.040.

[22] N. Blossom, "Copper in the Ocean Environment," *Am. Chemet Corp.*, no. 406, pp. 1–8, 2015, [Online]. Available: http://www.chemet.com/assets/1/6/Copper_and_the_Ocean_Environment.pdf.

*[23]* A. A. Al-Homaidan, H. J. Al-Houri, A. A. Al-Hazzani, G. Elgaaly, and N. M. S. Moubayed, "Biosorption of copper ions from aqueous solutions by Spirulina platensis biomass," *Arab.*

*J. Chem.*, vol. 7, no. 1, pp. 57–62, 2014, doi: 10.1016/j.arabjc.2013.05.022.

[24] F. E. Pinardag, "Modified Acrylic Hydrogels As Controlled Release Systems a Thesis Submitted To the Graduate School of Natural and Applied Sciences of Middle East Technical University By Fatma Esra Pinardağ in Partial Fulfillment of the Requirements for the Degree of Mast," no. May, 2006.

[25] A. G. Qaddoori, "Antimicrobial evaluation of selected medicinal plants using molecular approach," *Biomed. Res. Cent.*, p. 261, 2016, [Online]. Available: http://usir.salford.ac.uk/id/eprint/40178%0A.

[26] M. Said, Y. Atassi, M. Tally, M. Kattan, and L. Kouba, "Antimicrobial Chitosan-g-poly(AMPS-co-AA-co-AM)/Ground Basalt Composite Hydrogel: Synthesis and Characterization," *ChemRxiv*, no. 2, 2018, doi: 10.26434/chemrxiv.6233393.v2.

[27] N. Mohamed and A. E. Deghiedy, "Synthesis and Characterization of Superabsorbent Hydrogels Based on Natural Polymers: Kappa Carrageenan," 2016, doi: 10.15242/iae.iae1116411.

[28] L. Ren, "Production of Alginate Beads," pp. 1–81, 2009.

[29] Ý. A. Mørch, I. Donati, B. L. Strand, and G. Skjåk-Bræk, "Effect of Ca2+, Ba2+, and Sr2+ on alginate microbeads," *Biomacromolecules*, vol. 7, no. 5, pp. 1471–1480, 2006, doi: 10.1021/bm060010d.

[30] A. E. Odinaka, "Synthesis of Alginate-Graft-Poly ( benzyl methacrylate ) Copolymer by Chemical and UV Initiation," no. August, 2015.

[31] F. Yasmin, "D EVELOPMENT OF A LGINATE N ANOSPHERES AS A P ROTEIN D

ELIVERY D EVICE FOR C ARTILAGE T ISSUE E NGINEERING A Thesis Submitted to the College of Graduate and Postdoctoral Studies In Partial Fulfillment of the Requirements For the Degree of Master of Scienc," no. November, 2018.

[32] S. L. Huang and Y. S. Lin, "The Size Stability of Alginate Beads by Different Ionic Crosslinkers," *Adv. Mater. Sci. Eng.*, vol. 2017, 2017, doi: 10.1155/2017/9304592.

[33] R. J. Mumper, A. S. Huffman, P. A. Puolakkainen, L. S. Bouchard, and W. R. Gombotz, "Calcium-alginate beads for the oral delivery of transforming growth factor-β1 (TGF-β1): stabilization of TGF-β1 by the addition of polyacrylic acid within acid-treated beads," *J. Control. Release*, vol. 30, no. 3, pp. 241–251, 1994, doi: 10.1016/0168-3659(94)90030-2.

[34] N. G. Science, "Making Gel Beads."

[35] R. H. Sampieri, "No 主観的健康感を中心とした在宅高齢者における健康関連指標に関する共分散構造分析Title," p. 634.

[36] H. Majeed, H. N. Bhatti, and I. A. Bhatti, "Replacement of sodium alginate polymer, urea and sodium bicarbonate in the conventional reactive printing of cellulosic cotton," *J. Polym. Eng.*, vol. 39, no. 7, pp. 661–670, 2019, doi: 10.1515/polyeng-2019-0076.

[37] K. I. Draget and C. Taylor, "Chemical, physical and biological properties of alginates and their biomedical implications," *Food Hydrocoll.*, vol. 25, no. 2, pp. 251–256, 2011, doi: 10.1016/j.foodhyd.2009.10.007.

[38] N. Peng, X. Zhang, H. Xu, and Y. Liu, "Polymeric Hydrogels with Antimicrobial Activity-A Review of Their Progress," *Biomed J Sci Tech Res*, vol. 23, no. 5, pp. 17810–17823,

2019, doi: 10.26717/BJSTR.2019.23.003973.

[39] H. Kaygusuz, E. Torlak, G. Akın-Evingür, İ. Özen, R. von Klitzing, and F. B. Erim, "Antimicrobial cerium ion-chitosan crosslinked alginate biopolymer films: A novel and potential wound dressing," *Int. J. Biol. Macromol.*, vol. 105, pp. 1161–1165, 2017, doi: 10.1016/j.ijbiomac.2017.07.144.

[40] "All Rights Reserved Copper Ion Adsorption By Chitosan Gel Nanoparticles and Calcium-Alginate Gel Beads," 2014.

[41] G. Uyar, H. Kaygusuz, and F. B. Erim, "Methylene blue removal by alginate–clay quasi-cryogel beads," *React. Funct. Polym.*, vol. 106, pp. 1–7, 2016, doi: 10.1016/j.reactfunctpolym.2016.07.001.

[42] G. Balkız, E. Pingo, N. Kahya, H. Kaygusuz, and F. Bedia Erim, "Graphene Oxide/Alginate Quasi-Cryogels for Removal of Methylene Blue," *Water. Air. Soil Pollut.*, vol. 229, no. 4, 2018, doi: 10.1007/s11270-018-3790-5.

## APPENDIX A

The oral presentation by A.T. Belhi, B. Orhan and H. Kaygusuz, Preparation of Copper Crosslinked Alginate Quasi-Cryogel Beads and Investigation of Their Swelling Characteristics at 4th International Conference on Physical Chemistry & Functional Materials (PCFM 21) is published as an abstract (p. 124, ISBN: 978-605-80511-1-9) and is related to this thesis.