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GRADUATE SCHOOL OF SCIENCE AND ENGINEERING

SYNTHESIS AND CHARACTERIZATION OF ALTERNAN EXOPOLYSACCHARIDE-BASED HYDROGEL AND CRYOGEL BIOMATERIALS

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MASTER OF SCIENCE THESIS

Department of Bioengineering

Program of Bioengineering

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Sanem BULUT

Signature

To continually remind myself of my potential and to my cherished family, whose constant support and encouragement in all my endeavors means the world to me

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LIST OF SYMBOLS

ΔL	Change in Length
CFU/ml	Colony Forming Unit per Milliliter
C	Concentration
F	Force
ε_f	Fracture Strain
σ_f	Fracture Strenght
W_g	Gel Fraction
σ_{nom}	Nominal Stress
ε	Strain
q_w	Swelling Equilibrium Ratio
W	Toughness
w/v	Weight per Volume
E	Young's Modulus

LIST OF ABBREVIATIONS

ATR	Attenuated Total Reflectance
BHI	Brain Heart Infusion
ECH	Epichlorohydrin
EPS	Exopolysaccharides
FTIR	Fourier-Transform Infrared
GA	Glutaraldehyde
GRAS	Generally Recognized as Safe
MHA	Mueller-Hinton Agar
MHB	Mueller-Hinton Broth
NaOH	Sodium hydroxide
NMR	Nuclear Magnetic Resonance
PEGDE	Poly (ethylene glycol) diglycidyl ether
PTFE	Polytetrafluoroethylene
PVA	Poly (vinyl alcohol)
SEM	Scanning Electron Microscope

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ABSTRACT

Synthesis and Characterization of Alternan Exopolysaccharide-Based Hydrogel and Cryogel Biomaterials

Sanem BULUT

Department of Bioengineering

Master of Science Thesis

Supervisor: Prof. Dr. Mehmet Murat ÖZMEN

Exopolysaccharides have recently gained attention in the search for novel biomaterials for tissue engineering, and wound dressings, due to their biocompatibility, biodegradability and renewable nature. Hydrogels and cryogels are widely used in tissue engineering applications as they have unique mechanical properties and swelling capabilities. The exopolysaccharides can be utilized to produce hydrogels and cryogels with remarkable properties.

This study is the first to produce hydrogel and cryogel biomaterials using alternan exopolysaccharide derived from *Lactobacillus reuteri* E81. Alternan was cross-linked with epichlorohydrin (ECH) to create hydrogels and cryogels with a 20% alternan concentration, prepared at 20°C and -20°C, respectively. The samples were characterized by FTIR, SEM, gel fraction (W_g), swelling equilibrium (q_w), swelling kinetics, and mechanical tests.

The FTIR analysis results confirmed the successful synthesis of both hydrogels and cryogels. Swelling studies showed that cryogels had a q_w value of 14.01±0.19, compared to 10.03±0.09 for hydrogels. Cryogels reached swelling equilibrium within 1 minute, while hydrogels took approximately 24 hours. SEM images

confirmed the macroporous structure of cryogels, with an average pore size of 54.67±33.68 µm. Mechanical tests indicated that swollen cryogels could withstand a stress of 26.1±1.35 kPa, while hydrogels could withstand 53.85±4.75 kPa. Additionally, vancomycin was incorporated into cryogels to provide antibacterial properties. SEM images of vancomycin-containing cryogels showed an average pore size of 50.24±21.21 µm, and mechanical tests showed dried samples could withstand stress of 1505±98 kPa. Antibacterial tests demonstrated effective inhibition against *Staphylococcus aureus* strains. It was concluded that cryogels swelled faster, had higher water retention, exhibited more flexibility, and had larger pores than hydrogels, making them more suitable for wound dressings. Thus, alternan exopolysaccharide-based gels, specifically cryogels loaded with antibacterial agents, hold significant potential for tissue engineering and wound dressing applications.

Keywords: Alternan, cryogel, hydrogel, vancomycin, wound dressing.

ÖZET

Alternan Ekzopolisakkarit Tabanlı Hidrojel ve Kriyojel Biyomalzemelerin Sentezi ve Karakterizasyonu

Sanem BULUT

Biyomühendislik Anabilim Dalı

Yüksek Lisans Tezi

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Ekzopolisakkaritler, biyouyumlulukları, biyobozunurlukları ve yenilenebilir doğaları nedeniyle doku mühendisliği ve yara örtüsü uygulamalarında son zamanlarda dikkat çekmektedir. Hidrojeller ve kriyojeller, benzersiz mekanik özellikleri ve şişme yetenekleri nedeniyle doku mühendisliği uygulamalarında yaygın olarak kullanılmaktadır. Ekzopolisakkaritler, dikkat çekici özelliklere sahip hidrojel ve kriyojel üretmek için kullanılabilmektedir.

Bu çalışmada *Lactobacillus reuteri* E81 mikroorganizmasından elde edilen alternan ekzopolisakkariti kullanılarak literatürde ilk defa hidrojel ve kriyojel biyomalzemeler üretilmiştir. Alternan, epiklorohidrin (ECH) ile çapraz bağlanarak %20 alternan konsantrasyonuna sahip hidrojel ve kriyojeller 20°C ve -20°C'de hazırlanarak elde edilmiştir. Bu örnekler FTIR, SEM, jel fraksiyonu (W_g), şişme dengesi (q_w), şişme kinetiği ve mekanik testler ile karakterize edilmiştir.

FTIR analizleri, hem hidrojel hem de kriyojellerin başarılı bir şekilde sentezlendiğini doğrulamıştır. Şişme çalışmaları, kriyojellerin q_w değerinin 14.01±0.19 olduğunu, hidrojeller için ise 10.03±0.09 olduğunu göstermiştir.

Kriyojeller 1 dakika içinde tam şişme dengesine ulaşırken, hidrojellerin şişme dengesine ulaşması yaklaşık 24 saat sürmüştür. SEM görüntüleri, ortalama gözenek boyutu 54.67±33.68 μm olan kriyojellerin makrogözenekli yapısını doğrulamıştır. Mekanik testler, şişmiş kriyojellerin 26.1±1.35 kPa, hidrojellerin ise 53.85±4.75 kPa gerilime dayanabildiğini göstermiştir. Kriyojellere sonradan antibakteriyel özellik kazandırmak için vankomisin eklenmiştir. SEM görüntüleri vankomisin içeren kriyojellerin ortalama gözenek boyutunun 50.24±21.21 μm olduğunu göstermiş ve mekanik testler, bu kriyojellerin 1505±98 kPa gerilime dayanabildiğini ortaya koymuştur. Antibakteriyel testler, *Staphylococcus aureus* suşlarına karşı etkili inhibisyon göstermiştir. Kriyojellerin daha hızlı şiştiği, daha yüksek su tutma kapasitesine sahip olduğu, daha esnek bir yapı sergilediği ve hidrojellere göre daha büyük gözeneklere sahip olduğu, bu nedenle yara örtüleri için daha uygun olduğu sonucuna varılmıştır. Böylece, alternan ekzopolisakkarit bazlı jellerin, özellikle antibakteriyel ajanlarla yüklenmiş kriyojellerin, doku mühendisliği ve yara örtüsü uygulamaları için önemli bir potansiyele sahip olduğu ortaya çıkarılmıştır.

Anahtar Kelimeler: Alternan, hidrojel, kriyojel, vakonmisin, yara örtüsü.

1
INTRODUCTION

1.1 Literature Review

Currently, biopolymers contribute to the development of advanced materials in the field of tissue engineering and biomaterials due to their unique properties and advantages. However, alternative biopolymers are needed in terms of biobased economy, easier modification, renewability, and different functional properties. Of these biopolymers, exopolysaccharides play important roles in various industries, including medicine, food, environment, agriculture, and biotechnology. Their unique properties make them valuable for a variety of functions, from improving product quality to contributing to environmental sustainability and human health.

Exopolysaccharides are carbohydrate-based biopolymers secreted by microorganisms into the extracellular environment [1]. The structure and properties of exopolysaccharides vary depending on the microorganism from which they are secreted, but their general structure is divided into heteropolysaccharides or homopolysaccharides [2,3]. Homopolysaccharides contain either fructose or glucose monomers and form α-D-glucan, β-D-glucan, fructans, and galactans [4].

Alternan is a homopolysaccharide in the α-D-glucan class. It can be produced as a metabolite by lactic acid bacteria relatively cheaply and with high efficiency. Alternan is synthesized by the enzyme alternansucrase, containing branched α-(1→6) and α-(1→3) glycosidic bonds in different ratios depending on the type of bacteria in which it is produced [5,6,7]. When reviewing the literature, it becomes apparent that its application as a volume enhancer, low glycemic sweetener, thickener, and prebiotic has been investigated [3,8]. Apart from these features, it is observed that there are not enough studies on the usage areas of alternan, and the studies are mostly focused on the production of alternan from microorganisms and the characterization of the obtained alternan [9,10,11].

Hydrogels are three-dimensional polymer networks connected by cross-links, showing hydrophilic properties [12]. Macroporous hydrogels, so-called cryogels,

are formed when ice crystals form in solution at sub-zero temperatures and cross-linking reactions occur in semi-frozen regions around these crystals. After gelation reactions (cryogelation), a macroporous structure is obtained as a result of the melting of ice crystals [13]. Cryogels, unlike traditional hydrogels, have attracted attention, especially in the biomedical field due to their super-fast swelling ability and superior mechanical properties [14]. Depending on the structure of the polymer from which it is produced, biocompatible, highly biomimetic, antibacterial cryogels can be obtained. These properties make cryogels a good candidate as tissue scaffolds in tissue engineering and wound dressings in wound healing [15].

Wound dressings are materials that play a key role in optimizing the wound-healing process. The material intended for use in wound dressing must have certain properties. The most important among these features is biocompatibility; the material must be non-toxic and not cause adverse biological reactions. Having moisture retention is very important in creating a suitable environment for cell migration and proliferation. It is necessary to have permeability properties in order to facilitate optimal gas exchange and water vapor transfer. Having a structure that can adapt to the wound bed and flexibility to prevent distortion during patient movement and reduce trauma during removal is essential. Additionally, a wound dressing having antimicrobial properties is very advantageous in terms of preventing and alleviating infections. Features such as easy application and removal, economic suitability, and biodegradability are also features that play an important role in the preference of wound dressings. While hydrogels provide these desired properties, they can also be produced specifically for specific tissues. Hydrogels exhibit superior performance in terms of providing features such as absorbing excess liquid and providing moisture to dry wounds, which are essential for wound dressings. Cryogels are more suitable for use as wound dressings due to their superior mechanical properties compared to traditional hydrogels [16].

The use of exopolysaccharides in various fields of tissue engineering and in the search for new biomaterials to be used as wound dressings is an area of recent interest [17]. Exopolysaccharides are renewable resources that are cost-effective, easily modifiable, and highly biocompatible and biodegradable. Moreover, the

various functional groups in their structures increase the possibility of forming new and diverse cross-links in gel formation, making them suitable biopolymers for hydrogel synthesis. In addition, features such as antioxidant properties, especially low immunogenicity, which have an accelerating effect on damaged tissue repair, and the stability of their chemical structures, make exopolysaccharides very suitable for the production of hydrogel biomaterials in tissue engineering [17,18].

Although there are hydrogels made with different exopolysaccharides in the literature, this thesis is the first to report hydrogel and cryogel biomaterials using the α-D-glucan group exopolysaccharide alternan, which is an alternative to traditional biopolymers.

1.2 Objective of the Thesis

The aim of this study is to produce alternan by isolating it from *Lactobacillus reuteri* E81 bacteria and to produce cryogel and hydrogel biomaterials from the extracted alternan, which has the potential to be used as a wound dressing and can be loaded with antibacterial agents such as vancomycin.

For this purpose,

- Obtaining alternan by isolating it from *Lactobacillus reuteri* E81 bacteria,
- Producing novel functional cryogels and hydrogels based on alternan through crosslinking with various cross-linkers,
- Incorporating the antibacterial agent vancomycin into the synthesized cryogels,
- Carrying out the characterization of the gels obtained,
- Determining the potential usage of the obtained cryogels as wound dressings

were the primary objectives of this thesis.

1.3 Hypothesis

Biopolymers hold significant importance in biomaterial production and wound dressing applications. However, when the literature is examined, it becomes evident that there is a need for the use and applications of different biopolymers in terms of the production of new functional biomaterials. It appears that alternan, which is in the exo-homopolysaccharide class, has not yet been thoroughly examined in the literature regarding biomaterials and is believed to have substantial potential in biomaterial production.

2
GENERAL INFORMATION

2.1 Hydrogels and Cryogels

Hydrogel is a polymeric material that exhibits the ability to swell and retain a significant amount of water but is insoluble in water (Figure 2.1) [19]. The soft and elastic structure of hydrogels originates from the polymer networks from which they are formed [20]. Hydrogels are used in many branches of healthcare and industry due to their unique properties such as swelling and deswelling.

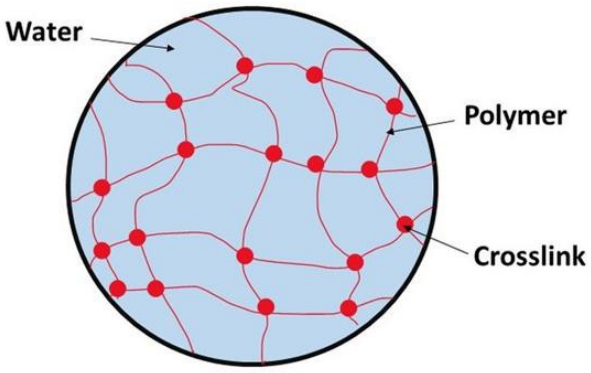


Figure 2.1 Schematic representation of the hydrogel structure [19]

Macroporous hydrogels, known as cryogels, are produced through a process in which ice crystals form within a solution at sub-zero temperatures. Cross-linking reactions take place in the semi-frozen areas surrounding these ice crystals. Following the gelation reactions, a macroporous structure emerges as the ice crystals melt (Figure 2.2) [21]. Cryogels have super-fast swelling ability and mechanically superior properties than traditional hydrogels due to their macroporous structure.

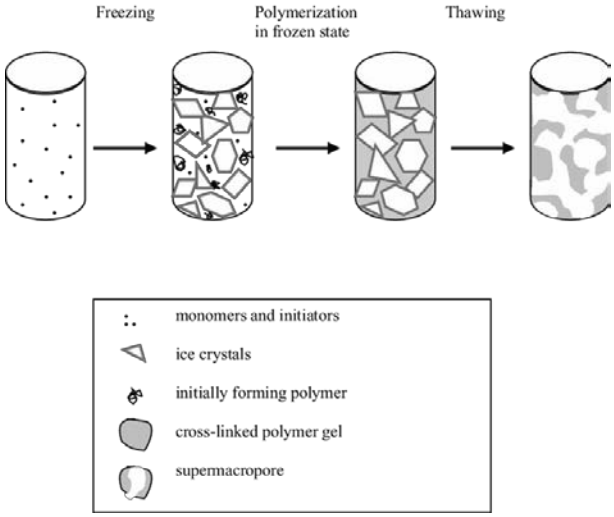


Figure 2.2 Schematic representation of cryogel formation [22]

Hydrogels are generally examined under seven headings; according to their source, cross-linking method, electrical charge, crystal structure, porous structure, synthesis method, and shape (Figure 2.3). The structural and mechanical properties of hydrogel vary depending on its place in this classification.



Figure 2.3 The classification of hydrogels [23]

2.1.1 Properties and Applications of Hydrogels and Cryogels

Hydrogels and cryogels are materials whose main property is swelling and de-swelling due to their hydrophilic structure. The properties of hydrogels and cryogels can be tailored depending on the polymer they are produced from and their intended use. As illustrated in Figure 2.4, hydrogels and cryogels find extensive applications in the biomedical field, including cell therapy, drug

delivery systems, tissue repair and regeneration, wound healing, and contact lens production. By using biopolymers, hydrogels, and cryogels can be produced that is biocompatible, non-toxic, and biodegradable [23]. For the intended use, such as use in tissue engineering, pore formation, and pore diameter in hydrogels can be controlled by changes in parameters such as production conditions, temperature, pH, and solvent type [24]. The mechanical properties of hydrogels and cryogels vary according to parameters such as cross-linking density and additive usage [25]. The important properties, such as biocompatibility, porosity and biodegradability, with applications of hydrogel and cryogels are examined in more detail in the next section.

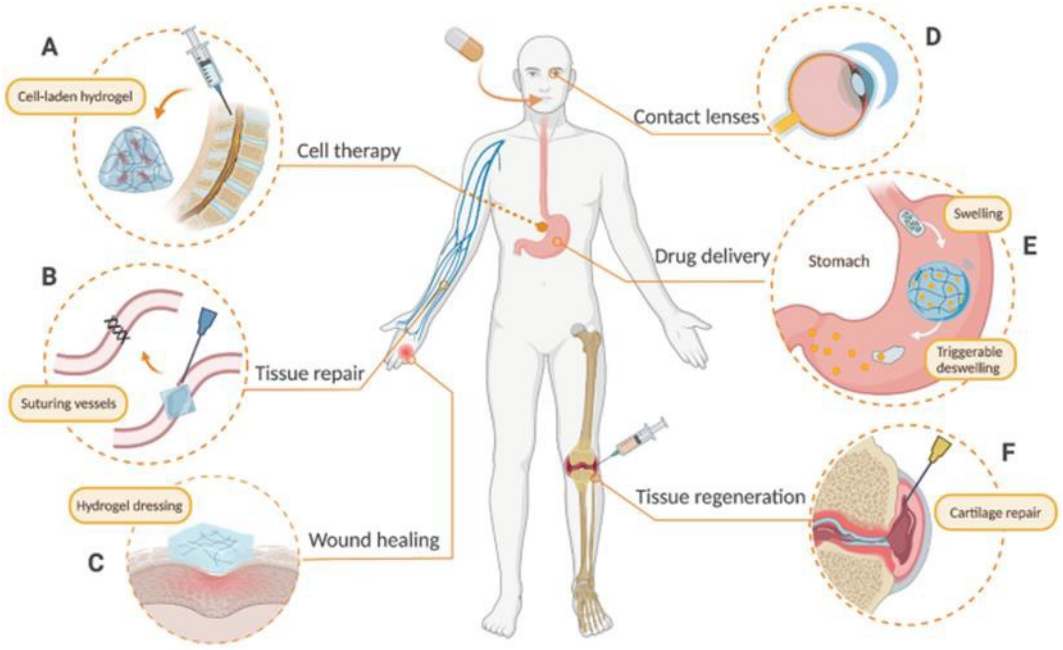


Figure 2.4 Application areas of hydrogels and cryogels in biomedical field [26]

2.1.1.1 Biocompatibility of Hydrogels and Cryogels

Biocompatibility is the ability of a material to be used in a specific application without provoking an adverse reaction from the host organism [27]. A biocompatible hydrogel should not have cytotoxic or carcinogenic effects and should not create an immune response in the area where it will be used. In particular, the source of the hydrogel to be used in wound dressing, scaffolding, and delivery systems should be selected accordingly [19]. To ensure the successful use of hydrogels and cryogels in medical and biotechnological fields, it is essential to enhance biocompatibility by carefully selecting materials,

modifying surfaces, and designing appropriate degradation profiles [28]. Hydrogels derived from biopolymers, especially polysaccharides, come to the fore in terms of biocompatibility [29].

2.1.1.2 Porosity of Hydrogels and Cryogels

The pores of hydrogels and cryogels can be tailored to the desired size and distribution during synthesis for specific applications, thereby improving their functionality and usefulness. Porosity in hydrogels is an important property in wound healing applications, wound dressings, for reasons such as allowing gas and fluid entry and exit and keeping the injured area moist [12]. In the context of tissue engineering, the porosity of hydrogels is a highly advantageous characteristic, as it significantly facilitates cell proliferation and growth. In drug delivery systems using hydrogels, therapeutic agents can be released in a controlled manner through pores [30].

2.1.1.3 Mechanical Properties of Hydrogels and Cryogels

The mechanical properties of hydrogels and cryogels are largely shaped by their swelling behavior [31]. Hydrogels show viscoelastic behavior and compress when a force is applied to them, and return to their old shape when this force is removed. Cryogels, on the other hand, have a stronger, more durable, and more elastic structure due to their macroporous structure [32]. Hydrogels and cryogels with improved mechanical properties by improving cross-linking density and network structures can be obtained according to the intended application.

2.1.1.4 Biodegradability of Hydrogels and Cryogels

Biodegradability is the ability of a material to break down into its small compounds by natural means, such as with the help of microorganisms [33]. In biomedical applications, biodegradability is a desired feature as there is no need for extra processing after the material has fulfilled its function. It is also important that the material does not create a toxic effect when broken into small pieces [34]. Biodegradability is an advantageous feature in hydrogel-based wound dressings. While the biodegradability of the wound dressing provides convenience for the patient, a controlled biodegradation has a positive effect by reducing the risk of infection during the wound healing process. With no need for re-application,

waste is reduced and injuries that may occur during re-application of the dressing are prevented.

2.2 Exopolysaccharides

Exopolysaccharides (EPS) are high molecular weight polymers consisting of monosaccharides such as glucose and fructose, secreted by microorganisms such as bacteria and archaea into their external environment [35]. The main reason why microorganisms secrete EPS is to form biofilm. Biofilm is a complex structure formed by microorganisms coming together and providing adhesion to the surface. While EPS within the biofilm provide protection to microorganisms, they also help cell-cell communication between them [36]. The structure and properties of EPS vary according to the microorganism they are secreted from. Although the areas of use vary depending on the type of EPS and the organism in which they are secreted, EPS are frequently used in different branches of industry, especially in the food industry. While EPS are used as thickeners, adhesives, and prebiotics in the food industry, they are also used as bioplastics, biofuels, and bioremediation agents in different industries. They are used as wound dressings and scaffolds in tissue engineering, as well as in drug delivery systems in pharmaceuticals due to their biocompatibility [35].

Exopolysaccharides are examined under two headings as homopolysaccharides and heteropolysaccharides, depending on the number of monomer types they contain (Figure 2.5) [37]. Homopolysaccharides and heteropolysaccharides are mentioned in more detail in the next section.

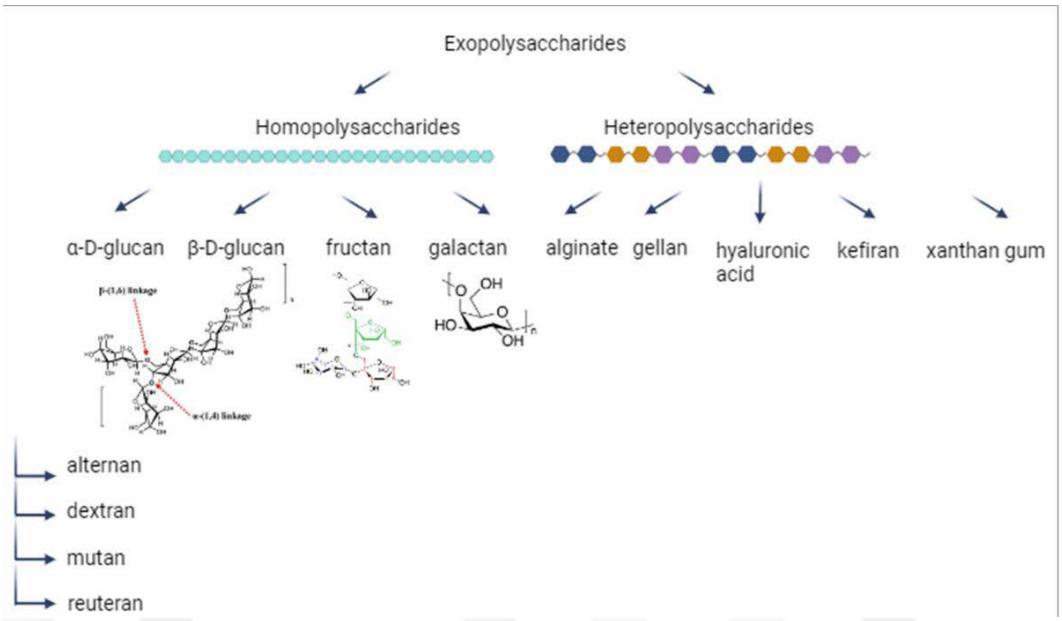


Figure 2.5 Classification of exopolysaccharides according to their structure

2.2.1 Exo-homopolysaccharides

Exo-homopolysaccharides consist of repeating a single type of monosaccharide and are examined under three subheadings according to the repeating unit: glucan, fructan, and galactan. Glucans are a type of EPS formed by glucose monomers bonding to each other via glycosidic bonds. They are divided into α-D-glucans and β-D-glucans according to the type of bonding of the glycosidic bond.

2.2.1.1 Glucans

The α-D-glucan class of homopolysaccharides mainly consists of alternan, dextran, mutan, and reuteran polymers (Figure 2.6). These exopolysaccharides can be produced as metabolites by lactic acid bacteria relatively cheaply and with high efficiency. α-D-glucan polysaccharides are synthesized by the glucansucrase enzyme, containing different amounts of glycosidic bonds depending on the type of bacteria in which they are produced [5]. Glucansucrase enzymes are alternansucrase, dextransucrase, reuteransucrase, and mutansucrase, depending on the type of glucan they synthesize. Alternansucrase produces alternan primarily containing α-(1→6) and α-(1→3) glycosidic bonds, dextransucrase produces dextran primarily containing α-(1→6) bonds, reuteransucrase produces reuteran primarily containing α-(1→4) bonds, and mutansucrase produces mutan containing α-(1→3) glycosidic bonds [6].

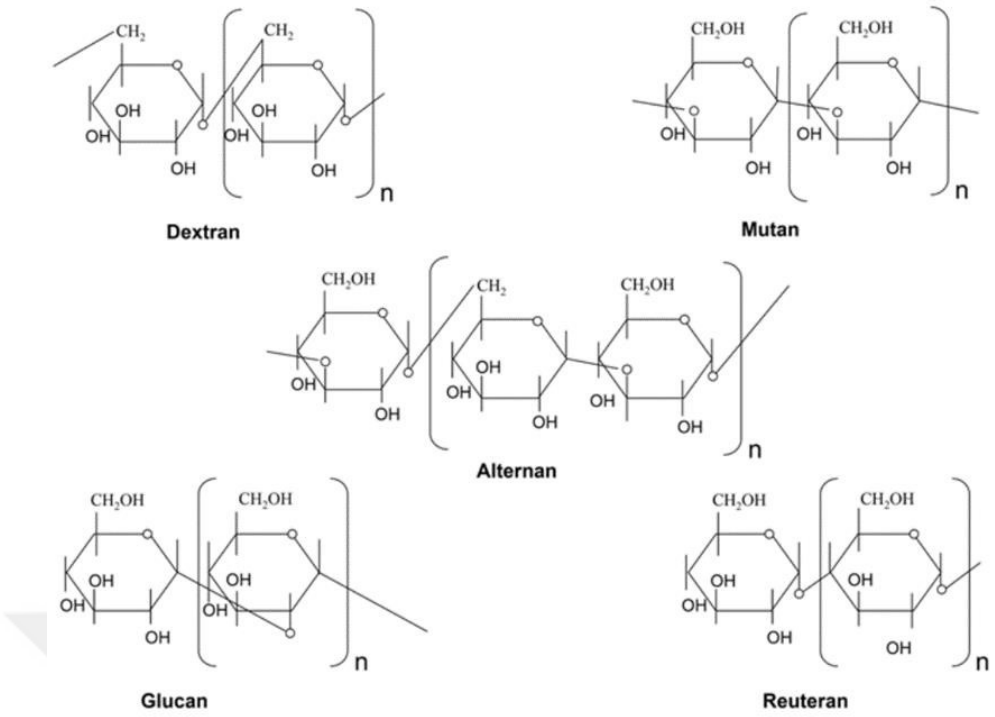


Figure 2.6 Structures of α-D-glucans [38]

Reuteran is a water-soluble α-D-glucan that consists primarily of α-(1→4) bonds but may also contain a small amount of α-(1→6) bonds. It has been reported in the literature that it is synthesized by different strains of *Lactobacillus reuteri* bacteria [39]. It is used in the food industry as a thickener, adhesive, and prebiotic [8,39].

Mutan is a type of α-D-glucan synthesized by *Streptococcal* group bacteria against acidic stress, is insoluble in water, contains predominantly α-(1→3) glycosidic bonds, but may also contain α-(1→6) bonds depending on its synthesis [40]. Some studies in the literature have shown that sulfated mutan becomes water-soluble and that this mutan type can potentially be used in the pharmaceutical industry by showing antibacterial activity [41].

Dextran is an α-D-glucan polymer which its main chain consisting of α-(1→6) glycosidic bonds, but may contain one or all of the α-(1→2), α-(1→3) or α-(1→4) branches in different proportions depending on its synthesis. The presence and ratio of these branches can change the physical and chemical properties of dextran [42]. When the literature is examined, it is observed that dextran with different branches is produced and characterized by bacterial genera such as *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Weissella*, but there is no production of

functional biomaterials from these characterized bacterial dextrans [9]. Dextran, which is used as a prebiotic, emulsifier, and stabilizer in the food industry due to its GRAS (generally safe) status [43], is also examined in the literature as a hydrogel material for use in drug delivery systems, scaffolds, wound dressings, biosensors, and bioprinting applications [44]. Ari et al. demonstrated that dextran-based cryogels, synthesized through the cross-linking of dextran with divinyl sulfone (DVS), are effective in the separation of toxic organic dyes [45]. As an example of wound healing applications, Ma et al. used dextran as a crosslinker by oxidizing it to obtain cryogel from chitosan, collagen, and polydopamine nanoparticles [46]. Pacelli et al. developed sponge-like cryogels using dextran methacrylate (DEX-MA) and polyethylene glycol dimethacrylate (PEG-DMA) polymers, intended for drug delivery systems [47]. Sugar et al. created scaffolds incorporating platelet-rich plasma (PRP) using oxidized dextran, designed for applications in bone tissue engineering [48].

Alternan is a homopolysaccharide synthesized by some lactic acid bacteria and containing both α-(1→3) and α-(1→6) glycosidic bonds. It is very soluble in water [49]. It has been reported that alternan has the ability to form a transparent film once dehydrated. A study conducted on alternan, produced using the alternansucrase enzyme isolated from the *Leuconostoc citreum* microorganism, showed that alternan forms nanoparticles at low concentrations and is soluble at higher concentrations, thus showing that alternan promises to be used in encapsulation applications and drug delivery systems [50]. Another study found that alternan supports growth, proliferation, and differentiation in human mesenchymal stem cells. This finding indicates that alternan has great potential in tissue engineering applications [51].

β-glucans are homopolysaccharides that can have a linear or branched structure containing one or more of β-(1→3), β-(1→4), and β-(1→6) glycosidic bonds. Bacterial-based β-glucans, such as the *Agrobacterium* species that synthesize curdlan, have a linear and β-(1→3) structure [52]. Curdlan is used in many areas in the food, pharmaceutical, and biotechnology industries due to its biodegradability and special gelling properties [53].

2.2.1.2 Fructan

Fructans are homopolysaccharides whose monomer is fructose and of two microbial origins: levan, synthesized by the enzyme levansucrase, and inulin, synthesized by inulosucrase [54]. Although fructans are widely used in the food industry, their use as biomaterials, especially as hydrogels, has also been examined in recent years. When levan-based hydrogels in the literature are examined, it can be seen that their potential for use as a wound dressing in the release of antifungal drugs, the composite of levan with hydroxyapatite as dental fillers, and the use of levan together with poly (vinyl alcohol) (PVA) as a virus-catching filter material have been investigated. When the usage areas of inulin are examined, it is seen that it has many applications in hydrogel form, especially in drug delivery systems. However, it appears that the inulin used in these applications is plant-originated, not microbial [55].

2.2.1.3 Galactan

Galactan is a homopolysaccharide composed of galactose monosaccharides. Structurally, they can be found in linear and branched structures. Galactans have an α-(1→6) glycosidic bond structure. They are used especially in the food industry as prebiotics. Galactan originates mostly from plants, and for this reason, there are limited studies in the literature on bacterial galactan [56,57].

2.2.2 Exo-heteropolysaccharides

Exo-heterosaccharides are polysaccharides formed by the combination of many monosaccharide types such as glucose, fucose, xylose, N-acetylglucosamine, galactose, galacturonic acid, N-acetylgalactosamine, rhamnose, and arabinose. Since they are formed by the combination of different monosaccharide types, they may contain many linear or branched combinations of α and β 1→4, 1→3 and 1→6 glycosidic bonds. The most well-known examples of exo-heteropolysaccharides used in various industries are alginate, xanthan gum, and gellan gum [58].

Among these biopolymers, alginate is an exo-heteropolysaccharide that is widely used in the biomedical field. Due to its ionic structure, it gels easily and is frequently preferred in wound dressings, drug delivery systems, scaffolding, and

encapsulation. There are many studies in the literature on hydrogel and cryogel using alginate alone or in composite form with various polymers [59].

Xanthan gum is an exo-heteropolysaccharide produced by the bacterium *Xanthomonas campestris*, consisting of glucose, mannose, and glucuronic acid monomers. There are many studies in which xanthan gum is used together with many biopolymers such as alginate, starch, cellulose, chitosan, and PVA to obtain hydrogels and cryogels for drug delivery systems and scaffolds [60].

Gellan gum, synthesized by the *Sphingomonas elodea* bacteria, finds its place especially in the food industry as a thickener and stabilizer. It is also used as a stabilizer in the cosmetic industry, in the production of edible biofilms and food packages due to its unique gelling and film-forming properties, in drug delivery systems in the forms of nanogels and microcapsules, in 3D bioprinting and in wound dressing applications [61].

2.3 Wound Dressings

Traditional wound dressing applications such as bandages, gauze, and woolen cotton have begun to be replaced by smart materials such as hydrogel, foam, and hydrocolloid films, which protect the wound, keep it moist and support wound healing. Traditional methods protect the wound against external influences, but since they prevent air and moisture passage to the wound, the healing time is long. Modern wound dressings, with the active agents they contain, both protect the wound more effectively from various infections and accelerate the wound healing process by supporting cell proliferation [62].

2.3.1 Wound Healing

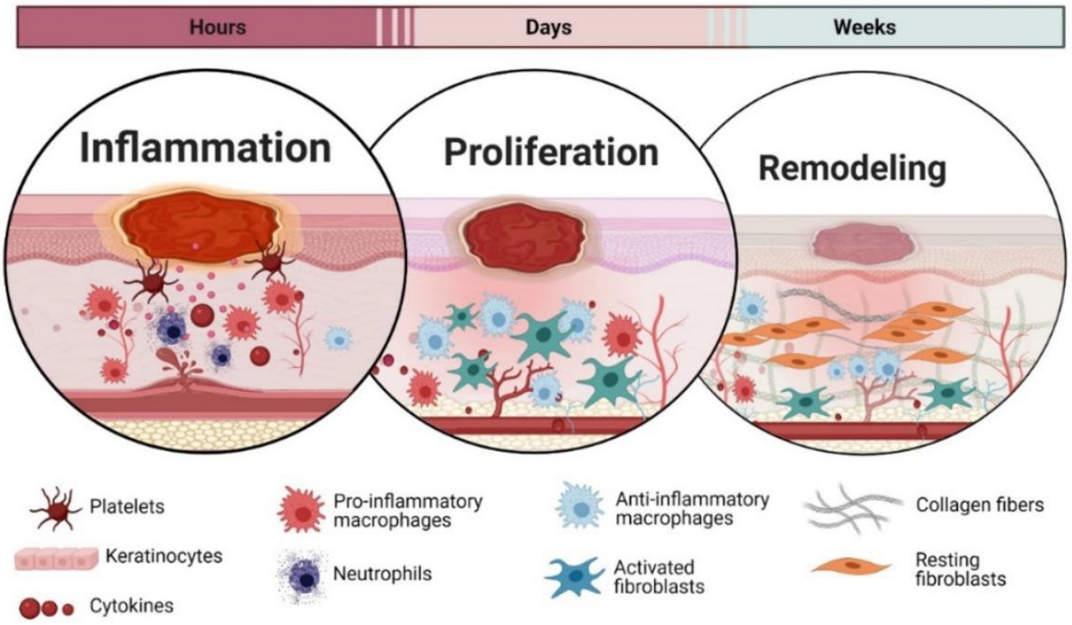


Figure 2.7 Stages of wound healing [63]

Figure 2.7 demonstrates that wound healing occurs in four stages: hemostasis, inflammation, proliferation, and remodeling. Bleeding occurs during the hemostasis phase and the first goal in wound dressing applications is to stop this bleeding. The inflammation phase begins within a few hours after wound formation occurs. At this stage, the body tries to prevent infection. It is a desirable feature for the wound dressing to support the cells in preventing infection. The proliferation phase is the phase in which cells begin to replace damaged tissue with new tissue with the help of fibroblasts. At this stage, the wound dressing must facilitate gas exchange for the new tissue cells and allow the passage of nutrients that promote cell proliferation to reach the newly formed cells. In the last stage, remodeling, epithelial tissue formation occurs. The tissue reconstructed during the proliferation phase is structurally strengthened at this stage and its properties are brought closer to the original tissue. It is a long phase that can last months or years [64]. Knowing the stages of wound healing is very important in designing effective wound dressings to support wound healing not only as a whole but also during the healing stages.

2.3.2 Properties of Wound Dressings

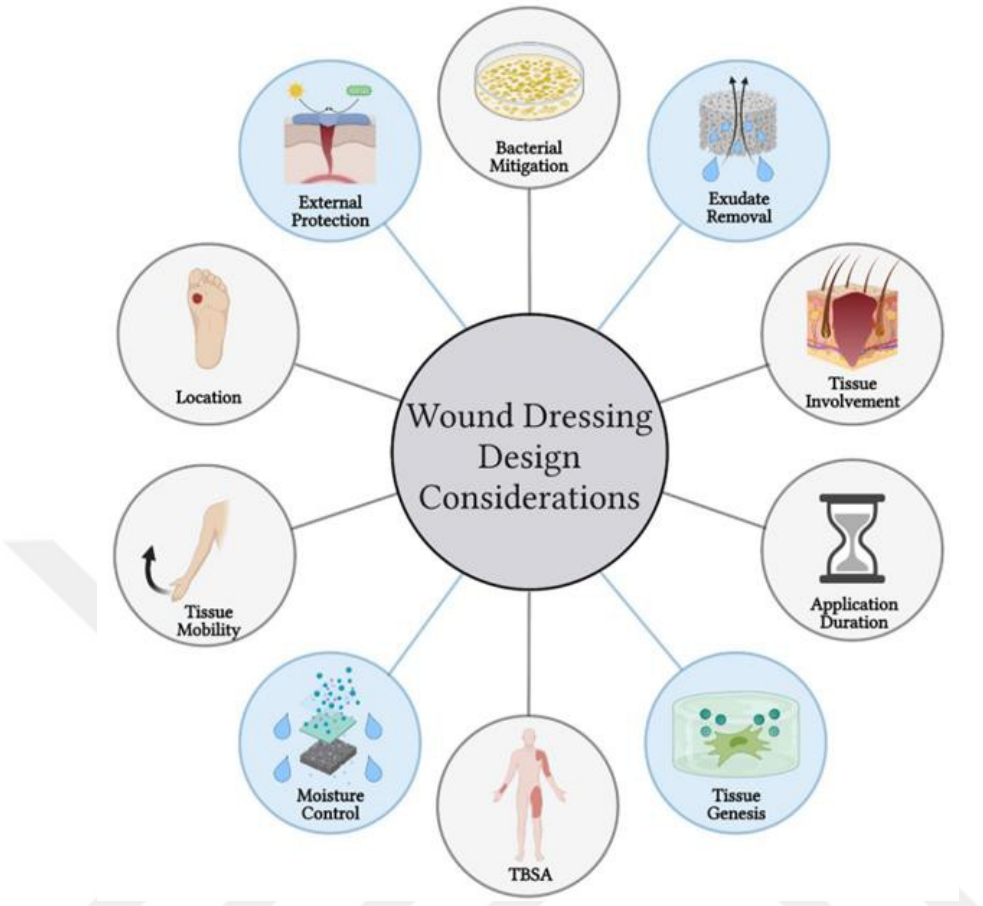


Figure 2.8 Parameters to consider when designing wound dressing [65]

When developing a wound dressing, it is crucial to carefully consider the material selection and its properties. The material to be used must be biocompatible, biodegradable, bioactive, elastic, allow gas entry and exit, provide moisture, and have hemostatic properties. It is a great advantage if the material has an antibacterial effect. Also, low material and production costs are a desirable feature when producing large-scale products [65].

As seen in Figure 2.8 the location and structure of the area where the wound dressing will be applied are also important parameters that should be evaluated when designing a wound dressing.

2.4 Exopolysaccharide-Based Hydrogels and Cryogels as Wound Dressings

Exopolysaccharides have been a subject of interest and have been researched for many years, but their application areas, especially in the health sector, have been expanded more recently [66]. In the examination of current literature, there has been a notable rise in research focusing on the application of EPS as materials for wound dressings, particularly within the last five years. Chang et al. reported the production of a hydrogel potentially suitable as a wound dressing, achieved through cross-linking welan gum derived from *Sphingomonas* bacteria with citric acid and incorporating the antibiotic ciprofloxacin into the resulting hydrogels. The study reported that while the hydrogels exhibited high biocompatibility, they required additional support from different materials due to insufficient mechanical properties [67]. Vazquez-Ayala et al. reported the production of hydrogel sponges through the cross-linking of chitosan with citric acid, incorporating an unspecified EPS sourced from *Porphyridium purpureum* microalgae. It has been reported that this material, which has the potential to be used as a wound dressing in diabetic wounds, showed a good antibacterial effect against *E. coli* and *S. aureus* bacteria [68]. In another study, the hydrogel was obtained by cross-linking EPS from *Cryptococcus laurentii* 70766, which consists of glucuronic acid, with horseradish peroxidase enzyme. This hydrogel was compared with its counterpart prepared with alginate. It has been reported that EPS-based hydrogel exhibits similar swelling and mechanical properties to alginate hydrogel and has the potential to be used as a wound dressing in humans [17]. In a study involving anionic EPS derived from *Nostoc* sp. PCC 7913 and PCC 7936 bacteria, it was reported that hydrogels accelerating wound healing were obtained using $FeCl_3$ [69]. It appears that there is a lack of sufficient studies on cryogels produced from microbial EPS for use as wound dressings. Our research will contribute to the literature by developing alternan cryogels, both with and without vancomycin.

In this thesis study, alternan exopolysaccharide-based cryogel and hydrogel biomaterials that can be used as wound dressings will be synthesized. This study is highly original as it marks the first time in the literature that cryogels and hydrogels have been synthesized using alternan. In the following sections, the preparation of alternan, the synthesis of gels, and the production of cryogels containing vancomycin were carried out.

3
MATERIALS AND METHOD

3.1 Materials

The materials used in this study are given in Table 3.1 along with their intended use and their supplier.

Table 3.1 Materials used in this study

Material	Supplier	Intended Use
Epichlorohydrin (ECH)	Fluka	Crosslinker
Poly(ethylene glycol) diglycidyl ether (PEGDE)	Sigma-Aldrich	Crosslinker
Glutaraldehyde (GA)	Merck	Crosslinker
Sodium hydroxide	Sigma-Aldrich	Solvent
Vancomycin hydrochloride	Sigma-Aldrich	Antibacterial agent
Milli Q pure water	YTU / Department of Bioengineering / Biopolymer and Vaccine Laboratory	Solvent / Washing /Swelling studies
MRS Broth	YTU / Department of Food Engineering	For obtaining alternan from *Lactobacillus reuteri* E81

Table 3.1 Materials used in this study (continued)

BHI (Brain Heart Infusion) Broth	YTU / Department of Food Engineering	For obtaining alternan from *Lactobacillus reuteri* E81
Ethanol	YTU / Department of Food Engineering	For obtaining alternan from *Lactobacillus reuteri* E81
Meat/beef extract	YTU / Department of Food Engineering	For obtaining alternan from *Lactobacillus reuteri* E81
Peptone casein	YTU / Department of Food Engineering	For obtaining alternan from *Lactobacillus reuteri* E81
Sodium acetate	YTU / Department of Food Engineering	For obtaining alternan from *Lactobacillus reuteri* E81
Tween 80	YTU / Department of Food Engineering	For obtaining alternan from *Lactobacillus reuteri* E81

Table 3.1 Materials used in this study (continued)

Magnesium Sulfate heptahydrate	YTU / Department of Food Engineering	For obtaining alternan from *Lactobacillus reuteri* E81
Sucrose	YTU / Department of Food Engineering	For obtaining alternan from *Lactobacillus reuteri* E81
Mueller-Hinton Agar (MHA)	YTU / Department of Molecular Biology and Genetics / Microbiology Laboratory	Antibacterial test medium
Mueller-Hinton Broth (MHB)	YTU / Department of Molecular Biology and Genetics / Microbiology Laboratory	Antibacterial test medium
Staphylococcus aureus (ATCC 25923) and 35OSSA, 37MORSA, 39MRSA, 87MSSA	YTU / Department of Molecular Biology and Genetics / Microbiology Laboratory	Bacterial strains for antibacterial testing

3.2 Instruments

The instruments used in this study are given in Table 3.2.

Table 3.2 Instruments used in this study

Instrument	Brand
Centrifuge	Himac
Freeze dryer	Heto / Drywinner DW 1.0-60e
FTIR	Bruker Tensor 27
Incubator	Biosan ES20
Magnetic stirrer	Heidolph MR300
NMR Spectroscopy	Agilent 400 MHz
SEM	ZEISS EVO 10
Ultra-pure water device	Millipore MilliQ Gradient
Vortex	Heidolph Reax Top
Drying oven	Binder
Refrigerator	Ariston
Cryostat	Kerman
Compression device	Zwick Roell Z0.5 TH

3.3 Methods

3.3.1 Exopolysaccharide Extraction

In this study, alternan, one of the exopolysaccharides of *Lactobacillus reuteri* E81 bacteria, was used as a polymer. To obtain alternan, as illustrated in Figure 3.1, initially, a medium was prepared containing BHI (Brain Heart Infusion) broth supplemented with meat/beef extract, peptone casein, sodium acetate, Tween 80, magnesium sulfate heptahydrate and sucrose to support the growth and proliferation of *Lactobacillus reuteri*. Subsequently, 50 ml of *Lactobacillus reuteri*, previously incubated in MRS broth, was transferred and inoculated into this prepared BHI medium. The bacteria were then incubated at 37°C for 24 hours. After incubation, the bacteria were centrifuged at 10300 rpm and 4°C for 20 minutes to collect the supernatant. The supernatant obtained was mixed with an equal volume of ethanol and incubated overnight at 4°C. The ethanol-supernatant mixture, which had been incubated overnight, underwent a second centrifugation under the same conditions as the initial process, resulting in the collection of pellets settled at the bottom. The pellets were dissolved in a certain amount of water, and ethanol was added to the mixture in double the amount of the resulting solution. The mixture was then left at 4°C overnight. The mixture was subjected to a final centrifugation and the pellet was collected once more. It was dissolved in water and stored at -20°C until drying with a lyophilizer [7].

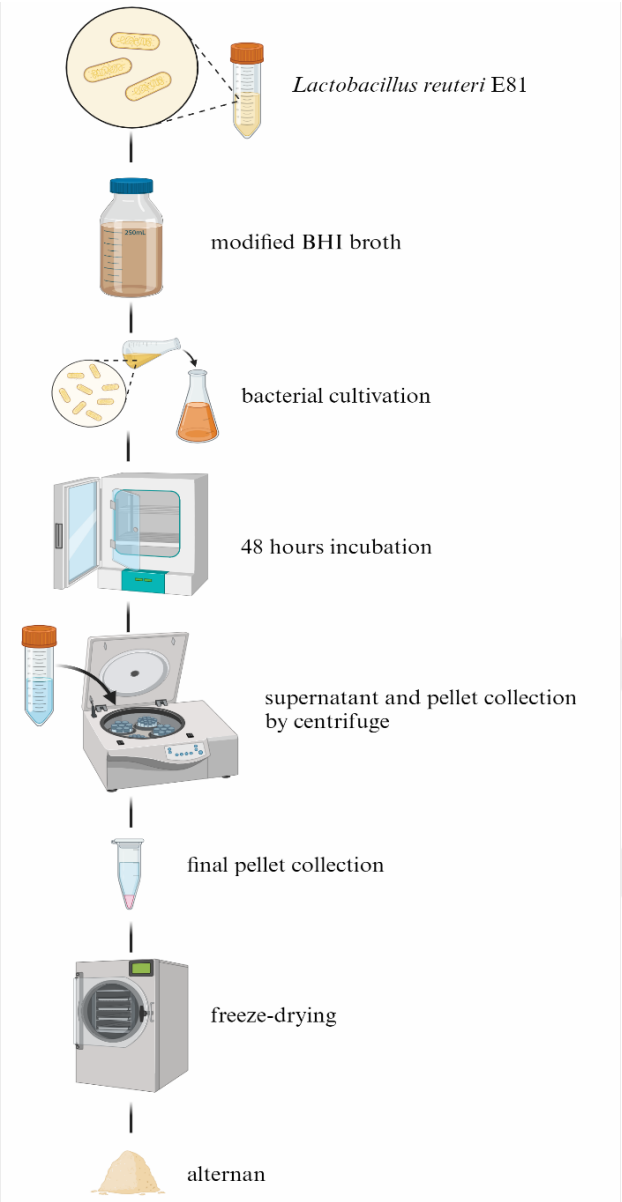


Figure 3.1 Alternan extraction from the bacterium *Lactobacillus reuteri*

3.3.2 Preliminary Experiments

Since alternan-based hydrogels and cryogels were to be produced for the first time, alternan cross-linking was attempted using epichlorohydrin (ECH), glutaraldehyde (GA), and polyethylene glycol diglycidyl ether (PEGDE).

After cross-linker selection was made, experiments were performed with different concentrations of alternan to optimize the gels. For this purpose, trials were initially carried out at 20%, 30%, and 40% alternan concentrations. The first experiments were carried out in round petri dishes coated with polytetrafluoroethylene (PTFE) to obtain cryogels in film form. Cryogels with different concentrations were produced in the proportions specified in Table 3.3,

by first mixing the polymer with NaOH solution at 500 rpm for 60 minutes, then adding ECH to the mixture, mixing for 5 minutes, pouring it into petri dishes and keeping it at -20°C for 24 hours in the freezer.

Table 3.3 Materials and quantities utilized for the preparation of alternan cryogels with different polymer concentrations

Polymer Concentration (%)	Polymer (g)	Polymer:Cross-linker ratio (w/w)	2.8 M NaOH (mL)
20	0.3	6:5	1.5
30	0.45	9:5	1.5
40	0.6	12:5	1.5

In the next stage, cross-linker ratio optimization was performed. Cryogels with 20% polymer concentration, prepared with 0.3 g alternan and 1.5 ml 2.8M NaOH, were produced with alternan/ECH (w/w) ratios of 6:3, 6:5 and 6:7.

Optimization was carried out by changing the reaction time, another effective parameter in the production of hydrogel and cryogel. 1, 3, and 7 days were chosen as the cryogelation time for 20% alternan cryogels prepared with 0.3 g alternan, 1.5 ml 2.8 M NaOH, and alternan/ECH (w/w) ratio of 6:3.

In order to see the effect of the freezing rate, 20% alternan cryogel was produced in a cryostat and refrigerator at -20°C for 24 hours. While the cryostat offers rapid freezing of the cryogel, the freezer facilitates a slower freezing process. Consequently, cryogels frozen using these two methods were subsequently examined.

Lastly, although the final alternan concentration was selected as 20%, cryogels with alternative concentrations of 10% and 15% were also examined in the next step to see the swelling behaviors of lower polymer concentrations. Cryogels were prepared using the proportions in Table 3.4.

Table 3.4 Materials and quantities utilized for the preparation of alternan cryogels with different polymer concentrations

Polymer Concentration (%)	Polymer (g)	Polymer:Cross-linker ratio (w/w)	2.8 M NaOH (mL)
10	0.15	2:2	1.5
15	0.225	3:2	1.5
20	0.3	4:2	1.5

Through the conducted trials, the optimal formulation was achieved.

3.3.3 Synthesis of Blank Alternan Hydrogel and Cryogels

In order to obtain alternan-based hydrogel and cryogels, as illustrated in Figure 3.2, firstly 2.8 M NaOH solution was prepared. Following that 0.3 grams of alternan was weighed and added into 1.5 ml of 2.8 M NaOH solution. The mixture was mixed in a magnetic stirrer at 500 rpm for 60 minutes. When a completely homogeneous mixture was obtained, the crosslinker ECH was added to the solution as a polymer/crosslinker (w/w) 2:1 ratio. After mixing for 5 minutes, the solution was drawn into the 1 ml syringe. While the syringes to be cryogel were placed in the freezer at -20 °C, the hydrogels were kept at room temperature of 20 °C for 24 hours. At the end of 24 hours, the syringes were cut and the obtained cylindrical gels were placed in containers containing 100 ml of pure water to remove impurities and then subjected to swelling. The gels were weighed and their water was changed periodically until the gels reached their swelling equilibrium weight. While the cryogels that reached swelling equilibrium were dried in the lyophilizer, the hydrogels were dried in the oven at 40 °C.

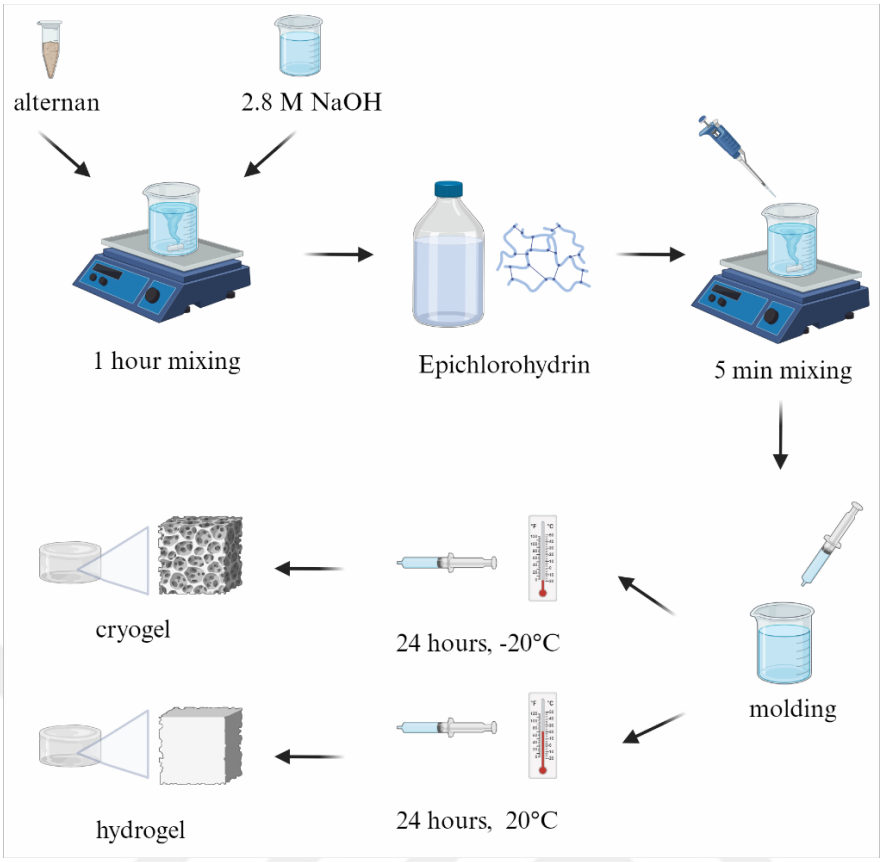


Figure 3.2 Synthesis of gels from the obtained alternan

3.3.4 Synthesis of Vancomycin-containing Hydrogel and Cryogels

Dried hydrogels and cryogels were cut into disc shapes. Vancomycin, an antibiotic, was weighed to 1 mg and then dissolved in 1 mL of distilled water, followed by vortexing to ensure complete dissolution. Afterward, 10 µL of the prepared vancomycin solution was pipetted and applied onto the surface of each disc-shaped cryo and hydrogel. The gels were then allowed to completely absorb the antibiotic solution. Finally, the vancomycin-loaded gels were placed in an oven at 40°C to ensure thorough drying.

3.4 Characterizations

3.4.1 Nuclear Magnetic Resonance (NMR) Spectroscopy

In order to determine the bond composition of alternan, ^{1}H and ^{13}C NMR spectroscopy analysis was carried out at 25 °C using an Agilent 400 MHz NMR device. For this purpose, 50 mg of alternan was dissolved in 1 mL D_2O and 600

µL of the solution was taken and analysis was performed. Interpretation of the data in the obtained spectra was made using MestReC software.

3.4.2 Fourier-Transform Infrared (FTIR) Spectroscopy

FTIR spectra of obtained alternan, crosslinker ECH, synthesized hydrogel and cryogel, and cryogel with vancomycin were acquired. The samples were analyzed with Bruker Tensor 27 FTIR spectrometer with attenuated total reflectance (ATR) apparatus at wavenumbers between 400 and 4000 cm^{-1}.

3.4.3 Swelling Studies

The swelling analysis of hydrogels and cryogels was determined by monitoring their weight from the initial state post-synthesis until they reached swelling equilibrium in pure water. The swelling ratio calculation was performed based on the change in weight as the gels absorbed water and expanded to their equilibrium state. For swelling analysis, the weights of the gels were measured at three stages using a precision scale: immediately following synthesis (m_0), after achieving swelling equilibrium in pure water ($m_{swollen}$), and after drying (m_{dry}). Using these measured values and polymer concentration (C), calculations for swelling equilibrium ratio (q_w) and gel fraction (W_g) were performed according to equations (3.1), and (3.2), respectively.

$$q_w = \frac{m_{swollen}}{m_{dry}} \tag{3.1}$$

$$W_g = \frac{m_{dry}}{m_0 \times C} \times 100 \tag{3.2}$$

$$M_{rel} = \frac{m_t}{m_{dry}} \tag{3.3}$$

For the swelling kinetics measurements dry gels with pre-determined weights, were immersed in distilled water and periodically removed at specified time intervals, including 1, 5, 10, 30, 60, and 120 minutes. Their weights (m_t) were recorded at each interval until they reached a state of equilibrium swelling. The kinetic swelling graph was drawn by calculating the time-dependent gravimetric swelling ratio (M_{rel}) from the data obtained according to equation (3.3).

3.4.4 Scanning Electron Microscope (SEM)

Morphological analyses of cryogels and hydrogels were performed using ZEISS EVO 10 SEM device. For the analysis firstly dry gels were coated with gold/palladium material for conductivity. Following the acquisition of images, the pore sizes were determined using the ImageJ software.

3.4.5 Compression Tests

Compression tests were conducted with Zwick Roell Z0.5 TH device which has a 500 N load cell. Tests were carried out at room temperature using cylindrical cryogels and hydrogels with a diameter of approximately 5 mm and the strain rate was adjusted to 1 min^{-1}. As a result of the test, nominal stress (σ_{nom}), strain (ε), fracture strength (σ_f), fracture strain (ε_f), Young's modulus (E), and toughness (W) values were obtained. These values were calculated by the instrument using the force applied to the gels (F), the original cross-sectional area of the gels (A_0), the change in length (ΔL), and the original length of the gels (L_0) with the equations shown in (3.4), (3.5), (3.6) and (3.7).

$$\sigma_{nom} = \frac{F}{A_0} \tag{3.4}$$

$$\varepsilon = \frac{\Delta L}{L_0} \tag{3.5}$$

$$E = \frac{\sigma_{nom}}{\varepsilon} \tag{3.6}$$

$$W = \int_0^{\varepsilon_f} \sigma d\varepsilon \tag{3.7}$$

3.4.6 Antibacterial Analysis

Antibacterial activity was assessed using the disk diffusion method. For this assay, 20% (w/v) alternan cryogels were fabricated into thin disk forms. Both surfaces of the gel disks were exposed to UV radiation for 30 minutes to ensure sterility. A 100 µl aliquot of a *Staphylococcus aureus* (ATCC 25923) suspension, with a concentration of 10^8 CFU/ml, was spread uniformly onto Mueller Hinton agar plates. Subsequently, 10 µL of aqueous solutions containing 10 µg, 20 µg, and 30 µg of vancomycin, corresponding to concentrations of 1 µg/µL, 2 µg/µL, and 3 µg/µL, respectively, were pipetted onto the cryogel disks. Following

absorption of the solutions by the disks, they were placed on the agar surface and incubated at 37°C overnight.

In the second stage, cryogels containing 1 µg/µL of vancomycin, were selected for testing against four different microorganisms with varying antibiotic resistances: 35OSSA, 37MORSA, 39MRSA, and 87MSSA. The antibacterial activity was evaluated using the same disk diffusion method as described above. Results were compared against those obtained using a commercial vancomycin disk.

4
RESULTS AND DISCUSSION

This study aimed to produce alternan-based hydrogel and cryogel biomaterials using the α-D-glucan group exopolysaccharide alternan for the first time in the literature. Alternan, a novel alternative to traditional biopolymers, was utilized to fabricate these materials, and their properties were compared. In this study, cryogels were synthesized at -20°C and subsequently dried using the freeze-drying method, whereas hydrogels were synthesized at 20°C and dried in an oven at 40°C. The differences between cryogels and hydrogels regarding the swelling, chemical, and mechanical properties of the resulting biomaterials were examined. In the final stage, vancomycin-containing cryogels were produced, and their potential as antibacterial wound dressings was evaluated. In the following sections, the characterization results are given in four parts: exopolysaccharide extraction results, preformulation experiment results, blank alternan hydrogel and cryogel results as well as vancomycin-containing cryogel results.

4.1 Structural Analysis of Extracted Alternan

4.1.1 NMR Spectroscopy Results

After extracting alternan exopolysaccharide from *Lactobacillus reuteri* E81 bacteria, NMR spectroscopy results were examined to elucidate the internal structure of alternan. In Figure 4.1, the sharp and intense peaks at the 4.8-5.0 ppm region are indicative of the anomeric protons of the sugar units in alternan. The anomeric proton typically appears downfield (at higher ppm) due to its attachment to an oxygen atom. Especially the peak seen between 5.3 ppm and 5.2 ppm indicates the presence of α-(1→3) glycosidic bond in the sample while the peak seen at 4.9 ppm indicates the presence of α-(1→6) glycosidic bond in the structure. The presence of these peaks suggests the characteristic glycosidic linkages in the polysaccharide structure of alternan.

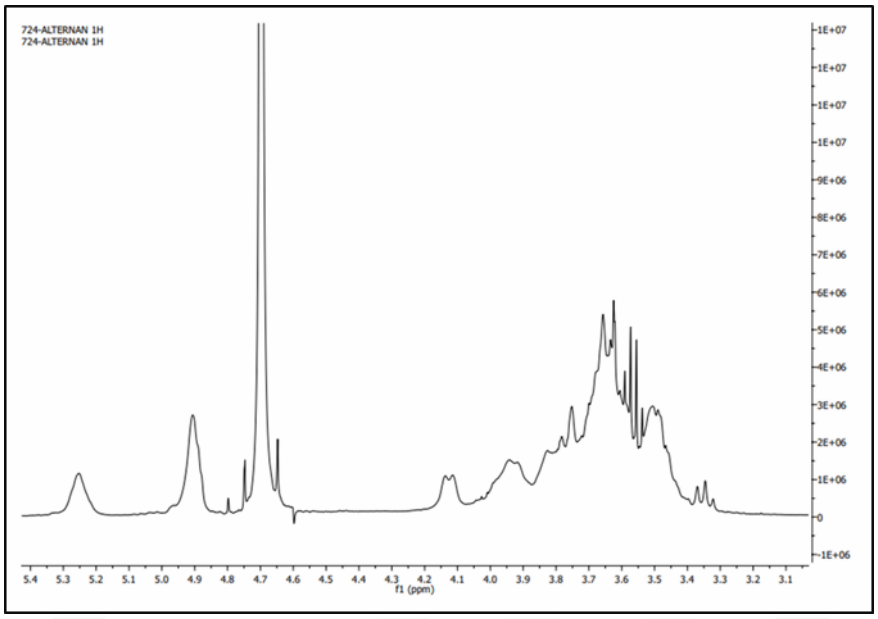


Figure 4.1 NMR spectrum of alternan

The multiple peaks at 3.5-4.0 ppm region are likely due to the various protons in the carbohydrate ring, such as those on carbon atoms 2, 3, 4, and 5. These protons experience different chemical environments, leading to multiple signals. This region is consistent with the typical chemical shifts for protons in polysaccharides, further supporting the identification of the material as alternan.

The peaks at 3.1-3.4 ppm are likely due to the methylene (CH2) protons in the sugar ring. The presence of multiple peaks in this region indicates a complex chemical environment, which is typical for polysaccharides with various branching patterns or different types of linkages. The NMR spectrum results of alternan from this study are consistent with the findings of Dertli et al., which focus on the characterization of α-glucan from Lactobacillus reuteri E81, later identified as alternan [6].

In conclusion, the NMR spectrum confirms the presence of alternan and its structural characteristics.

4.1.2 FTIR Spectroscopy Results

The FTIR spectrum of alternan is seen in Figure 4.2. The spectrum displays transmittance (%) on the x-axis and wavenumber (cm-1) values on the y-axis. In the spectrum, the broad peak around 3400 cm^{-1} indicates O-H stretching vibrations, which are typical of polysaccharides and suggest the presence of hydroxyl groups. Peaks around 2900 cm^{-1} correspond to C-H stretching, further

confirming the polysaccharide structure. The peaks around 1100-1000 cm^{-1} are indicative of C-O-C stretching vibrations, which are common in polysaccharides..

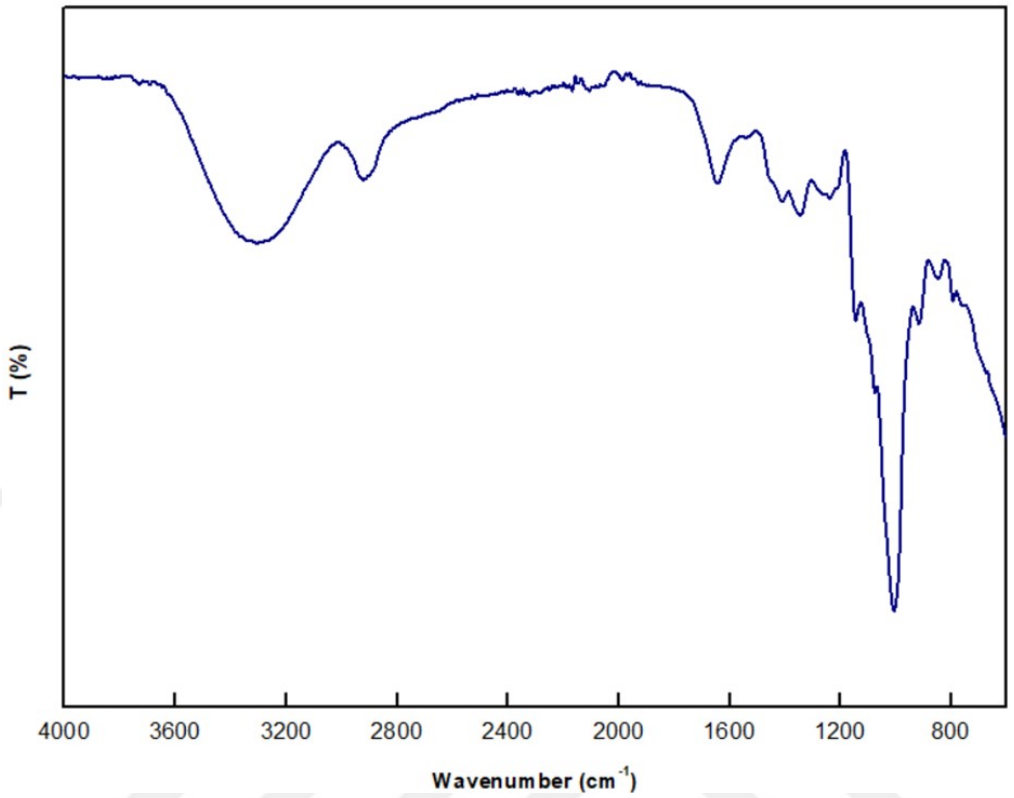


Figure 4.2 FTIR spectra of the alternan

NMR and FTIR results confirmed that the polymer extracted from *Lactobacillus reuteri* E81 was an alternan exopolysaccharide, leading to the initiation of gel preparation from alternan.

4.2 Preliminary Experiment Results

Several experiments have been conducted to optimize cryogels and hydrogels. Initially, efforts were undertaken to cross-link the alternan with PEGDE, GA, and ECH. As a result of many attempts using GA in different ratios, alternan could not be successfully cross-linked with GA. Cryogels obtained using PEGDE could not maintain their integrity even though they were initially cross-linked. It was observed that the cryogels and hydrogels produced using ECH were successfully cross-linked, and as a result, the decision was made to continue with ECH. The trials were initially carried out based on cryogel production and characterizations were carried out by examining the swelling behavior of the produced cryogels.

Firstly, the swelling behavior and gel fractions of the cryogels with 20%, 30%, and 40% alternan concentrations were examined. Swelling equilibrium ratios (q_w) of 20%, 30%, and 40% alternan cryogels were 14.60±0.1, 14.98±0.37 and 8.90±0.16, respectively (Figure 4.3a). The q_w values for 20% and 30% alternan cryogels are observed to be high and nearly identical, indicating that these cryogels have a high capacity to absorb water. It is seen that cryogel with 40% alternan concentration has low water absorption capacity due to its low q_w value. It is expected that the swelling equilibrium ratio decreases with increasing polymer concentration in hydrogels and cryogels due to the cross-linking density. This higher crosslink density restricts the polymer chains' mobility and reduces the space available for water to infiltrate and swell the cryogel. This can be explained by the fact that the cryogel with 40% alternan concentration has a lower q_w value than the cryogel with 20% and 30% alternan concentration.

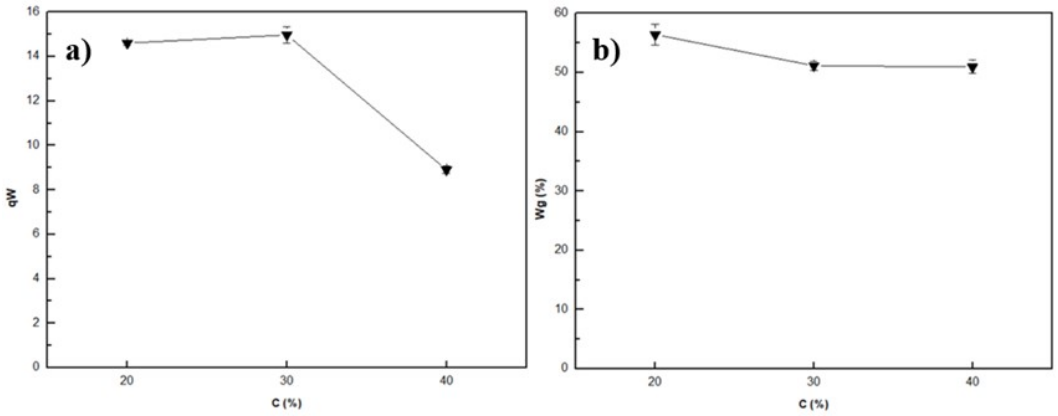


Figure 4.3 a)Swelling equilibrium ratios (q_w) of cryogels with different alternan concentrations b)Gel fraction (W_g) of cryogels with different alternan concentrations

When the gel fractions of cryogels (W_g) were examined it was seen that 20%, 30%, and 40% alternan cryogels have W_g values of 56.4±1.73, 51.15±0.74 and 50.95±1.12, respectively. As the polymer concentration in cryogels increases, the gel fraction is generally expected to increase. However, an inspection of Figure 4.3b reveals that the exact opposite situation is occurring. The reason for this behavior can be explained as the viscosity becomes too high for 30% and 40% polymer concentrations and the cross-linking reaction does not occur completely. The higher gel fraction value at 20% polymer concentration could be explained by

the fact that more cross-linking reactions occur while a homogeneous formation occurs.

At %20 alternan concentration, the cryogel showed a relatively high gel fraction and swelling ratio. This indicates a well-balanced network in which cross-linking is efficient enough to form a stable gel but the network remains open enough to allow significant water absorption. Due to the high gel fraction and swelling ratio with the ease of preparation, it was decided to continue with 20% alternan cryogel.

In the next stage, cross-linker ratio optimization characterization was performed at 20% alternan polymer concentration. Swelling behavior and gel fractions of cryogels with 20% polymer concentration, prepared with 0.3 g alternan and 1.5 ml 2.8M NaOH, which produced with alternan/ECH (w/w) ratios of 6:3, 6:5, and 6:7 were investigated.

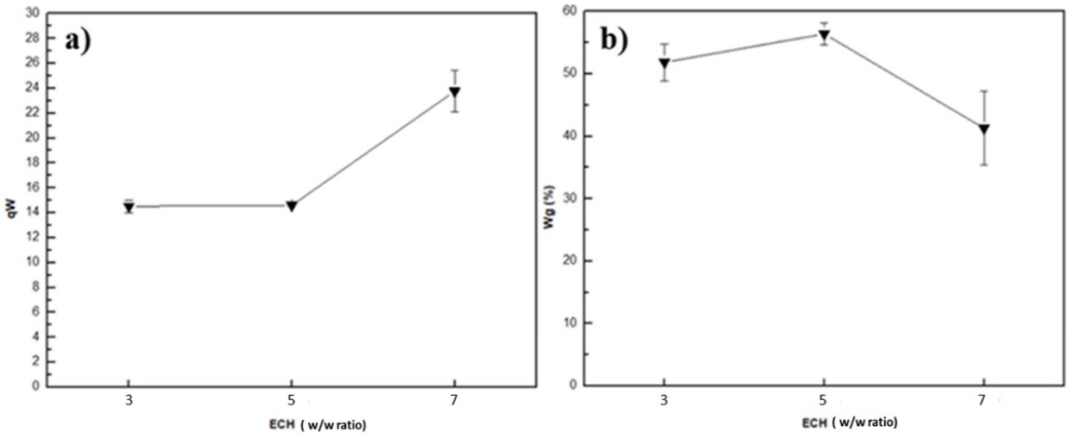


Figure 4.4 a)Swelling equilibrium ratios (qw) of 20% alternan cryogels with different amounts of ECH b)Gel fractions (Wg) of 20% alternan cryogels with different amounts of ECH

An examination of Figure 4.4a reveals that the swelling equilibrium values for ECH ratios of 3, 5, and 7 are 14.52±0.5, 14.6±0.1 and 23.76±1.67, respectively. When the gel fractions of these gels were examined, (Figure 4.4b) it was seen that values of 51.86±2.93, 56.39±1.73 and 41.28±5.91 were obtained for 3, 5, and 7 ECH ratios, respectively. When these two graphs are examined, it is seen that a more stable polymer network is formed for ECH ratios of 3 and 5. Lower gel fraction and higher swelling ratio values at the highest ECH concentration suggests that the network formed is less uniform with regions that are highly

cross-linked and others that are not. This could result in a structure that swells more due to the presence of loosely crosslinked or un-crosslinked regions.

Due to its optimal balance between gel fraction and swelling the alternan/ECH (w/w) ratio 6:3 was chosen.

Following that swelling studies of reaction time parameters, 1,3, and 7 days, were investigated for 20% alternan cryogels prepared with 2:1 alternan/ECH (w/w). It was observed that the swelling equilibrium ratio decreased as cryogelation time increased. A swelling equilibrium ratio of 18.34±1.79 was obtained for 1 day of reaction time, 16.84±0.72 for 3 days, and 13.55±0.47 for 7 days (Figure 4.5a).

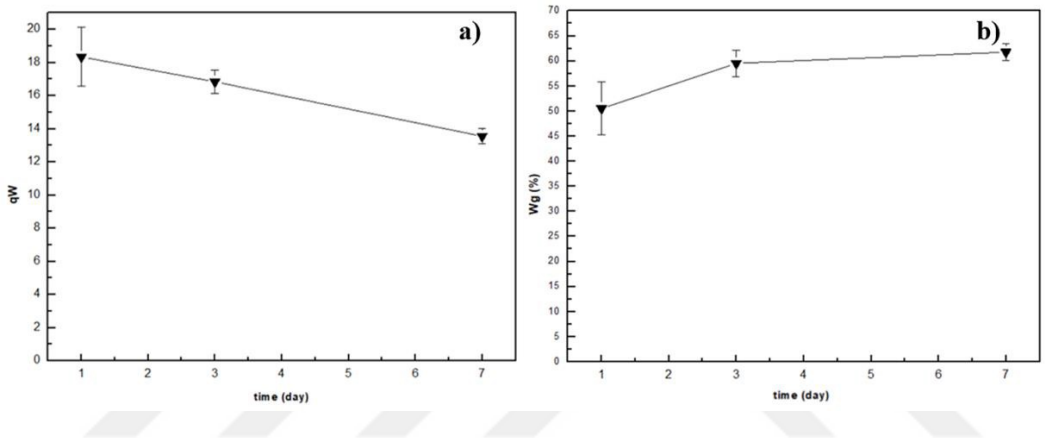


Figure 4.5 a)Swelling equilibrium ratios (q_w) of 20% alternan cryogels with different cryogelation time b)Gel fractions (W_g) of 20% alternan cryogels with different cryogelation time

As expected, the gel fractions of cryogels with a high swelling equilibrium value and a reaction time of 1 day were lower (Figure 4.5b). A gel fraction of 50.51±5.28 was achieved after 1 day of reaction time, 59.53±2.67 after 3 days, and 61.76±1.74 after 7 days. Although increasing the reaction time decreased the swelling equilibrium ratio and the gel fraction, no significant difference was observed between the 1 and 3-day cryogelation times. The 7-day cryogelation time was not preferred because it would be costly and time-consuming when considered on a large scale in production, and 1 day was chosen as the final cryogelation time.

To determine the impact of freezing rate on gelation, 20% alternan cryogel was produced in both cryostat and refrigerator at -20°C for 24 hours. While the cryogels prepared using the refrigerator were cross-linked within 24 hours, it was

observed that the gels prepared using the cryostat required at least 48 hours to cross-link.

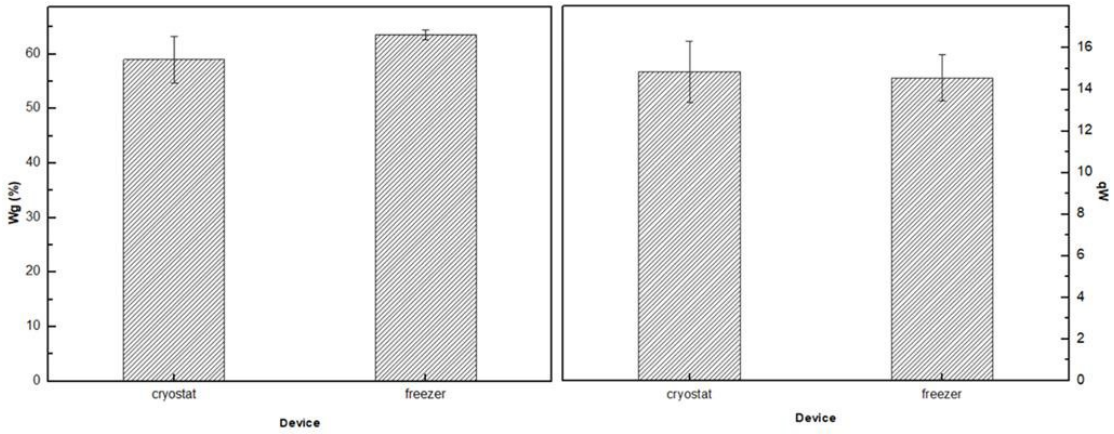


Figure 4.6 Gel fractions and swelling equilibrium ratios of 20% alternan cryogels according to the cryostat and refrigarator usage

When the use of cryostat and refrigerator was examined, as seen in Figure 4.6, no major difference was observed in terms of swelling behavior and gel fractions of the cryogels formed. The refrigerator continued to be used due to the longer reaction time required for cryogel synthesis in the cryostat.

Finally, swelling studies of cryogels with 10%, 15%, and 20% alternan concentrations (given in Table 3.4) were conducted.

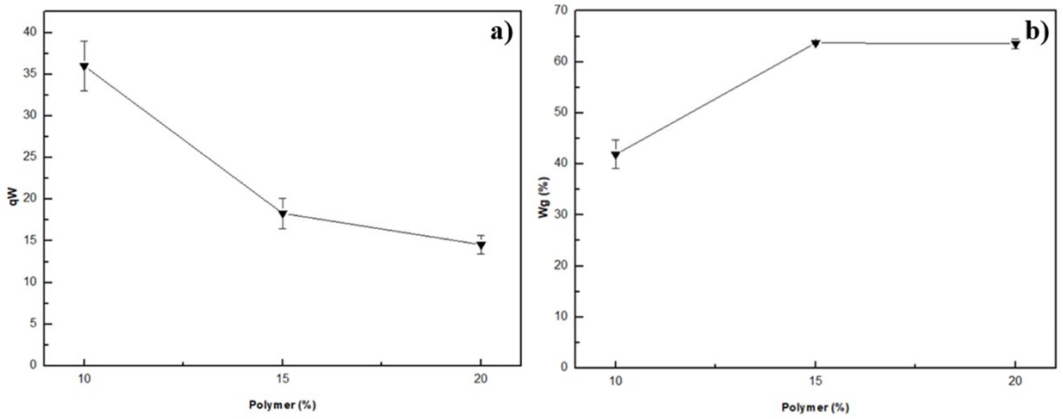


Figure 4.7 a)Swelling equilibrium ratios (q_w) of cryogels with different alternan concentrations b)Gel fractions (W_g) of cryogels with different alternan concentrations

It is expected that the swelling equilibrium ratio decreases with the increase in the polymer concentration. As can be seen in Figure 4.7a, cryogels with 10%, 15%, and 20% alternan concentrations behave as expected. The swelling equilibrium

ratio in 10% alternan cryogel is notably high with 35.99±2.97. In contrast, 15% and 20% alternan cryogels exhibit swelling equilibrium ratios close to each other, with values of 18.3±1.81 and 14.56±1.11, respectively. On the contrary, cryogels with a high swelling equilibrium ratio are expected to have lower gel fractions. When Figure 4.7b is observed, it was determined that these cryogels showed an expected behavior with the gel fraction values of 41.87±2.8, 63.72±0.29 and 63.55±0.96 for the alternan concentrations of 10%, 15% and 20%, respectively.

To observe the swelling behavior of the cryogels after drying, the cryogels were thrown into pure water, and measurements were made at certain periods. As a result, a swelling kinetics graph was drawn (Figure 4.8). All three cryogels with different concentrations began to swell as soon as they were placed in water. The cryogels with alternan concentrations of 15% and 20% reached swelling equilibrium in approximately 20 seconds. Cryogel with a 10% alternan concentration swelled at a value above its own swelling equilibrium ratio.

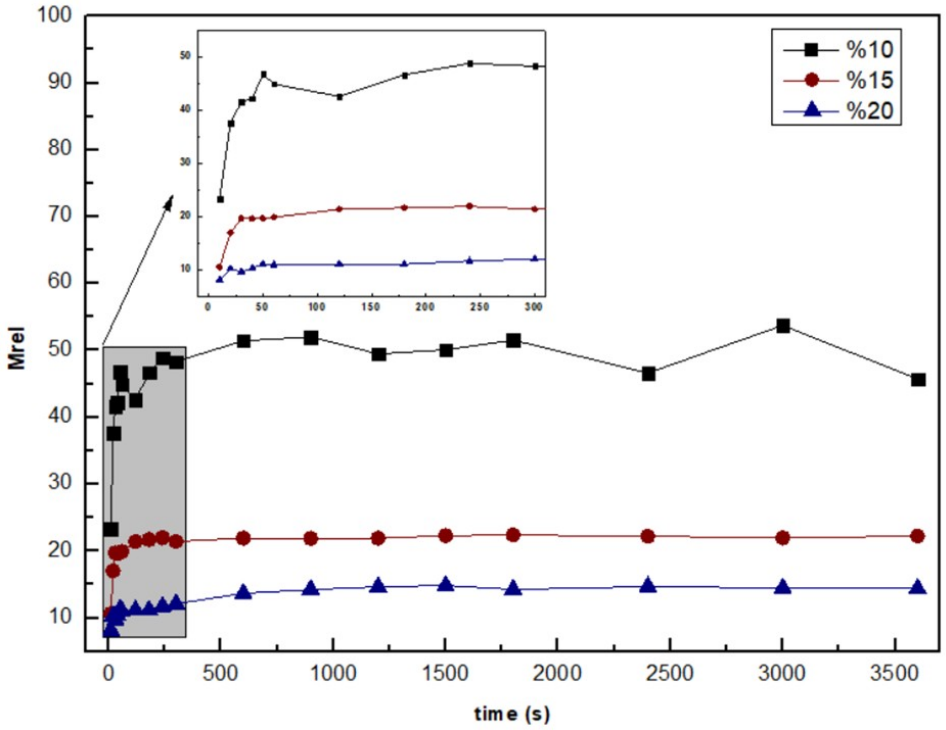


Figure 4.8 Swelling behavior of 10%, 15% and 20% alternan cryogel. The graph shows the swelling kinetics values between 0-3600 seconds.

As a result of these last trials, it was determined that 10% alternan concentration was not sufficient for cryogel production, a minimum 15% alternan concentration

was required and 20% alternan concentration was the optimal alternan concentration among the trials.

In light of the preliminary trials, it was decided to proceed to the next stage with a 20% alternan concentration formulation prepared with polymer to crosslinker ratio of 2:1 (w/w) and with a reaction time of 24 hours in the refrigarator.

4.3 Blank Alternan Hydrogel and Cryogel Results

20% alternan cryogel and hydrogels with optimized formulation in dry form are shown in Figure 4.9. The characterization of these gels and their comparison results are given in the following sections.

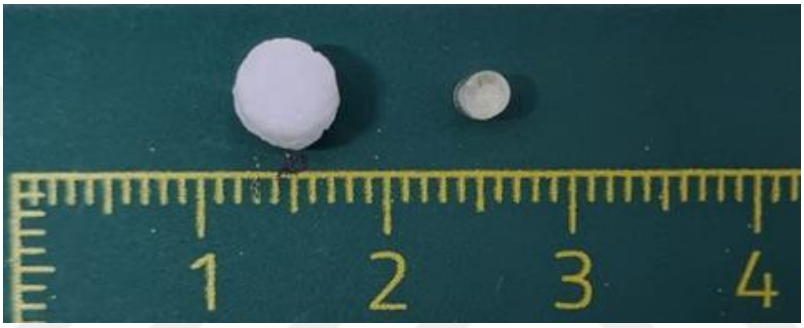


Figure 4.9 20% alternan cryogel (left) and hydrogel (right) in dry state

4.3.1 FTIR Spectroscopy Results

To illuminate the internal chemical structures of the obtained materials, FTIR analyses were first conducted on the ECH crosslinker used in their production. Then, the produced cryogels and hydrogels were also examined. As seen in Figure 4.10, it is observed that alternan-based cryogel and hydrogels give absorption peaks in the same ranges. The ECH spectrum shows sharp peaks around 1200-1000 cm^{-1}, suggesting C-O-C stretching. These peaks indicate the successful incorporation of crosslinking agent. The presence of peaks in the region of 1700-1600 cm^{-1} suggests carbonyl (C=O) stretching, indicative of ester or carboxylic acid groups. These groups are likely introduced through the crosslinking process, confirming successful crosslinking.

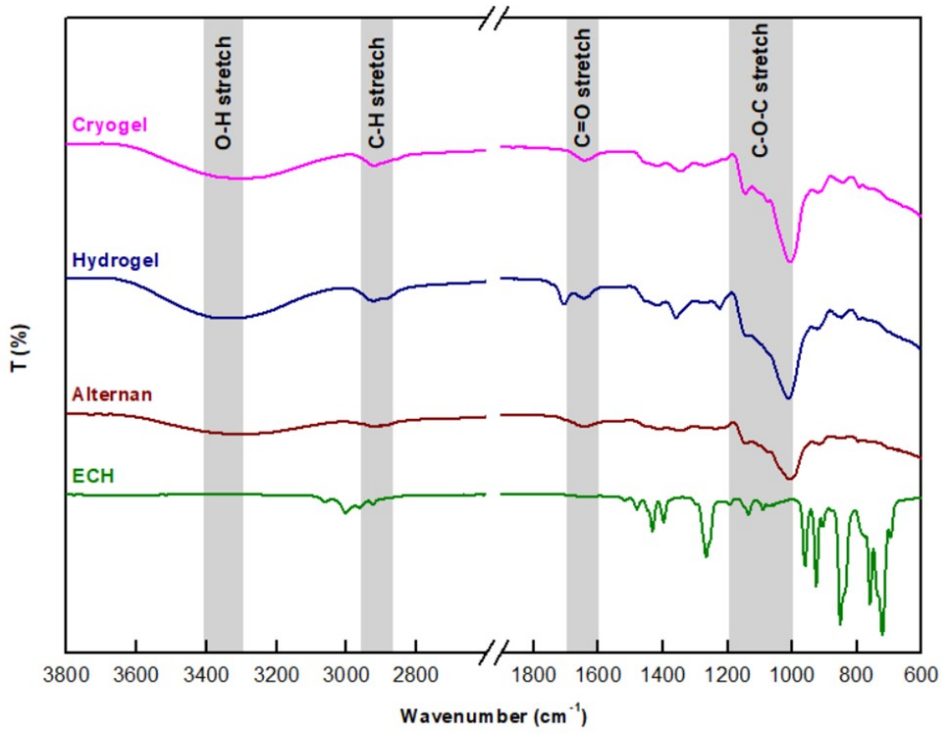


Figure 4.10 FTIR spectra of the produced gels, as well as alternan and ECH used in their production

Hydrogel and cryogel spectra show characteristic O-H stretching around 3400 cm^{-1} and C-H stretching around 2900 cm^{-1}, similar to alternan. Notably, the peaks in the 1700-1600 cm^{-1} region indicate carbonyl (C=O) stretching, suggesting the presence of crosslinked ester or carboxylic acid groups. Peaks around 1100 cm^{-1} suggest C-O-C stretching vibrations.

It can be concluded that the FTIR spectra confirm the successful crosslinking of alternan gels, as evidenced by the presence of specific peaks corresponding to the functional groups introduced through the crosslinking process. The noticeable peaks around 1700-1600 cm^{-1} (C=O stretching) and 1100-1000 cm^{-1} (C-O-C stretching) in the ECH, hydrogel, and cryogel spectra indicate the incorporation of crosslinking agents and the formation of new chemical bonds, leading to the successful crosslinking of the alternan gels.

4.3.2 Swelling Studies Results

To investigate the swelling behavior of the produced gels, which is a key characteristic of hydrogels and cryogels, swelling studies were conducted in ultra-pure water at room temperature. The swelling equilibrium ratios (q_w) of the gels were calculated using the equation (3.1). Figure 4.11 was drawn by calculating the

q_w values of three samples with similar weights for each gel type. As seen in Figure 4.11, the q_w value of cryogels was 14.01±0.19, while the value of hydrogels was 10.03±0.09, which was lower than cryogels. Figure 4.11 shows that the cryogel exhibits a significantly higher swelling equilibrium value (approximately 14) compared to the hydrogel (approximately 10). The differences in swelling behavior reflect the underlying differences in the internal morphology of the cryogels and hydrogels. A higher q_w value for cryogels indicates that they can absorb and retain more water compared to hydrogels. This is due to the fact that cryogels have a macroporous structure. The lower q_w value for hydrogels indicates a comparatively lower water absorption capacity, which may be due to a denser network structure. The higher swelling capacity of cryogels makes them suitable for applications where high water absorption is required, such as in wound dressings that need to manage a significant amount of exudate. Hydrogels, with their lower swelling capacity, might be more suitable for applications requiring a more controlled and moderate level of water absorption. Thus, the swelling equilibrium data clearly shows that cryogels have a higher capacity for water absorption compared to hydrogels. This characteristic makes cryogels particularly advantageous for applications that benefit from high water retention, such as wound dressings. In a hydrogel study conducted by Alves et al. using dextran, it was observed that the swelling ratios of hydrogels with various compositions ranged between 15 and 5 [70]. Our study corroborates these findings, demonstrating that gels derived from alternan, which possesses a structure similar to dextran, also exhibit swelling ratios within this established range. This alignment with existing literature values underscores the consistency and reliability of our experimental results.

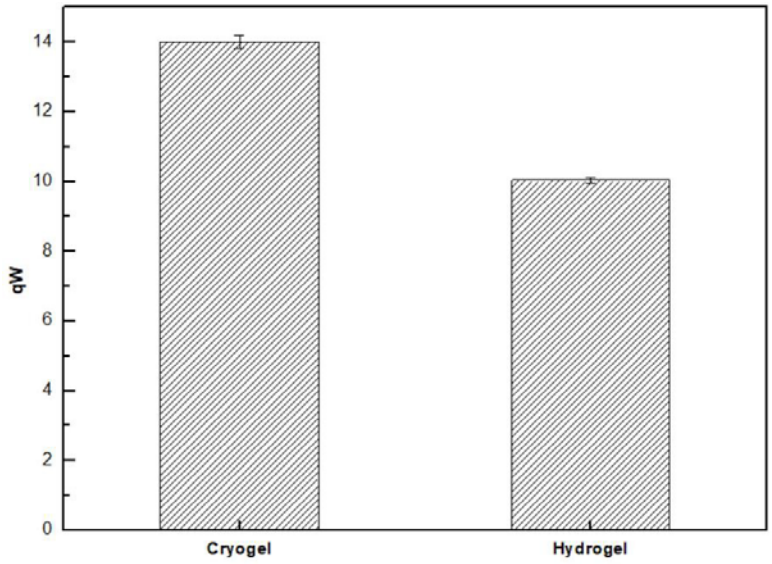


Figure 4.11 Swelling equilibrium of cryogel and hydrogels

Gel fraction (W_g) represents the proportion of polymer networks in a hydrogel that are cross-linked. This value is critical because it directly determines the hydrogel's mechanical strength and chemical stability thus potential uses in biomaterial science. As seen in Figure 4.12, the percentage W_g value is calculated as 63.94±0.66% in cryogels, while it is 72.09±0.91% in hydrogels. The observed higher gel fraction value in the hydrogel compared to the cryogel was thought to be due to the differences in the cross-linking reactions and structural formation of the gels. In the hydrogel, the cross-linking reaction occurs in a homogeneous and dense manner, resulting in a more uniformly cross-linked network. Whereas the cryogel formation involves a heterogeneous cross-linking process due to the ice crystal formation and thawing, leading to a porous structure.

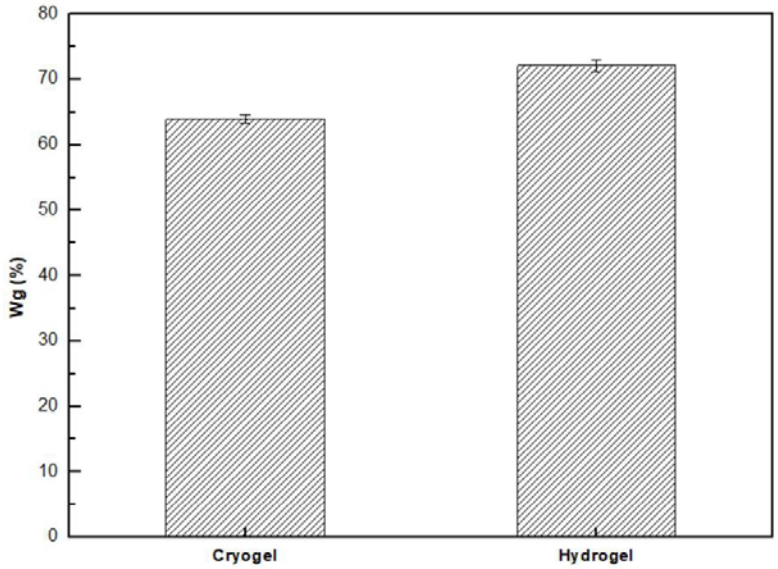


Figure 4.12 Gel fraction of cryogel and hydrogels

When the swelling kinetics of the gels were examined as shown in Figure 4.13, it was determined that the cryogels swelled rapidly from the first moment they were thrown into water due to their macroporous structure and reached complete equilibrium in a short time of 1minutes. The maximum swelling ratio for cryogels is around 14, indicating a high water absorption capacity as well as confirming the equilibrium swelling ratio given above. Due to their less porous, denser structure, the hydrogels took longer to swell and were observed to reach full equilibrium only after 24 hours. Also, the hydrogels do not reach the same level of swelling as cryogels, with a maximum swelling ratio of around 9-10.

The cryogels swell and reach their swelling equilibrium much faster compared to the hydrogels, as indicated by the steep initial rise in the swelling ratio. Hydrogels continue to absorb water slowly over an extended period, indicating a prolonged swelling process. Thus, the swelling kinetics graph clearly demonstrates the superior swelling properties of cryogels compared to hydrogels. Cryogels not only absorb more water but also do so at a much faster rate, reaching equilibrium quickly. These properties make cryogels particularly suitable for applications where rapid and high water absorption is critical, such as in wound dressings. In contrast, the slower and lower swelling capacity of hydrogels suggests they may be better suited for applications requiring controlled and sustained water absorption.

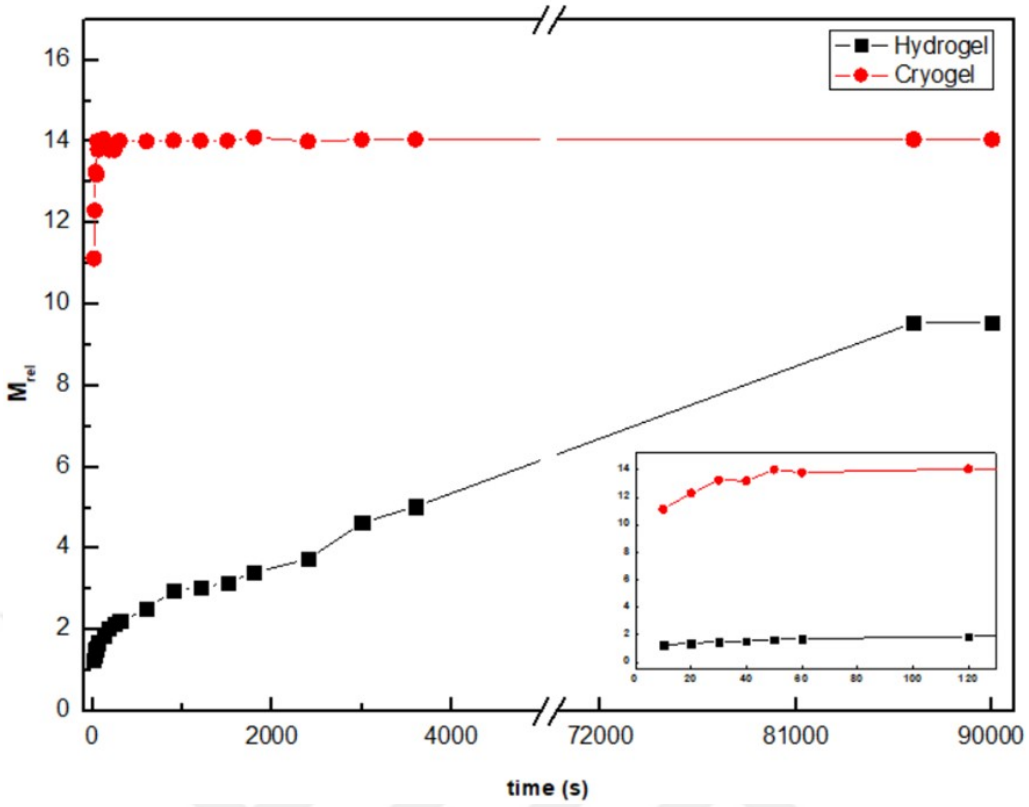


Figure 4.13 Swelling kinetics of cryogel and hydrogels depending on time

4.3.3 SEM Results

The internal morphological structures of the gels were analyzed by examining their cross-sections using SEM. As seen in Figure 4.14b, obtained SEM images revealed that the cryogels showed a macroporous structure, while the hydrogels displayed a non-porous, smooth internal structure. Calculations performed using the Image J program determined that the cryogels have an average pore size of 54.67±33.68 µm.

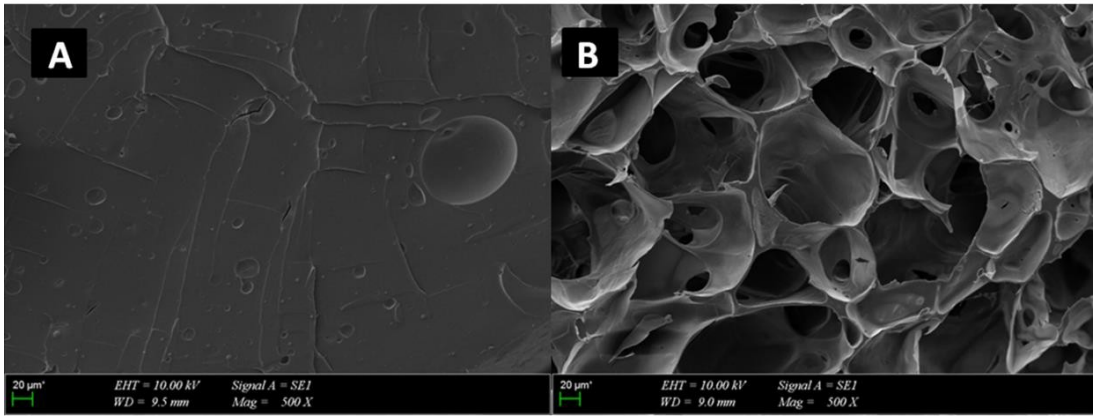


Figure 4.14 SEM images of the 20% alternan gels at 500x a) hydrogel, b) cryogel

The SEM images showcase the notable microstructural distinctions between the hydrogel and cryogel forms of 20% alternan gels. As seen in Figure 4.15 the hydrogel's compact and less porous formation is associated with its diminished swelling capacity and slower swelling kinetics. On the other hand, the cryogel's highly porous and interconnected network structure, as seen in Figure 4.16, accounts for its superior swelling properties, making it more suitable for applications that demand quick and significant water absorption, such as wound dressings.

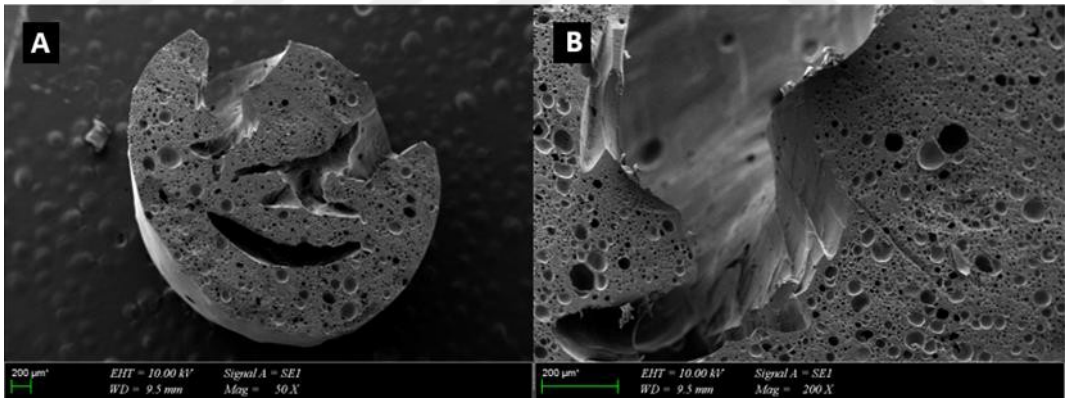


Figure 4.15 SEM images of the 20% alternan hydrogel at a)50x b)200x magnifications

In a study on welan gum, a type of EPS, hydrogels were found to have an average pore size of 55 μm in the swollen state [67]. Comparing this with the pore sizes of alternan cryogels obtained in our study, it appears that the alternan cryogels, with pore sizes approaching this value in the dry state, exhibit larger pore sizes.

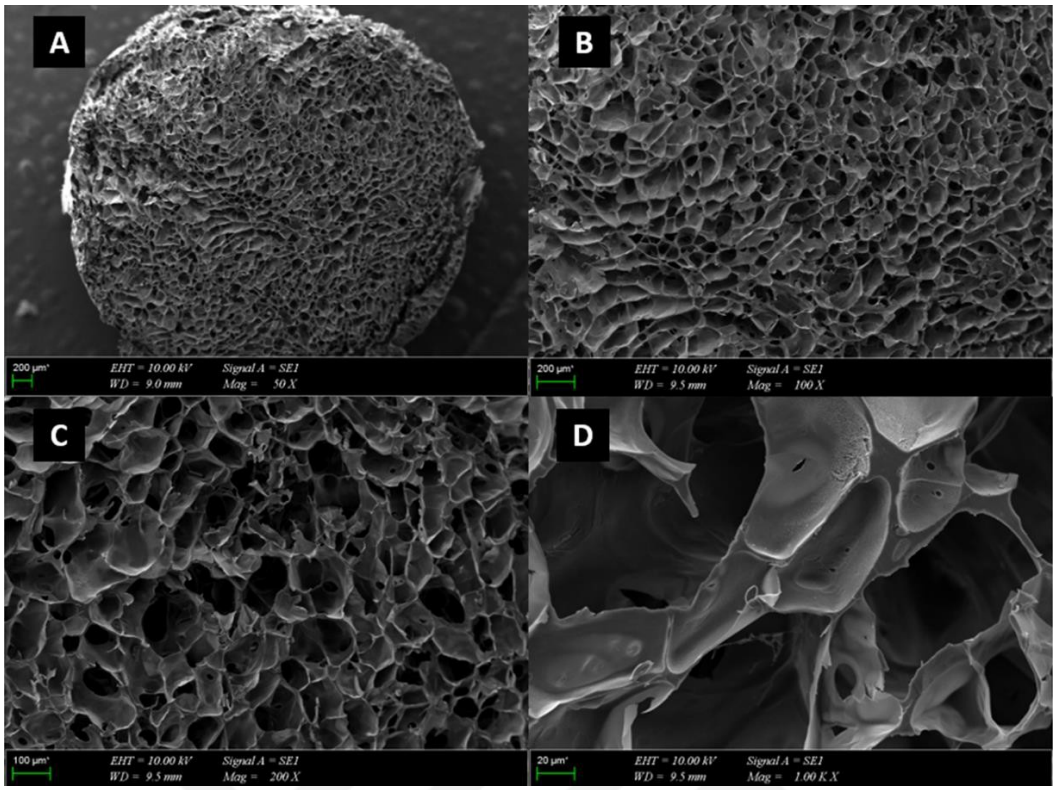


Figure 4.16 SEM images of the 20% alternan cryogel at a)50x b)100x c)200x d)1000x magnifications

4.3.4 Compression Test Results

Compression testing was firstly performed on wet hydrogels and cryogels.

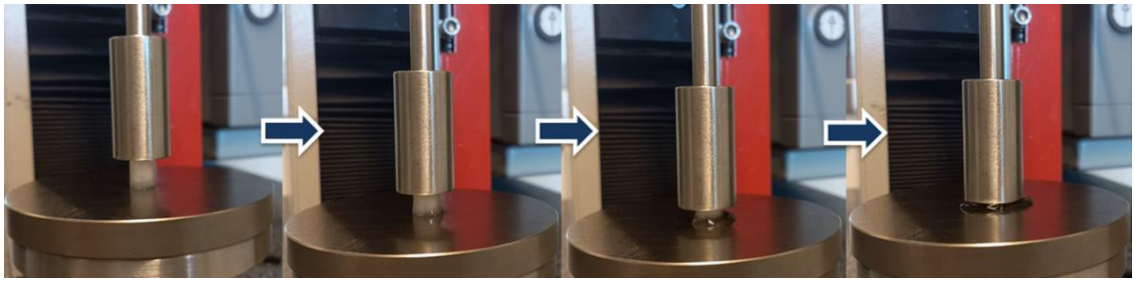


Figure 4.17 Compression test of swollen 20% alternan cryogel from its initial state to compressed state

As illustrated in Figure 4.17, the swollen 20% alternan cryogels began releasing water from their macropores immediately upon compression. Disintegration commenced once the cryogels had completely expelled their water content.

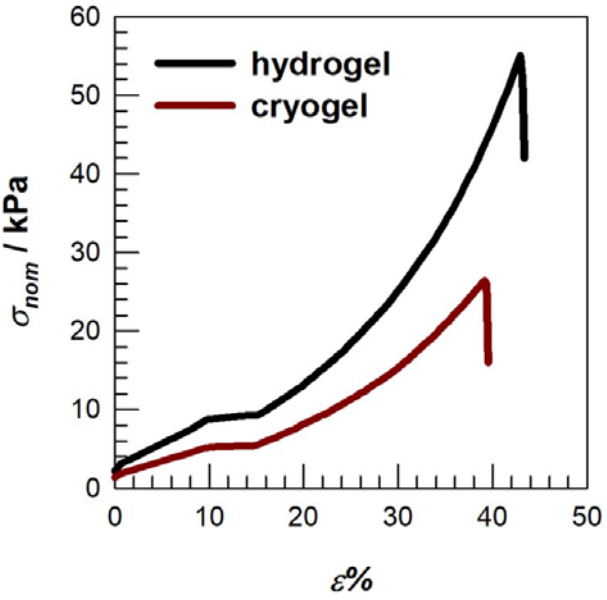


Figure 4.18 Stress-strain graph of swollen hydrogel and swollen cryogels. X-axis is nominal stress (σnom) in kilopascal while y-axis is strain (ε) as percentage.

Swollen cryogel and hydrogel stress-strain graph showed (Figure 4.18) that the swollen hydrogels withstood a stress of 53.85±4.75 kPa and approximately 43% could be compressed without completely breaking down while the swollen cryogel was compressed withstanding a stress of 26.1±1.35 kPa, with ~41% of it completely compressed without breaking down. Pacelli et al. reported a roughly ten-fold difference in compressive modulus between dextran-based hydrogels and cryogels, with the cryogels demonstrating greater deformation under compression [47]. In a study by Sharma et al., it was found that hydrogels and cryogels made from carrageenan exhibited a significantly lower compressive modulus in the wet state compared to the dry state [71].

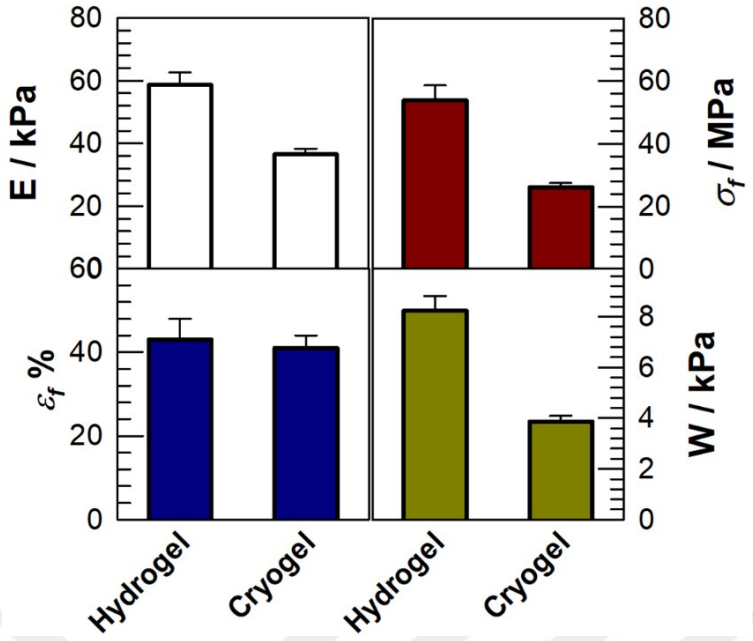


Figure 4.19 Comparison of swollen hydrogels and cryogels according to their Young's modulus (*E*), fracture strength (σ_f) , fracture strain (ε_f) and toughness (W)

When Figure 4.19 is examined, it is seen that the swollen hydrogels have Young's modulus of 58.62±4.03 kPa, according to the slope calculated in the 5-15% deformation range, whereas based on the slope calculated in the 3-5% deformation range, swollen cryogels show Young's modulus of 36.48±1.87 kPa. The breaking point of the gels, which can be observed in the stress-strain graph in Figure 4.18, is given as fracture strength (σ_f) in Figure 4.19. Figure 4.19 shows that the swollen hydrogel and cryogel both show nearly identical fracture strains of ~43% while the toughness of swollen hydrogel is twice of swollen cryogel.

According to the characterization results of hydrogels and cryogels with 20% alternan concentration, it was determined that the produced cryogels were suitable for use in wound dressings.

4.4 Vancomycin-containing Cryogel Results

In order to provide antibacterial properties, vancomycin was incorporated to the cryogels with 20% alternan concentration, and their antibacterial effects were examined. At first, to determine the optimal amount of vancomycin 1 µg/µl, 2 µg/µl, and 3 µg/µl were absorbed into the gels and tested against the *Staphylococcus aureus* 25923 bacteria using the disk diffusion method. A vancomycin-free cryogel was also tested to determine whether the alternan

cryogel alone showed any antibacterial effect. As shown in Figure 4.20, all the cryogels containing vancomycin created inhibition zones, while the cryogel without vancomycin exhibited no antibacterial effect on its own.

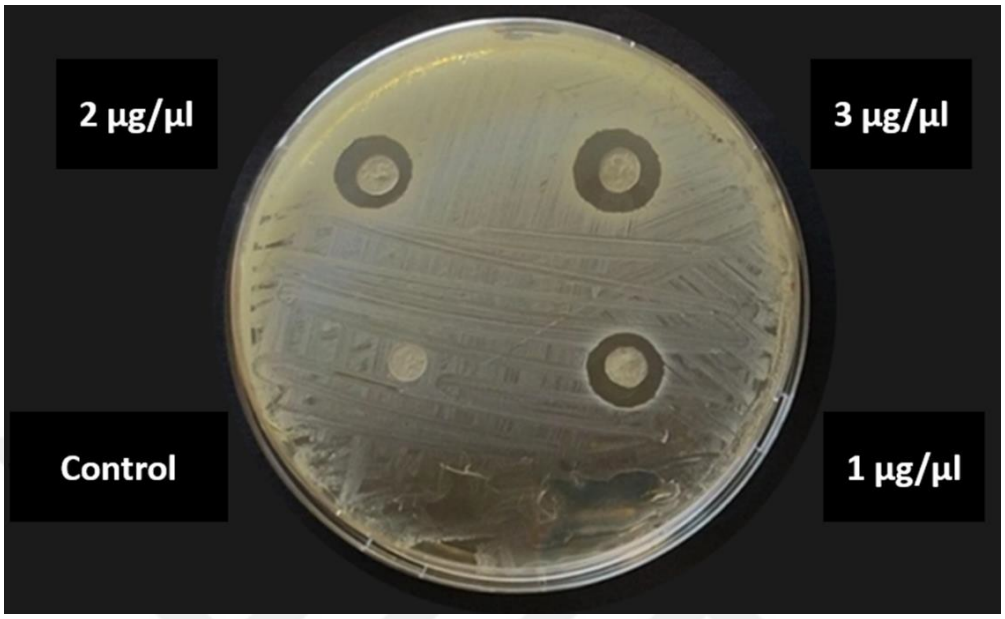


Figure 4.20 Antibacterial effect of alternan cryogels containing different amounts of vancomycin on *S.aureus*

The cryogel containing 1 µg/µl of vancomycin exhibited a zone diameter of 11±0.5 mm, while the one with 2 µg/µl showed 12±0.5 mm, and the cryogel with 3 µg/µl of vancomycin displayed 14±0.5 mm. An increase in the concentration of vancomycin also resulted in an increase in the zone diameters. However, since no significant increase was observed, it was decided to continue with 1 µg/µl vancomycin.

After determining the amount of vancomycin, characterization analyses of the cryogels were performed.

4.4.1 FTIR Results

The FTIR spectrum of the produced vancomycin-containing cryogels was examined.

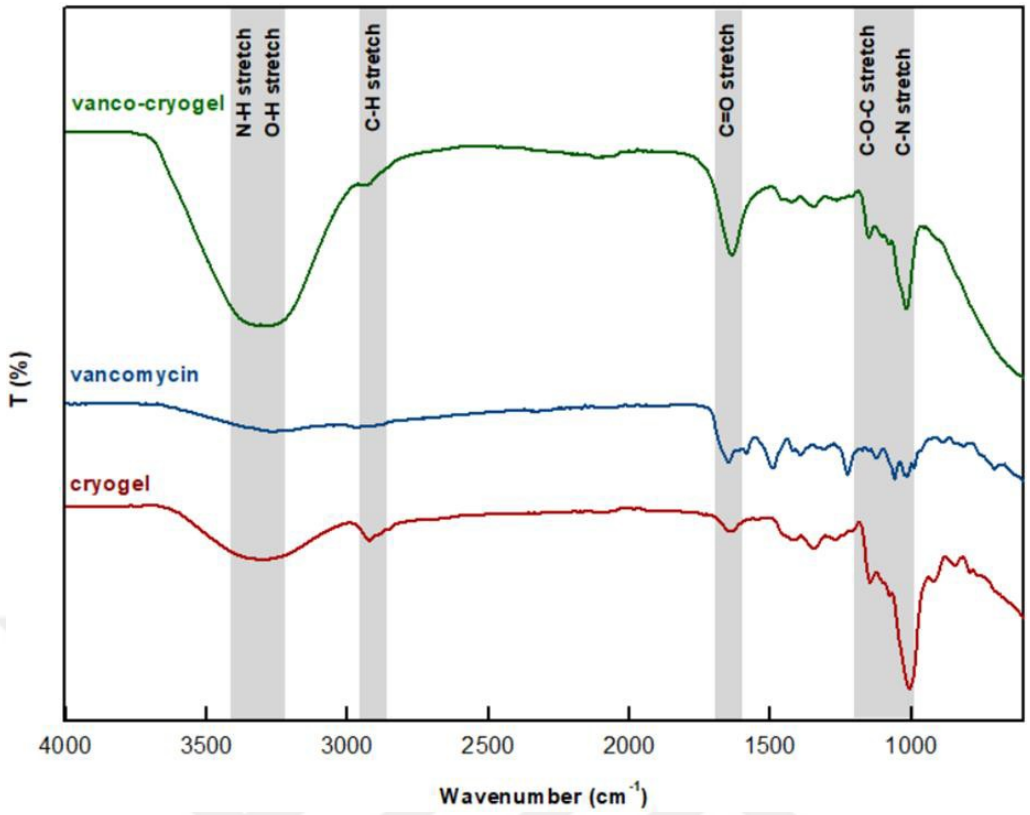


Figure 4.21 FTIR spectra of the vancomycin-containing cryogel, as well as the vancomycin and vancomycin-free cryogel used in their production

As seen in Figure 4.21 cryogel spectrum shows characteristic peaks around 3400 cm^{-1} for O-H stretching, indicating hydroxyl groups. Peaks around 2900 cm^{-1} correspond to C-H stretching. The region around 1700-1600 cm^{-1} shows C=O stretching, suggesting the presence of carbonyl groups. Peaks around 1100 cm^{-1} are indicative of C-O-C stretching, typical of polysaccharides. The vancomycin spectrum shows distinct peaks that differ from those of the cryogel. Notable peaks around 3400 cm^{-1} for N-H and O-H stretching. Peaks around 1600 cm^{-1} indicate aromatic ring vibrations and C=O stretching. The region around 1100 cm^{-1} shows C-O and C-N stretching vibrations.

The vanco-cryogel spectrum combines features from both vancomycin and cryogel. The broad peak around 3400 cm^{-1} indicates overlapping O-H and N-H stretching vibrations from both components. Peaks around 2900 cm^{-1} for C-H stretching remain present. The region around 1700-1600 cm^{-1} shows intensified peaks, indicating the presence of both C=O stretching from the cryogel and aromatic ring vibrations from vancomycin. Peaks around 1100 cm^{-1} are more

pronounced, suggesting enhanced C-O-C and C-N stretching due to the incorporation of vancomycin.

Thus, the FTIR spectra confirm the successful incorporation of vancomycin into the cryogel. The vanco-cryogel spectrum displays combined features of both vancomycin and cryogel, particularly in the regions corresponding to O-H/N-H stretching, C=O stretching, and C-O-C/C-N stretching. The presence of vancomycin-specific peaks in the vanco-cryogel spectrum indicates that the drug is effectively embedded within the cryogel matrix, potentially imparting antibacterial properties to the cryogel. This analysis supports the conclusion that vancomycin-containing cryogels have been successfully synthesized, merging the structural features of both components.

4.4.2 SEM Results

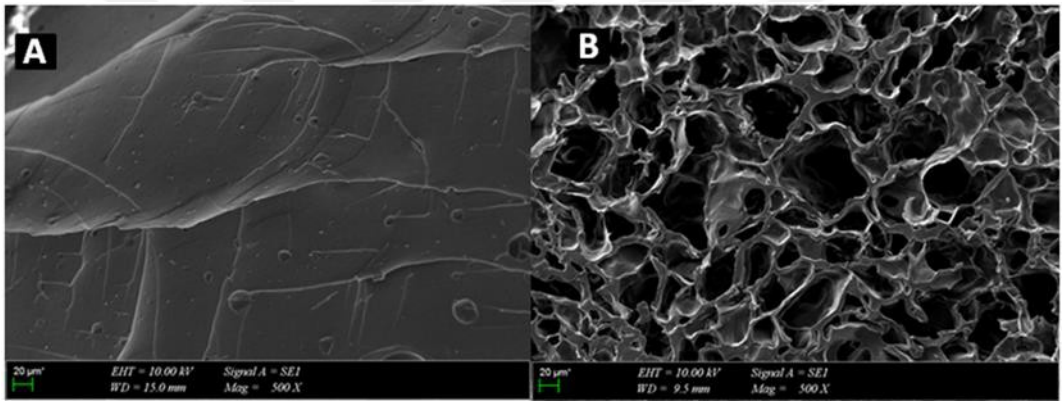


Figure 4.22 SEM images of the vancomycin-containing gels a) hydrogel, b) cryogel

The internal morphological structures of the vancomycin-containing hydrogel and cryogels in Figure 4.22 were analyzed by examining their cross-sections with a SEM. When these vancomycin-containing gels were investigated, no difference was found in their internal structures compared to vancomycin-free ones. It was concluded that vancomycin did not cause any change in the structure of the gels. Vancomycin-containing gels had an average pore size of 50.24±21.21µm.

4.4.3 Compression Test Results

Compression testing was conducted on swollen and freeze-dried cryogels with vancomycin-containing cryogels and they were compared with each other.

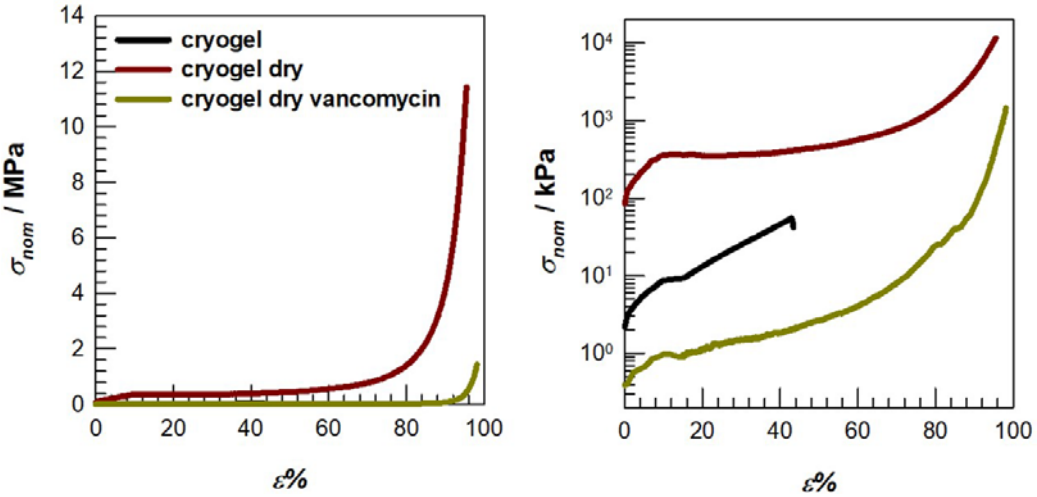


Figure 4.23 Stress-strain graph of swollen cryogel, dry cryogel and vancomycin-cointaining cryogel. X-axis is nominal stress (σ_{nom}) while y-axis is strain (ε). On the left graph nominal stress values were shown as kPa.

When Figure 4.23 is examined, it is observed that all of the cryogels exhibit a linear behavior in the 3-5% deformation range and maintain their plateau state until their fracture point. Swollen cryogel, dry cryogel, and vancomycin-containing cryogel stress-strain graph showed (Figure 4.23) that 41±3% of the swollen cryogel could be compressed without completely breaking down while both the dry cryogel and the vancomycin-containing cryogel were 100% compressed.

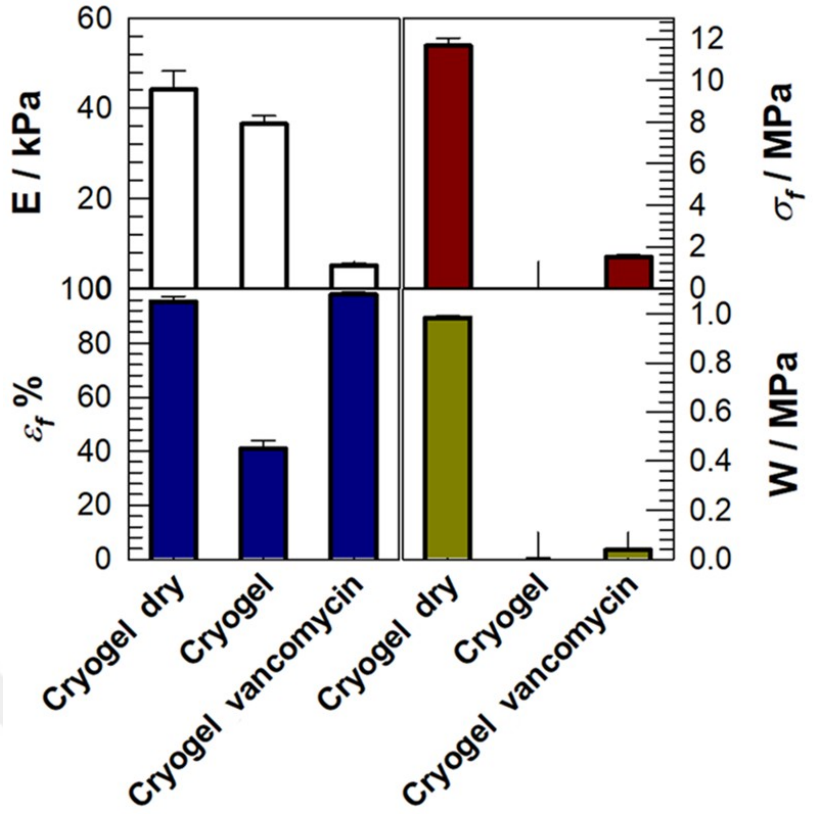


Figure 4.24 Comparison of dry cryogel, swollen cryogel and vancomycin-containing cryogel according to their Young's modulus (*E*), fracture strenght (σ_f) , fracture strain (ε_f) and toughness (W)

When Figure 4.24 is examined, according to the slope calculated in the 3-5% deformation range, it is seen that the dry cryogels have Young's modulus of 44.15±4.14, while swollen cryogels have 36.48±1.87 kPa and vancomycin-containing cryogels have 5.04±0.45. The fracture stress of dry cryogels, swollen cryogels, and vancomycin-containing cryogels were 11675±385 kPa, 26.1±1.35 kPa, and 1505±98 kPa, respectively. In the same order, the fracture strain of the cryogels are 95.5±1.9%, 41±3%, and 98±0.85%. When the toughness graph is examined (Figure 4.24), it is observed that the dry cryogel is quite tough, while the swollen cryogel has a brittle structure.

4.4.4 Antibacterial Analysis Results

Finally, the antibacterial effect of cryogel containing 1 µg/µl of vancomycin was tested on four different microorganisms coded as 35OSSA, 37MORSA, 39MRSA, and 87MSSA. For comparison, the same amount of vancomycin was applied to commercial discs, and vancomycin-free cryogel was used as a control.

Table 4.1 The antibacterial effect of vancomycin-containing cryogels on different microorganisms

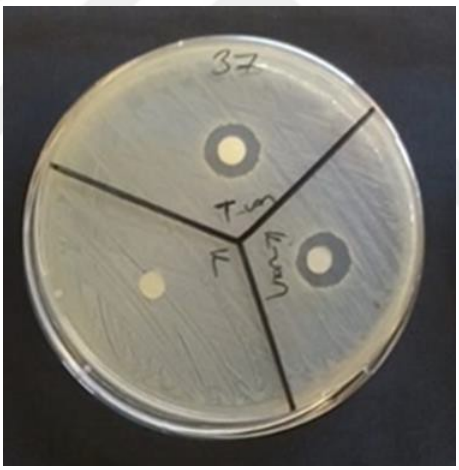
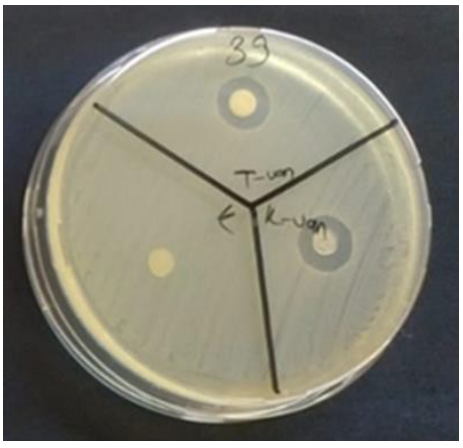
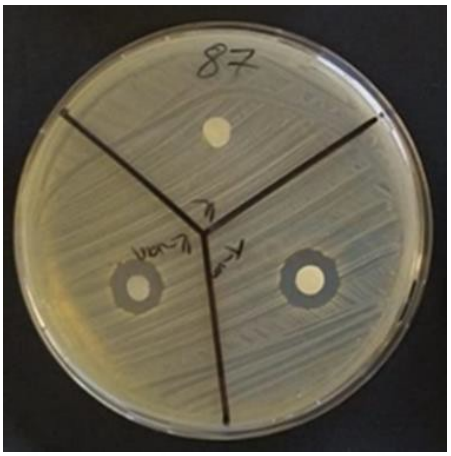
Microorganism	Sample
35OSSA	
37MORSA	
39MRSA	

Table 4.1 The antibacterial effect of vancomycin-containing cryogels on different microorganisms (continued)

87MSSA	

The control cryogel exhibited no antibacterial effect against any of the tested microorganisms, whereas, both the vancomycin-containing cryogel and the vancomycin-infused commercial disc exhibited antibacterial activity against all five tested bacteria. According to zone diameters in Table 4.2, it was concluded that the antibacterial effects of the vancomycin-containing cryogel and vancomycin-infused commercial disc were almost the same.

Table 4.2 Zone inhibition diameters of the vancomycin-containing cryogel and commercial disk against different microorganisms

Microorganism	Cryogel	Commercial Disk
35OSSA	13±0.5	11.5±0.5
37MORSA	13±0.5	12±0.5
39MRSA	13.5±0.5	12±0.5
87MSSA	11.5±0.5	12±0.5

5
CONCLUSION

Biopolymers, especially polysaccharides, have been used in the production of biomaterials in various industries for years due to their properties such as biocompatibility and biodegradability, as well as being cheaper and more reliable. However, there is a need to investigate polymers whose potential has not been fully explored in order to develop new biomaterials by improving the literature and scientific research. This need arises especially in the development of hydrogel/cryogel-based wound dressing, which is an application area that has attracted much attention recently.

In this study, to our knowledge, alternan-based hydrogel and cryogel biomaterials were obtained for the first time. Based on the experiments conducted in this study, the following conclusions have been reached:

- Firstly, alternan exopolysaccharide was obtained from the microorganism *Lactobacillus reuteri.* With the NMR and FTIR characterization results, it was confirmed that the obtained polymer was alternan.
- Then, PEGDE, GA, and ECH cross-linkers were tried to cross-link the alternan. While alternan was successfully cross-linked with ECH, gel formation could not be obtained with the other two cross-linkers.
- With the optimization studies, hydrogels and cryogels with 20% alternan concentration, polymer-to crosslinker ratio of 2:1 (w/w), and a reaction time of 24 hours were obtained. The FTIR spectrum confirmed the successful synthesis of these gels.
- Swelling behavior studies on optimized 20% alternan gels showed that cryogels have higher water retention capacity than hydrogels. In addition, while cryogels swelled very quickly in less than a minute due to their macroporous structure, hydrogels swelled slower and in a longer time due to their less porous structure.

- The obtained SEM images proved the macroporous structure of cryogels and the non-porous structure of hydrogels. It was observed that the average pore size of the produced cryogels was 54.67±33.68 μm.
- Compression test results showed that approximately 40% of both the cryogel and the hydrogel could be compressed in the wet state without disintegration. It was observed that in the dry state, the cryogel could be compressed up to 100%, and when thrown into the water, it swelled and returned to its previous form.
- Based on the results obtained, it was concluded that cryogels would be more advantageous as wound dressings. Therefore, in order to obtain an antibacterial wound dressing, the obtained cryogel was loaded with vancomycin.
- FTIR spectrum confirmed that vancomycin was successfully loaded into the cryogel.
- Mechanical tests performed on vancomycin-loaded cryogels showed that the vancomycin-containing cryogel was more flexible than the non-vancomycin-containing cryogel. This flexibility is an advantage for wound dressing applications, as it allows the material to conform to the wound shape without causing discomfort.
- Antibacterial tests have shown that vancomycin-containing cryogels are effective on *Staphylococcus aureus*, a pathogen that is found especially in the skin and has a negative effect on wound healing, strains with different resistances coded as 35OSSA, 37MORSA, 39MRSA, and 87MSSA.

Upon examining all the results, it was determined that alternan-based hydrogel and cryogel biomaterials could be produced, these gels could be loaded with antibacterial agents such as vancomycin, and particularly, the cryogels showed great potential for use as wound dressings.

In order to further examine the potential of alternan-based cryogels as wound dressings biocompatibility tests, biodegradation tests, drug release studies, and scratch assays are planned as future studies.

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PUBLICATIONS FROM THE THESIS

Conference Papers

1. Bulut S., Taşdurmazlı S., Özbek T., Su E., Korkmaz K., Dertli E., Özmen M. M., "Alternan Ekzopolisakkarit Tabanlı Hidrojel ve Kriyojel Biyomalzemelerin Sentezi ve Karakterizasyonu", VIII. Ulusal Biyomühendislik Kongresi, Manisa, Türkiye, May 16-18, 2024.