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Formulation and Properties of Antibacterial Hydrogels Using Cellulose Infused with Silver Ions.

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DECLARATION

I declare that this thesis entitled Formation and Properties of Antibacterial Hydrogels Using Cellulose Infused with Silver Ions is the result of my own research except as cited in the references.

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ABSTRAK

Kajian ini menandakan kemajuan penting dalam penciptaan hidrogel berasaskan selulosa yang diperkaya dengan ion perak, bertujuan untuk mengatasi cabaran utama dalam aplikasi bioperubatan. Kajian ini memilih rumput Napier sebagai sumber selulosa kerana kandungan selulosa yang tinggi dan manfaat alam sekitar. Proses pengekstrakan selulosa melibatkan rawatan alkali dan pemutihan. Satu aspek unik kajian ini adalah pembuatan hidrogel menggunakan proses pengikatan silang fizikal yang baru yang termasuk 5% glisin dalam 2M NaOH, dipilih kerana keupayaannya membentuk ikatan bukan kovalen dan kesan ke atas kestabilan mekanikal hidrogel. Ion perak dimasukkan menggunakan larutan nitrat perak 3%, dengan berhati-hati menyeimbangkan keberkesanan antibakteria dan sitokompatibiliti. Hidrogel yang dihasilkan menunjukkan kemampuan antibakteria dan anti-radang yang ketara, yang terakhir dinilai melalui ujian penghambatan denaturasi protein, mencadangkan penggunaan potensial mereka dalam penyembuhan luka dan regenerasi tisu. Teknik analisis seperti spektroskopi FTIR dan SEM digunakan untuk memahami ciri-ciri struktur dan fungsi hidrogel. Secara menarik, analisis EDS mendedahkan kehadiran silikon, menunjukkan kawasan untuk penyelidikan lanjut. Walaupun hasilnya menggalakkan, kajian ini mengenal pasti cabaran seperti pencapaian pengagihan ion perak yang seragam dan keperluan untuk ujian sitokompatibiliti yang menyeluruh. Kerja ini menawarkan perspektif baru dalam pembangunan hidrogel, terutamanya dalam penggunaan bahan lestari seperti rumput Napier dan penggabungan elemen fungsional seperti glisin dan ion perak. Arah penyelidikan masa depan termasuk meneroka sumber selulosa yang berbeza, memperhalusi formulasi hidrogel, dan menjalankan ujian biokompatibiliti dan biodegradabiliti yang komprehensif, yang boleh mengembangkan penggunaan bahan-bahan ini dalam aplikasi bioperubatan secara signifikan.

Kata kunci: Hidrogel, Pengekstrakan Selulosa, Rumput Napier, Ion Perak, Aplikasi Bioperubatan.

ABSTRACT

This research marks a significant advancement in the creation of cellulose-based hydrogels, enhanced with silver ions, aimed at addressing key challenges in biomedical applications. The study chose Napier grass as the source of cellulose due to its high cellulose concentration and environmental benefits. The process of extracting cellulose involved alkaline and bleaching treatments. A unique aspect of this study was the fabrication of hydrogels using a novel physical crosslinking process that included 5% glycine in 2M NaOH, selected for its non-covalent bond formation and impact on the hydrogels' mechanical stability. Silver ions were incorporated using a 3% silver nitrate solution, carefully balancing antibacterial effectiveness and cytocompatibility. The resulting hydrogels demonstrated considerable antibacterial and anti-inflammatory capabilities, the latter evaluated through protein denaturation inhibition tests, suggesting their potential use in wound healing and tissue regeneration. Analysis techniques such as FTIR spectroscopy and SEM were employed to understand the structural and functional characteristics of the hydrogels. Interestingly, EDS analysis revealed the presence of silicon, indicating areas for further investigation. While the outcomes were encouraging, the study identified challenges like achieving even distribution of silver ions and the need for thorough cytocompatibility testing. This work offers new perspectives in hydrogel development, especially in using sustainable materials like Napier grass and incorporating functional elements such as glycine and silver ions. Future research directions include exploring different cellulose sources, refining hydrogel formulations, and conducting comprehensive biocompatibility and biodegradability tests, which could significantly expand the use of these materials in biomedical applications.

Keywords: Hydrogels, Cellulose Extraction, Napier Grass, Silver Ions, Biomedical Applications.

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DNA	Deoxyribonucleic Acid	1
SEM	Scanning Electron Microscopy	5
EDS	Energy Dispersive X-ray Spectroscopy	5
FTIR	Fourier Transform Infrared Spectroscopy	5
NaOH	Sodium Hydroxide	5
E. coli	*Escherichia coli*	6
BSA	Bovine Serum Albumin	6
LCST	Lower Critical Solution Temperature	14
UCST	Upper Critical Solution Temperature	15
AgNPs	Silver Nanoparticles	18
$AgNO_3$	Silver Nitrate	18
Ag+	Silver Ions	18
APS	Ammonium Persulfate	20
GCE	Glassy Carbon Electrode	20
PEG	Polyethylene Glycol	20
TEM	Transmission Electron Microscopy	24
TLR	Toll-Like Receptor	27
TNF-α	Tumour Necrosis Factor Alpha	27

ROS	Reactive Oxygen Species	28
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs	29
H_2O_2	Hydrogen Peroxide	37
PBS	Phosphate-Buffered Saline	37
$NaClO_2$	Sodium Chlorite	40
ZOI	Zones of Inhibition	46
OH	Hydroxyl groups	52
C–H	Alkyl groups	52
C=O	Carbonyl	53
NH_2	Amine	53
C-O-C	Ether	53
CH_2	Methylene	53
O	Oxygen	70
C	Carbon	70

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Hydrogels have emerged as crucial components in biomedical fields, renowned for their remarkable water absorption capabilities, biocompatibility, and their imitation of human tissue characteristics. These materials are composed of hydrophilic networks of crosslinked polymer chains, characterized by their high-water content and flexibility, making them versatile for a range of applications from tissue engineering to drug delivery (Cui et al., 2019; Ho et al., 2022).

Research in hydrogels distinguishes between natural and synthetic varieties. Natural hydrogels, derived from biological materials such as proteins, DNA, and polysaccharides, are increasingly favoured for their biocompatibility, low toxicity, and eco-friendliness, especially cellulose-based hydrogels, which are highlighted for their suitability in biomedical applications (Ahmed, 2015; Cui et al., 2019). On the other hand, synthetic hydrogels can be engineered to possess specific mechanical properties and controlled degradation rates, but they encounter issues with biocompatibility and toxicity, which constrains their biomedical applications (Ni et al., 2023).

Cellulose is a key material in natural hydrogels. It is a natural polymer found abundantly in wood, plants, algae, and bacterial fibres. Its widespread availability highlights its sustainable and environmentally gentle nature. This abundance positions it as a prime candidate for extensive application in the biomedical field. The biocompatibility and versatility of cellulose make it highly effective, particularly in the realm of hydrogels, such as in the creation of wound dressing materials (Seddiqi et al., 2021). Cellulose-based hydrogels exhibit crucial antibacterial activity and moisture retention, essential for the healing process, fostering faster and more effective recovery (Su et al., 2021).

In the realm of tissue engineering, cellulose-based hydrogels play a pivotal role, acting as scaffolds that support cell adhesion, differentiation, and integration into native tissues. Their ability to mimic the extracellular matrix is indispensable for tissue regeneration (Mantha et al., 2019). Research conducted by Hafezi et al. (2021) demonstrates their efficacy in enhancing cell interactions and promoting healing, especially in applications like cartilage repair.

A significant advancement in this field is the incorporation of antibacterial agents like silver ions into cellulose hydrogels. This addition significantly improves their antibacterial and anti-inflammatory properties, presenting a promising approach to address chronic wounds and infections in the face of rising antibiotic resistance (Banti et al., 2021; Gunasekaran et al., 2011; Li et al., 2019). Naomi & Fauzi (2020) emphasized the potential of cellulose hydrogels in drug delivery, acting as carriers for bioactive substances and forming a protective barrier against microbial invasion.

In summary, cellulose-based hydrogels, especially those infused with silver ions, are revolutionizing biomaterial applications. Their contributions to wound healing, tissue engineering, and drug delivery highlight their potential in addressing key challenges related to bacterial infections, inflammation, and tissue regeneration. Future research should focus on optimizing these materials, exploring new biomedical applications, and enhancing their therapeutic effectiveness.

1.2 Problem Statement

In the field of biomedical research, the importance of hydrogels is being increasingly recognized for their substantial water content, compatibility with biological tissues, and similarity to tissue characteristics. This acknowledgment coincides with progress in personalized medicine and the management of wound care (Cui et al., 2019; Ho et al., 2022). These hydrogels are pivotal in meeting the stringent medical standards while concurrently tackling contemporary challenges such as antibiotic resistance and the imperative for environmental sustainability.

Furthermore, the economic implications associated with traditional sources of hydrogels, notably illustrated by cost analyses revealing significant expenditures in cotton production amounting to US$1,524,609.75, underscore the urgent need for sustainable and cost-effective alternatives (Neto et al., 2022). In response to these

challenges, the current study proposes Napier grass as a feasible alternative for hydrogel synthesis, presenting an innovative approach that aims to mitigate both environmental impact and economic costs.

Napier grass represents a promising cellulose source, attributed to its considerable cellulose and hemicellulose concentrations (46.58% and 34.14%, respectively), alongside a comparatively low lignin percentage (22.25%) (Kamarullah et al., 2015). Its rapid growth, substantial biomass yield, and minimal nutritional demands underscore its potential as an environmentally friendly and cost-effective source for hydrogel production, effectively addressing sustainability concerns and the environmental impacts associated with conventional cellulose sources (Negawo et al., 2017; Takara & Khanal, 2015).

Despite the biocompatibility and biodegradability of cellulose-based hydrogels, their lack of inherent antimicrobial properties poses a significant limitation, especially in the context of rising antibiotic-resistant bacteria (Kamarullah et al., 2015; Naomi et al., 2020). This limitation prompts the exploration of additives and modifications to impart effective antibacterial capabilities to these hydrogels. Current research is concentrated on integrating metal ions, specifically silver ions, into cellulose hydrogels as a solution to this challenge. By incorporating silver ions, cellulose hydrogels can be developed with improved antibacterial properties, effectively addressing antibiotic resistance (Forero-Doria et al., 2020).

However, the investigation into the integration of silver ions within cellulose hydrogels, particularly their antibacterial efficiency, necessitates a thorough assessment of potential toxicity and environmental repercussions. The influence of silver ions on human health warrants significant attention, especially considering variables such as the amount of silver used, the duration of exposure, and the different pathways through which these ions can be encountered. High levels of silver ions have been shown to adversely affect various human cell types, including monocytes, T-lymphocytes, and human mesenchymal stem cells, underscoring the importance of precise and cautious application of silver ions in hydrogels (Elga Editorial Team, 2022).

The environmental impact of silver ion release, notably their accumulation in soil, are of comparable concern. Silver ions have the potential to alter soil properties, thereby affecting microbial communities and disrupting ecological balance (Calderón-

Jiménez et al., 2017). The accumulation of this substance may lead to significant consequences for soil health and, by extension, the broader ecosystem. Therefore, it is imperative to meticulously weigh the benefits of incorporating silver ions into hydrogels against these potential ecological risks. This approach demands a balanced perspective, ensuring that the use of silver ions is effective in providing antibacterial properties while also being mindful of minimizing adverse environmental and health impacts.

Overall, as the demand for hydrogels in the biomedical sector grows, there is a pressing need to embrace sustainable materials like Napier grass and innovative approaches such as the integration of silver ions. Nevertheless, the potential toxicity and environmental implications of silver ions must be carefully considered in this context. The intersection of these needs and challenges highlights the paramount importance of developing novel and sustainable hydrogel technologies in the biomedical industry, addressing healthcare challenges ethically and environmentally friendly.

1.3 Objectives

The aims and objectives of this study are as follows:

1. To extract cellulose from Napier grass via alkaline and bleaching techniques and analyse its chemical composition using Fourier-transform infrared spectroscopy (FTIR) to understand its characteristics for hydrogel development.
2. To evaluate the morphological characteristics of cellulose hydrogel infused with silver ions using scanning electron microscopy (SEM) and assess the elemental composition of the hydrogel through energy-dispersive X-ray spectroscopy (EDS).
3. To synthesize cellulose hydrogel infused with silver ions, assess their antibacterial effectiveness against *Escherichia coli* by evaluating inhibition zones, and evaluate their anti-inflammatory properties using the inhibition of protein denaturation.

1.4 Scope of Study

This research investigates the formulation and properties of cellulose-based hydrogels, enhanced with silver ions for their recognized antibacterial and anti-inflammatory properties. In the initial phase, high-quality cellulose is extracted from Napier grass using precise alkaline and bleaching techniques, a critical step in ensuring the efficacy of the resulting hydrogels. The subsequent phase involves the careful addition of glycine at a 5% concentration in 2M NaOH, playing a vital role in facilitating physical cross-linking within the hydrogel. This process not only refines the structural integrity of hydrogel but also significantly enhances its mechanical stability and functionality, making it suitable for various applications.

A key aspect of this study, the uniform distribution of silver ions within the hydrogel matrix is achieved through a combination of chemical reduction and controlled diffusion, a process essential for the hydrogel's enhanced and consistent antibacterial activity. This meticulous integration of silver ions is a critical aspect of the design of hydrogel, as it directly impacting its ability to effectively against bacterial infections.

Fourier-transform infrared spectroscopy (FTIR) is utilized to identify specific functional groups and molecular interactions within the cellulose, such as hydroxyl and ether bonds, which are essential in evaluating the cellulose’s compatibility for hydrogel fabrication. Additionally, scanning electron microscopy-energy dispersive X-ray (SEM-EDS) analysis will be executed to inspect the structural attributes of hydrogel, particularly focusing on verifying the uniform dispersion of silver ions within the hydrogel matrix. This analysis is fundamental in confirming the effective incorporation of the antimicrobial agent.

Moreover, the antimicrobial efficacy of the hydrogels is rigorously tested against Escherichia coli (E. coli) via the disk diffusion method. Variables, including the hydrogel concentration and the size of the inhibition zone are carefully measured to determine the capability of hydrogel to prevent bacterial proliferation. The anti-inflammatory properties of the hydrogels are also assessed by examining their ability to prevent Bovine Serum Albumin (BSA) denaturation, with absorbance values at 660nm providing a quantitative basis for comparing the effectiveness of hydrogels, both with and without silver ion enhancement.

This research follows a structured approach: cellulose extraction optimizes methods; hydrogel formation involves silver ion incorporation and cross-linking; assays

evaluate hydrogel efficacy. The study aims to combat bacterial infections, offering a sustainable alternative to antibiotics with silver ions-incorporated cellulose-glycine hydrogels. Emphasizing controlled release, it aligns with targeted delivery, reducing antibiotic resistance risks in wound healing and tissue engineering, particularly bone cartilage repair. This work advances antimicrobial materials, addressing antibiotic resistance challenges in medical applications.

1.5 Significances of Study

This research represents a significant contribution to green chemistry, medical applications, and the management of bacterial infections, bridging the gap between healthcare and environmental sustainability. A key aspect of the study is its use of Napier grass as a renewable cellulose source, aligning with the principles of green chemistry. This approach not only reduces the environmental impact usually associated with cellulose extraction but also fosters the creation of eco-friendly materials. Utilizing Napier grass for hydrogel synthesis is indicative of a shift towards more sustainable practices, aiming to lower the carbon footprint and minimize resource depletion.

In medical fields, the introduction of silver ions into hydrogels is set to transform wound care and surface coating technologies. These enhanced hydrogels demonstrate superior antibacterial qualities, which is particularly relevant given the rising concern over antibiotic resistance. The comprehensive evaluation of these hydrogels, especially against E. coli, reflects a dedication to improving healthcare practices and patient outcomes. The application of silver-ion-enriched hydrogels in medical settings ranges from faster wound healing to developing antibacterial coatings, potentially reducing infection rates and elevating patient care standards. This research's broader significance lies in its holistic approach, tackling challenges across materials science, antimicrobial resistance, and sustainability. By diversifying cellulose sources using Napier grass, the study contributes to sustainable technology and material innovation, standing at the nexus of environmental stewardship, healthcare improvement, and scientific inquiry.

Moreover, the transformative potential of this research lies in its enhancement and broadening of the applications for cellulose-silver-ion hydrogels. By improving the antimicrobial properties of cellulose-based materials, the study paves the way for their

use in diverse areas, from biomedical devices to environmental engineering. Future research could focus on scaling this process, examining the long-term implications of silver ion release, and integrating these hydrogels in various medical and environmental scenarios.

In conclusion, this study is a significant step forward in addressing the dual challenges of contemporary healthcare and environmental sustainability. It innovatively combines sustainable cellulose sourcing with advanced antimicrobial methods, positioning itself as a forerunner in scientific progress. The research not only forges new paths in materials science and antimicrobial studies but also promotes green technology. Its findings provide a robust foundation for further research, charting a course towards environmentally friendly and medically beneficial materials, and setting a new standard for future innovations in these domains.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Antibiotic resistance presents a formidable threat to global health, as forecasted by the World Health Organization, predicting nearly 10 million annual deaths from antibiotic-resistant infections by 2050. In this context, the development of antibacterial hydrogels, especially those enhanced with silver ions, emerges as a crucial innovation. These hydrogels are lauded for their wide-ranging applications, from treating chronic wounds and aiding in tissue engineering to serving as protective layers on implants, thereby facilitating quicker healing and reducing the risk of postoperative infections.

The objective of this literature review is to explore comprehensively the structural composition, as well as the antibacterial and anti-inflammatory properties, of silver ion-infused cellulose-based hydrogels. The review pays special attention to the synergistic effects of combining cellulose with silver ions to create potent antibacterial agents and scrutinizes various methods of incorporating these ions into the hydrogels. A pivotal tool in this exploration is Fourier-transform infrared spectroscopy (FTIR), which provides essential insights into the chemical makeup and structural framework of the cellulose, thereby unravelling the molecular constitution of the hydrogel.

Furthermore, his review explores the advanced methodologies for embedding silver ions into cellulose-based hydrogels, highlighting the impact of these processes on the overall structure and antibacterial properties of hydrogels. Techniques such as Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS), alongside Fourier-transform Infrared Spectroscopy (FTIR), provide a comprehensive morphological analysis and map the distribution of silver ions within the hydrogel matrix.

Additionally, this review synthesizes findings from various studies and empirical data to evaluate the efficacy of these hydrogels against common bacterial

strains, such as *Escherichia coli*. Furthermore, the review examines the anti-inflammatory capabilities of hydrogels, emphasizing their importance in protein structure stabilization and denaturation prevention, which is vital for numerous medical applications.

In conclusion, the incorporation of silver ions into cellulose-based hydrogels marks a notable progress in combating antibiotic resistance. Combining the historical medical use with modern medical technology produces hydrogels that play a critical role in wound care, tissue engineering, and enhanced antibacterial action. The use of advanced analytical methods such as FTIR and SEM-EDS offers provides deep insights into the structural integrity and efficacy of these hydrogels. This innovative approach not only offers substantial medical benefits but also highlights the significance of sustainability and healthcare impact, representing a forward leap in biomedical research.

2.2 Cellulose-based hydrogels

In the field of biomedical research, cellulose, a naturally abundant polysaccharide, is garnering significant attention for its biodegradable, biocompatible, and non-toxic nature, as emphasized by (Naomi et al., 2020). The molecular structure of cellulose, characterized by D-glucose units connected through 1→4 glycosidic bonds, results in a polymeric chain with the formula $(C_6H_{10}O_5)$ n. The presence of numerous hydroxyl groups in cellulose facilitates strong inter- and intra-molecular hydrogen bonding, thus enhancing its mechanical stability and making it an excellent candidate for hydrogel production. These hydrogen bonds, clearly depicted in Figure 2.1, not only reinforce the glycosidic linkages but also support the orderly arrangement of cellulose chains, a critical factor for maintaining its structural integrity (Liyanage et al., 2021).

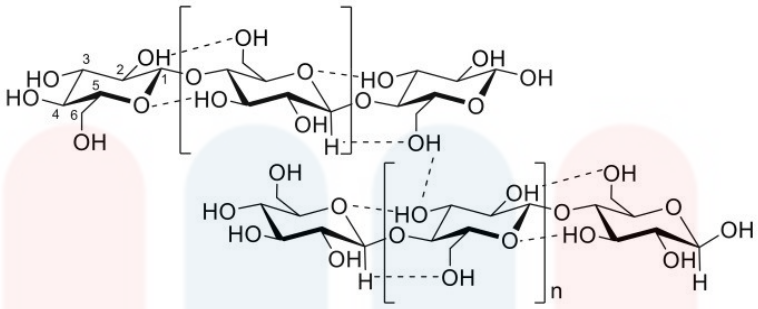

Figure 2. 1: Cellulose intra- and inter-chain hydrogen bonds.

(Source: Liyanage et al., 2021)

The structure of the cellulose polymer, marked by a high degree of crystallinity and a multi-layered composition, stems from its intrinsic supramolecular architecture. Furthermore, the nanoscale cellulose microfibrils, due to these supramolecular interactions, particularly hydrogen bonding, exhibit remarkable mechanical properties. The cellulose's unique makeup, encompassing both amorphous and crystalline segments, varies depending on its source and the processing it undergoes (D. Hu et al., 2021). This crystalline framework, combined with its ability to form a three-dimensional matrix, renders cellulose-based hydrogels highly effective in absorbing wound fluids, reducing inflammation, and inhibiting bacterial growth. This efficacy is supported by studies conducted by Ko & Liao, 2023; Mantha et al. (2019). The remarkable water-holding capacity of these hydrogels, attributed to the cellulose nanostructure, greatly enhances their utility in biomedical applications, especially in wound healing scenarios.

The scope of cellulose-based hydrogels is considerably expanded by incorporating antibacterial agents such as silver ions. Silver, available in forms like nanoparticles, oxides, or ions, is celebrated for its extensive antimicrobial properties, encompassing antibacterial, antifungal, and antiviral activities, while maintaining a relatively low toxicity profile for mammalian cells (Shin et al., 2018; Yin et al., 2020). The integration of such agents into cellulose hydrogels not only amplifies their antibacterial effectiveness but also capitalizes on the biocompatibility of cellulose, thus creating a synergistic effect that presents a robust solution against microbial threats.

Cellulose-based hydrogels, despite their structural adaptability, inherently lack antibacterial properties, necessitating the enhancement of this attribute. Research by Dharmalingam & Anandalakshmi (2020), have demonstrated significant improvements in wound healing and antibacterial efficacy following the incorporation of zinc oxide and silver nanoparticles. Zinc oxide is increasingly favoured in dermatological treatments for its ability to accelerate healing and prevent infections, providing lasting antibacterial benefits at wound sites.

Comparative analyses with other biopolymers, such as alginate or chitosan, indicate that cellulose-based hydrogels possess superior properties, notably in mechanical strength and biodegradability (Sánchez-Cid et al., 2022). Their optimal combination of porosity, absorbency, and structural integrity makes them highly suitable for various medical applications, including tissue engineering and drug delivery systems.

The incorporation of antibacterial agents, particularly silver ions, significantly enhances their potential in medical applications. Silver ions, recognized for their wide-ranging antimicrobial properties, enhance the ability of hydrogels to prevent infections, proving particularly useful in wound care (Shin et al., 2018; Yin et al., 2020). This integration not only increases the antimicrobial efficacy but also preserves the biocompatibility of cellulose, offering a strong defence against microbial contamination.

Additionally, the flexibility of cellulose-based hydrogels in tissue engineering highlights their importance. Serving as scaffolding materials, these hydrogels support tissue regeneration by simulating the extracellular matrix. This capability is further improved by using sustainable sources, such as Napier grass-derived cellulose, thereby advancing the environmental sustainability of hydrogel production (Dutta et al., 2019).

In conclusion, the combination of silver ions with cellulose-based hydrogels represents a significant breakthrough in biomaterials. Their advanced nanostructure and crystallinity give them an edge over other biopolymers, rendering them highly effective for diverse biomedical applications, from wound care to tissue engineering. This review not only underscores the potential of cellulose-based hydrogels but also emphasizes their increasing importance in the fields of biomedicine and material science research.

2.2.1 Crosslinking for cellulose-based hydrogel.

Advancements in biomedical research are highlighting the importance of developing cellulose-based hydrogels, particularly those enhanced with antibacterial agents such as silver ions. At the core of their development is the crosslinking process, which is crucial for enhancing their mechanical strength and ensuring structural integrity. The process begins with the extraction of cellulose fibers from sustainable sources, like Napier grass, using alkaline and bleaching methods. This step is foundational for the subsequent creation of hydrogels that are infused with silver, thereby significantly improving their antimicrobial properties (Danial et al., 2020; Khorairi et al., 2022).

These hydrogels are then structured into a three-dimensional network utilizing various crosslinking agents. These agents can be chemical, forming permanent covalent bonds, or physical, establishing reversible non-covalent interactions such as ionic and hydrogen bonds. The molecular structure of the resulting network is critical to the functionality of hydrogel (Nasution et al., 2022). Crosslinking agents, derived from natural or synthetic sources, play an essential role in the synthesis of these hydrogels, as illustrated in Figure 2.2.

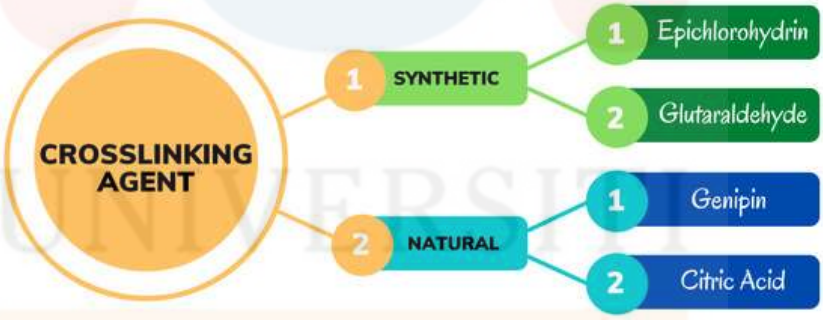

Figure 2. 2: Types of crosslinking agents for cellulose-based hydrogel.

(Source: Nasution et al., 2022)

Figure 2.2 demonstrates the differences between synthetic and natural crosslinking agents and their impact on hydrogel properties. Synthetic agents, including epichlorohydrin and glutaraldehyde, provide strong crosslinking but might challenge biocompatibility. In contrast, natural agents like genipin and citric acid are linked with

better biocompatibility and support cellular processes, making them more suitable for certain biomedical uses (Nasution et al., 2022).

Hydrogels are characterized by their crosslinking method (Figure 2.3). Physical hydrogels form through interactions like ionic and hydrogen bonding, and hydrophobic associations, influenced by temperature changes around LCST (Lower Critical Solution Temperature) /UCST (Upper Critical Solution Temperature). Chemical hydrogels emerge from methods like photopolymerization and "click" chemistry processes, including the Michael addition and Diels-Alder reactions. This overview highlights both physical and chemical crosslinking techniques essential for creating biomedical hydrogels with superior structures and properties (W. Hu et al., 2019).

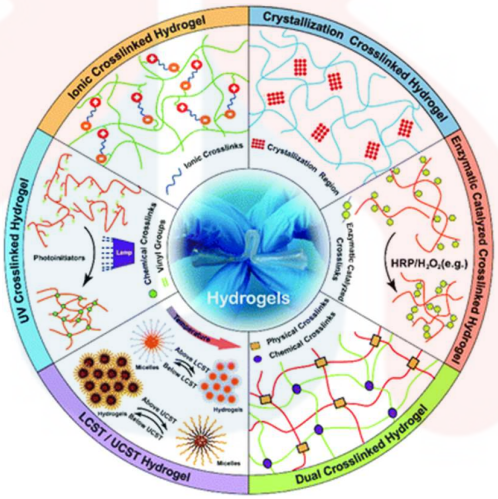

Figure 2. 3: Crosslinking strategies illustrating the chemical and physical techniques used to structure hydrogels.

(Sources: Hu et al.,2019)

Chemical crosslinking is a versatile technique for hydrogel development. However, it can compromise the integrity of encapsulated substances, such as proteins and cells. Some crosslinkers, like glutaraldehyde, are toxic and may interact negatively with bioactive substances in the hydrogel matrix. To avoid these drawbacks, physical crosslinking methods, which do not use toxic agents like GA, are employed. These include chain entanglement, hydrogen bonding, and hydrophobic interactions, sufficient for creating water-insoluble hydrogels (Reyna-Urrutia et al., 2019).

Physically crosslinked hydrogels form through reversible intermolecular interactions, including ionic/electrostatic, hydrogen bonding, polymerized entanglements, and hydrophobic/hydrophilic interactions. They offer substantial biomedical safety benefits, eliminating the risk of cytotoxicity from unreacted chemical crosslinkers. These hydrogels also exhibit stimuli-responsiveness, self-healing, and injectability at room temperature, and can be tailored for bioactive matrix applications, containing living cells and releasing therapeutic compounds (W. Hu et al., 2019).

In conclusion, the production of cellulose-based hydrogels generally favors the physical crosslinking method using natural agents over chemical crosslinking with synthetic agents. This preference stems from the enhanced biocompatibility, absence of toxic chemicals, and the capability of forming hydrogels that are stimuli-responsive and possess self-healing properties. Natural crosslinkers, which are sustainably sourced, promote cellular activity, rendering them particularly suitable for biomedical applications. Consequently, physical crosslinking with natural agents is a more advantageous choice, particularly in scenarios that require materials to be safe, efficient, and environmentally friendly.

2.2.2 Glycine in Physical Crosslinking for cellulose based hydrogel

Glycine, recognized as the simplest amino acid, is characterized by a primary amine (-NH2) and a carboxylic acid (-COOH) group. In the central nervous system, glycine functions as a neurotransmitter and plays multiple roles, including acting as an antioxidant, anti-inflammatory agent, cryoprotective, and immunomodulatory substance in both peripheral and nervous tissues (Razak et al., 2017). Additionally, glycine aids in tissue repair and is essential for synthesizing other biological compounds. These properties highlight its biological significance and establish its importance in the crosslinking of cellulose-based hydrogels.

In the development of hydrogels, glycine's functional groups provide chemical versatility. They partake in condensation reactions to form vinylic monomers, crucial for creating a variety of polymeric materials. Patra et al. (2020) utilized the functionality of glycine to produce monomers with either basic or acidic properties, significantly affecting the swelling behaviour of the resultant hydrogels.

The adaptability of glycine is also highlighted by its amphoteric nature, as pointed out by Palantöken et al. (2020), which facilitates the effective fabrication of hydrogels across various pH ranges. This flexibility extends the hydrogels' usability in different environments, thereby increasing their potential in biomedical applications.

Additionally, the role of glycine in anti-inflammatory, anti-apoptotic, and antioxidative stress responses, as detailed by Y. Zhang et al. (2022), further emphasizes its importance in the physical crosslinking process. These properties enhance the physiological compatibility and therapeutic efficacy of the hydrogels, establishing glycine as a crucial ingredient in their composition.

In conclusion, the involvement of glycine in the physical crosslinking of cellulose-based hydrogels is significant due to its dual role in chemical modification and biological functions. This dual capability not only assists in creating hydrogels with desired features but also improves their biocompatibility and efficiency in medical contexts. The innovative use of glycine in hydrogel production promises the development of materials that are both functionally diverse and biologically synergistic, paving the way for advancements in medical applications.

2.3 Ionic Silver

Silver ions (Ag+) and silver nanoparticles (AgNPs) represent two distinct and potent forms of silver, each with unique properties and antibacterial capabilities. Ag+ ions, generated by removing an electron from a silver atom, are highly reactive and water-soluble, facilitating the formation of compounds with various elements. This monatomic version of silver is celebrated for its antimicrobial prowess, both historically and in modern research (Hamad et al., 2020).

The antimicrobial attributes of silver ions have been exploited for centuries, showcasing their effectiveness against bacterial infections. Derived from compounds such as silver nitrate (AgNO3) and silver sulfadiazine, silver ions are effective against both Gram-positive and Gram-negative bacteria. However, their heightened reactivity may lead to diminished antimicrobial efficiency due to the creation of insoluble precipitates or interactions with specific proteins (Kędziora et al., 2018).

Studies indicate that silver ions possess more potent antibacterial activity compared to silver nanoparticles, primarily because they can disrupt cell membrane permeability, bind to bacterial proteins, trigger oxidative stress, and hinder DNA replication. This multifaceted assault on bacterial cells leads to their destruction (Lotlikar et al., 2019; Mohamed et al., 2020; Z. Xu et al., 2021). The controlled and prolonged release of ionic silver is ideal for applications requiring extended antibacterial action, such as in hydrogels, ensuring effective bacterial growth inhibition while minimizing potential cytotoxic effects on human cells (Shin et al., 2018; L. Wang et al., 2021).

Unlike silver nanoparticles, the antibacterial efficacy of silver ions is not influenced by particle size, which is a significant factor in the activity of AgNPs. This size independence allows silver ions to maintain uniform antibacterial effectiveness across various applications. Moreover, the predictable and manageable release patterns of silver ions in hydrogels offer advantages over silver nanoparticles, making them a more reliable option for certain medical and industrial applications (Ferrag et al., 2021).

Therefore, silver ions (Ag+) are highly effective antimicrobial agents, differing from nanoparticle forms in terms of reactivity, action mechanism, and application. Nevertheless, their application, especially at high concentrations, requires careful management to prevent side effects like argyria, ensuring their safe and effective use in various antibacterial applications (Hamad et al., 2020).

2.4 Incorporation of silver ions into cellulose-based hydrogels

Research have focused on the incorporation of silver ions into cellulose-based hydrogels, considering their distinctive properties and potential applications of these composite materials. This review examines the antibacterial efficacy of cellulose-based hydrogels infused with silver ions, detailing the methods used for their integration and the elements that affect these methods.

2.4.1 Methods of incorporation of silver ions into cellulose-based hydrogels

Researchers have successfully enhanced the antibacterial capabilities of hydrogels by integrating silver ions, employing substances such as silver nitrate (AgNO3) and silver sulfadiazine (Kędziora et al., 2018). This integration utilizes

diverse approaches, specifically designed for various types of hydrogels and their specific applications.

Research has particularly highlighted methods for attaching silver ions to cellulose-based hydrogels. A technique devised by Masood et al. (2019) involves mixing prefabricated nanoparticles with a polymer solution prior to its crosslinking and gelation. In this method, silver nanoparticles (AgNPs) are created by adding silver nitrate (AgNO3) to a combination of polyethylene glycol (PEG) and chitosan, where PEG acts as both a reducer and stabilizer. Following the creation and stabilization of the silver nanoparticles, glutaraldehyde is introduced to chemically link the chitosan and PEG polymers, thereby producing the hydrogel.

Another approach involves infusing metal ions into pre-cross-linked polymer. Ul-Islam et al. (2019) produced a gelatin hydrogel embedded with silver nanoparticles through a chemical reduction process. To generate silver nanoparticles within the gelatin hydrogel, they utilized a 1mM solution of silver nitrate. The pre-formed hydrogel was soaked in the silver nitrate solution to absorb the silver ions, which were then converted into nanoparticles by transferring the hydrogel into a sodium tetrahydroborate solution.

In addition, T. Xu et al. (2023) produced conductive hydrogels containing silver nanoparticles for electrochemical detection of hydroquinone. Firstly, a solution of hydrogel precursor was prepared. In situ, silver nanoparticles were deposited onto the surface of the modified glassy carbon electrode (GCE) via hydrogel, in which the APS solution was combined with the hydrogel precursor solution and dropped with care. The hydrogel was created through the process of gelation. The GCE was promptly submerged in a 0.05M silver nitrate solution after a thorough washing process. The GCE was then removed from the silver nitrate solution and thoroughly rinsed with deionized water to remove any remaining silver nitrate that might have adhered to the hydrogel surface. Finally, a sodium citrate solution (0.05 M) was added to the gel to ultimately reduce silver ions within silver nanoparticles.

The purpose of incorporating silver ions into hydrogels is to develop materials with antimicrobial properties. In this context, the reduction of silver ions (Ag^{+}) to silver nanoparticles (Ag^{0}) is essential for the formation of silver nanoparticles. However, in this research main purpose is to incorporate silver ions into the hydrogel, so no reducing agent is utilized in this specific study. As the literature review shows, silver nitrate

dissolves in the solution, and silver ions become dispersed throughout the cellulose matrix. Without a reducing agent, these ions do not form silver nanoparticles.

As a result, loading metal ions into an already cross-linked polymer is more suitable for incorporating silver ions into hydrogels because it allows for more controlled and uniform distribution of the ions within the established hydrogel network. In this method, the structure and properties of the hydrogel are already defined, and the subsequent introduction of silver ions does not interfere with the polymer matrix formation. This approach ensures the stability of the hydrogel's physical structure while effectively incorporating the silver ions, which can then impart their desired antimicrobial properties. Additionally, this method can help prevent the aggregation of silver ions, ensuring enhanced dispersion and effectiveness of the antimicrobial activity within the hydrogel.

2.4.2 Factors affecting the incorporation of silver ions into cellulose-based hydrogels.

Several crucial factors influence the efficacy of incorporating silver ions into cellulose-based hydrogels. Optimizing these factors is key to maximizing the antibacterial and anti-inflammatory properties of the hydrogels:

- The concentration of silver ions is pivotal for their effective integration into cellulose-based hydrogels. Higher concentrations typically lead to better incorporation due to the enhanced diffusion process (X. F. Zhang et al., 2016). However, excessive concentrations can cause adverse effects, including cytotoxicity. to both bacteria and mammalian cells. While silver ions have antimicrobial properties, they can also be toxic to mammalian cells. Thus, it is vital to find a balance between effective incorporation and minimizing the potential cytotoxic effects of high silver ion concentrations (Aldakheel et al., 2023).
- The conditions of incorporation, such as temperature, pH, and reaction time, significantly impact the efficiency of silver ion integration into the hydrogels (Murali Mohan et al., 2010). Systematic studies are essential for identifying optimal conditions for controlled and efficient incorporation without compromising the quality of the hydrogel. Higher temperatures may facilitate faster integration, but too much heat can lead to degradation or alteration of the

hydrogel's properties (L. Wang et al., 2021). Similarly, the pH level can influence the ionization and complexation of silver ions, affecting their integration into the hydrogel matrix (Lustosa et al., 2017). Optimizing reaction time is crucial for ensuring thorough incorporation while avoiding prolonged exposure that could lead to undesirable side reactions or degradation of the hydrogel structure.

- The porosity of the hydrogel matrix significantly influences the efficiency of silver ion integration (Agnihotri et al., 2012; Dong et al., 2022; Pangli et al., 2021; Rozmysłowska-Wojciechowska et al., 2020). Increased porosity enhances the diffusion of silver ions, thereby improving their integration. Furthermore, a porous matrix provides a greater surface area for the interactions between the silver ions and the hydrogel, facilitating a more effective integration process (Agnihotri et al., 2012).
- Essential for prolonged antibacterial action, the controlled release of silver ions from cellulose-based hydrogel matrices balances ion retention with their gradual dispersal. This balance guarantees ongoing release over time, vital for uses such as wound dressings or implants. The retention of silver ions averts their rapid diminishment, preserving the hydrogels' antibacterial efficiency (Shin et al., 2018). Acting as a reservoir, the hydrogel matrix's composition and the surrounding environmental conditions are key to the kinetics of ion release (Dutta et al., 2019).
- The crosslinking process is crucial for bolstering the stability and mechanical strength of the hydrogels (Zainal et al., 2021). A variety of cross-linking techniques, including physical, chemical, and polymerization methods, are utilized. The hydrogel's ultimate properties are significantly affected by processing conditions such as temperature, pH, and reaction time (Thoniyot et al., 2015).

In summary, integrating silver ions into cellulose-based hydrogels involves a complex interplay of factors, including ion concentration, conditions of incorporation, the matrix's porosity, controlled ion release, and crosslinking techniques. Each aspect is vital in shaping the hydrogels' effectiveness and applicability, underlining the importance of meticulous attention in the creation of sophisticated biomedical materials designed for optimized antibacterial and anti-inflammatory functions.

2.5 In Vitro Antibacterial Activity of Silver Ions Incorporated Cellulose-Based Hydrogel.

A fundamental technique in antimicrobial research is the disk diffusion assay, also known as the Kirby-Bauer method. It is playing a crucial role in evaluating the efficacy of substances like silver-ion-embedded cellulose-based hydrogels against bacteria. This assay involves the placement of disks saturated with a test substance on an agar plate that has been inoculated with a target bacterium. As the substance diffuses through the agar, it inhibits bacterial growth, creating clear zones of inhibition around the disks. The diameter of these zones is measured to evaluate antimicrobial efficacy of the materials (Hudzicki, 2009).

This method is particularly advantageous for the development of antimicrobial surfaces, facilitating the modification of existing materials or the integration of antimicrobial agents with specific substrates. Its simplicity, combined with the rapidity with which results can be obtained, renders it an invaluable tool for identifying optimal antimicrobial combinations and conditions (van Rensburg et al., 2021).

In the context of silver-ion incorporated cellulose-based hydrogels, the disk diffusion assay is instrumental. These hydrogels have shown significant promise, attributed to the synergistic effects of cellulose's biocompatibility and structural integrity with the potent antibacterial properties of silver ions. This innovative combination offers a promising approach to addressing the challenge of antibiotic-resistant bacterial strains, an escalating concern in the domain of medical research (Bruna et al., 2021).

A notable study by Chartarrayawadee et al., (2020) highlighted the antibacterial activity of synthesized silver nanoparticles (AgNPs) against E. coli, utilizing the disk diffusion assay. Their findings revealed that AgNPs, particularly those synthesized with a low concentration of lemongrass extract, exhibited superior antibacterial effects in comparison to traditional treatments. The enhanced antibacterial activity was attributed to the smaller size of these nanoparticles, as confirmed by Transmission Electron Microscopy (TEM), which resulted in a greater surface area for bacterial interaction.

This comprehensive assessment of silver-ion incorporated hydrogels via the disk diffusion assay underscores their potential in addressing the challenges posed by antibiotic-resistant bacteria. By integrating established microbiological techniques with advanced material science, researchers are forging new pathways in the development of

effective antimicrobial therapies. This integration of traditional methods with innovative materials signifies a significant progression in combating bacterial infections that are resistant to standard antibiotic treatments.

2.5.1 Mechanism of Antibacterial Activity of Silver Ions Incorporated Cellulose-Based Hydrogels.

Silver ions are integral to enhancing the antibacterial properties of cellulose-based hydrogels, providing a vital defence against a broad spectrum of bacterial pathogens. These hydrogels harness the potent antibacterial and anti-inflammatory actions of silver ions, making them promise for advanced wound care and infection control applications.

Silver ions target bacteria through multiple mechanisms: they compromise cell membranes, bind to intracellular molecules to inhibit vital functions, and induce oxidative stress, leading to cell death. Initially, silver ions destabilize bacterial cell walls, disrupting vital processes. Internally, they interfere with DNA replication and protein synthesis, while also inducing reactive oxygen species that damage cellular components.

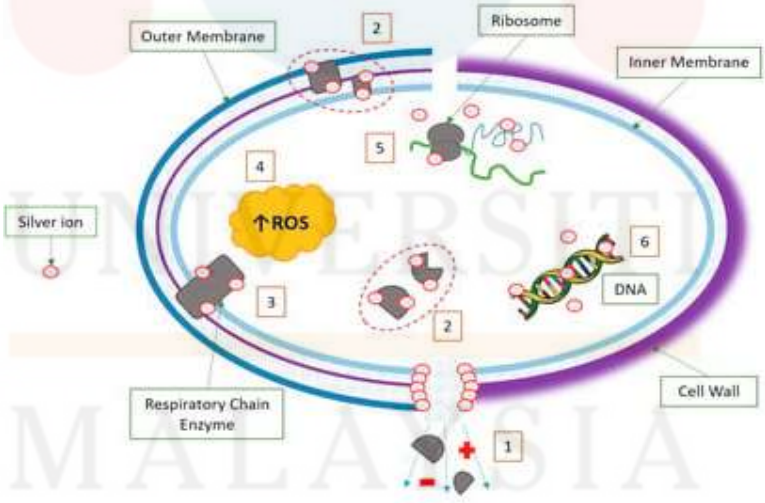

Figure 2. 4: Silver ion in the mode of action to Gram negative (left) and Gram-positive (right) bacteria.

(Source: Kędziora et al., 2018)

The figure 2.4 provides a visual representation of the antibacterial action of silver ions, showing how they disrupt the cell wall (1), affect the respiratory chain enzymes (2), bind to ribosomes (5), and inhibit DNA replication (6), among other targets, in both Gram-negative and Gram-positive bacteria. This multifaceted attack underscores the efficacy of silver ions as an antimicrobial agent (Kędziora et al., 2018).

The oligodynamic effect ensures the efficacy of silver ions at low concentrations, but their potency can vary depending on factors such as ion production methods and environmental pH. These factors must be optimized for each specific application. In wound dressings and medical implants, the controlled release of silver ions from cellulose-based hydrogels facilitates sustained antimicrobial activity without cytotoxicity, highlighting the importance of tailoring ion concentration for the intended therapeutic use.

Ultimately, incorporating silver ions into cellulose-based hydrogels marks a significant advancement in the development of antimicrobial materials, demonstrating strong effectiveness against a wide range of bacterial threats. Such innovation is pivotal for the progression of healthcare solutions. The ability of these hydrogels to effectively combat diverse microorganisms, along with their reduced propensity for resistance development, highlights their importance for both medical and industrial (Hamad et al., 2020; Kędziora et al., 2018).

2.6 In Vitro Anti-Inflammatory Activity of Silver Ions Incorporated Cellulose-Based Hydrogel

The critical role of silver ions in modulating anti-inflammatory activities within cellulose-based hydrogels has profound implications for medical applications. These ions enhance the ability of hydrogels regulate inflammation, a key factor in the healing of chronic wounds and in ensuring successful tissue integration during medical treatments. Chronic wounds, such as diabetic ulcers or severe burns, often experience exacerbation from an overactive inflammatory response, which impedes the natural tissue repair mechanisms. The incorporation of silver ions into hydrogels offers a promising strategy to mitigate prolonged inflammatory activity, thus facilitating the healing process (Huang et al., 2022).

According to Ninan et al. (2020) identified several mechanisms through which silver ions achieve their anti-inflammatory effects. One primary mechanism is the modulation of immune cell activities, including macrophages and T lymphocytes, which prevents an exaggerated immune response. Additionally, silver ions influence cytokine action either by disrupting cytokine-receptor interactions or by suppressing the expression of cytokine genes, which is pivotal in lowering the levels of pro-inflammatory cytokines such as TNF-α and various interleukins (IL-1β, IL-6).

Another significant mechanism involves the inhibition of toll-like receptor (TLR) signalling. TLRs play an essential role in the immune system's recognition and response to pathogens. By attenuating TLR-mediated pathways, silver ions moderate the inflammatory response, which is advantageous in scenarios like implant transplantation and drug delivery systems, where reducing immune reactions is crucial for therapeutic efficacy (Ninan et al., 2020).

Additionally, silver ions are instrumental in preventing the excessive recruitment of inflammatory cells to injury sites, an important aspect in diminishing inflammation in autoimmune conditions and aiding post-operative recovery. They also play a role in reducing levels of reactive oxygen species (ROS), thereby preventing immune cell dysfunction and immunosuppression. This antioxidative property of silver ions is a key contributor to their anti-inflammatory action, particularly relevant in the central nervous system, where they modulate the activity of microglial cells, vital for the immune system defence from body (Ninan et al., 2020).

In conclusion, the incorporation of silver ions into cellulose-based hydrogels offers a comprehensive approach to mitigating inflammation at the cellular level. By influencing immune cell function, cytokine production, TLR signalling, and oxidative stress, silver ions enhance the therapeutic potential of hydrogels, especially in applications requiring meticulous wound care and tissue integration.

2.6.1 Inhibition of Protein Denaturation by Silver Ions Incorporated Cellulose-Based Hydrogel

The role of silver ions in cellulose-based hydrogels in inhibiting protein denaturation emerges as a vital aspect of anti-inflammatory therapy. Protein denaturation, characterized by the loss of structural integrity and functional capacity of proteins due to environmental stresses such as heat, pH, and other denaturing agents,

significantly contributes to tissue damage and inflammation. This process is notably critical in chronic inflammatory diseases like rheumatoid arthritis, where denatured proteins may act as autoantigens, exacerbating autoimmune responses. Understanding and mitigating protein denaturation thus hold substantial promise in anti-inflammatory strategies (Anokwah et al., 2022).

In exploring the protective effects against protein denaturation, Dikkumburage and Madhuranga (2023) utilize the in vitro egg albumin denaturation assay. This assay utilizes egg albumin as a model protein, subjecting it to extreme heat or pH conditions to induce denaturation. The ability of agents, including those in hydrogels, to maintain the native conformation of egg albumin indicates their potential as effective anti-inflammatory compounds. Agents that substantially reduce the denaturation of egg albumin are considered to have significant anti-inflammatory properties (Dikkumburage & Madhuranga, 2023).

Further insights are provided by, Sharifi-Rad et al. (2020) who demonstrated that green synthesized silver nanoparticles (AgNPs) exhibit considerable anti-inflammatory activity. This effect is attributed to the secondary metabolites from plant extracts, which can inhibit the release of lysosomal contents at inflammation sites. These metabolites, similar to the role of silver ions in hydrogels, likely prevent protein denaturation, thus reducing inflammation and tissue damage (Sharifi-Rad et al., 2020).

In conclusion, the strategic incorporation of silver ions into cellulose-based hydrogels presents a novel and effective approach to combating inflammation, primarily by inhibiting protein denaturation. This innovative method combines principles of protein biochemistry with the pharmacological action of non-steroidal anti-inflammatory drugs (NSAIDs), paving the way for new therapeutic interventions in anti-inflammatory treatments.

2.7 Analytical and Characterization Technique

In the field of material science, focusing on the study of hydrogels and cellulosic materials, the application of Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray Spectroscopy (EDS) is pivotal. These analytical techniques are instrumental in providing intricate details about

the chemical composition and surface morphology of materials, which are vital for comprehending and modifying their functionalities.

Fourier Transform Infrared Spectroscopy (FTIR) especially, is paramount for assessing alterations in chemical composition across various processing stages. The research conducted by Somseemee et al. (2021) provides a comprehensive analysis of the FTIR spectral characteristics of untreated Napier grass stems. This study delineates distinct spectral peaks, depicted in Figure 2.5, at wavenumbers 3200–3500cm^{-1}, 2898 cm^{-1}, 1638 cm^{-1} and 1161 cm^{-1} among others. These peaks symbolize different structural constituents, such as cellulosic material, water absorption, and the crystalline structure of cellulose, thereby providing a thorough investigation of the material's chemical framework.

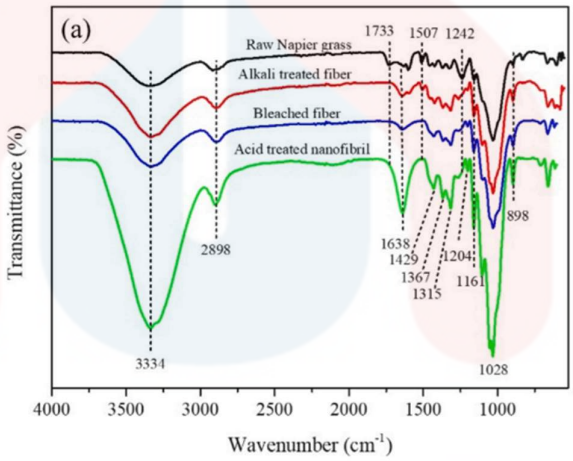

Figure 2. 5: FTIR spectra of samples.

(Source: Somseemee et al., 2021)

This Figure 2.5 displays the FTIR spectra of cellulose, highlighting the vibrational bands corresponding to various functional groups. It aids in understanding the interaction between cellulose and crosslinking agents. The study further delineates peaks representative of hemicellulose and lignin, notably at 1733 cm^{-1}, 1507 cm^{-1}, and 1242 cm^{-1}. These peaks, indicative of specific structural components within the grass fibers, were found to diminish significantly following alkali treatment and virtually disappear after the bleaching process. This observation underpins the effective removal of lignin and hemicelluloses through chemical treatments. Moreover, the intensity increase of the cellulose characteristic peak at 1161 cm^{-1} post-treatment corroborates an

augmentation in cellulose content. The presence of a small peak at 1204 cm^{-1} in the acid-treated CNF spectrum, attributed to S–O vibration, indicates the insertion of sulphate groups during the hydrolysis process, as noted by Somseemee et al. (2021).

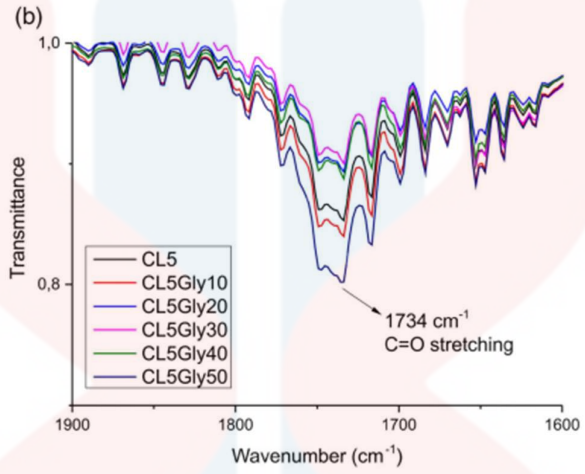

Figure 2. 6: FTIR of cellulose hydrogels

(Source: Palantöken et al., 2020)

Besides, Palantöken et al. (2020) investigated cellulose hydrogels crosslinked by glycine, utilizing FTIR spectra to discern the characteristic vibrational bands of cellulose depicted Figure 2.6. These include O─H, CH, and C─O stretching vibrations at specific wavenumbers. The absence of distinct shifts in the carbonyl stretching at 1734 cm^{-1} and the lack of an amide or ester band around 1680 cm^{-1} suggest that glycine functions as a physical crosslinker without forming chemical crosslinks with the cellulose chains. This study expands the understanding of cellulose's interaction with crosslinking agents, emphasizing the non-covalent nature of the interaction with glycine.

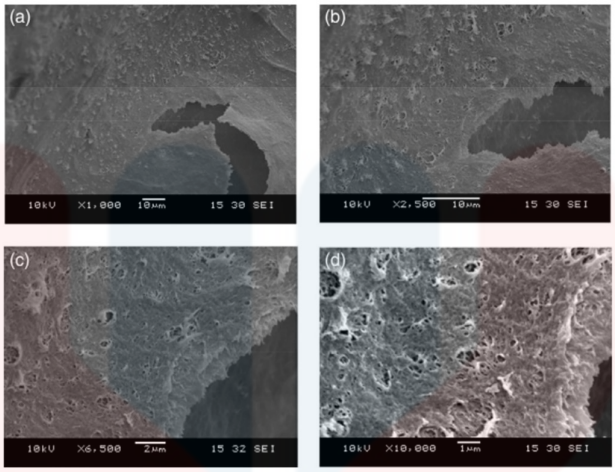

Figure 2. 7: Different magnification SEM images of CL5Gly30 hydrogels (a) ×1000, (b) ×2500, (c) ×5000, (d) ×10 000

(Source: Palantöken et al., 2020)

Additionally, Scanning Electron Microscopy (SEM) has been employed to analyse the surface morphology and microstructure of these cellulose hydrogels. Palantöken et al. (2020) illustrate through SEM images demonstrate as Figure 2.7 the three-dimensional network structure of cellulose–glycine hydrogels that shown in different magnification of image to emphasize their potential application in tissue engineering due to their conducive microstructure for cellular adhesion and tissue formation.

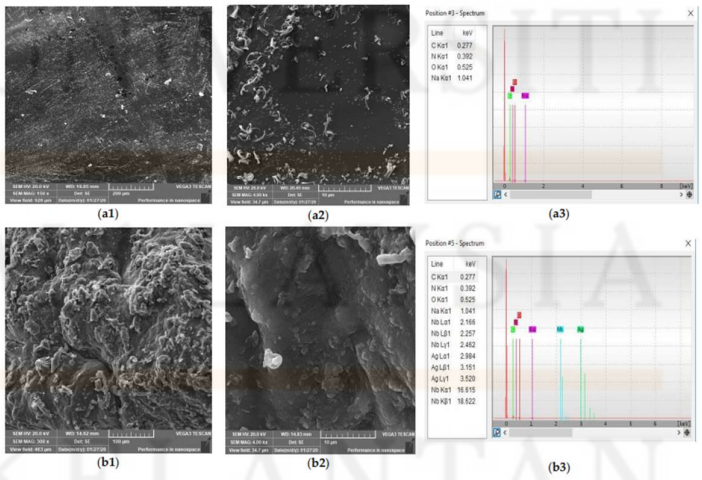

Figure 2. 8: SEM images of hydrogel and their EDS spectra of hydrogel.

(Source: Abd El-Hady & El-Sayed Saeed, 2020)

Furthermore, Abd El-Hady & El-Sayed Saeed (2020) utilized SEM which shown in Figure 2.8 to analyse chitosan and chitosan/silver–curcumin hydrogels. Their findings revealed a flat, clear, and uniform surface topology for chitosan hydrogels. The presence of specific elements, such as C, O, N, Ag, and Nb, was confirmed through Energy Dispersive X-ray Spectroscopy (EDS) analysis, corroborating the formation of silver nanoparticles within the hydrogel matrix.

In conclusion, the incorporation of Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDS) in material science research has been pivotal, as evidenced by the studies of Somseemee et al. and Palantöken et al. The ability of FTIR to detect and track functional groups and compositional changes, in conjunction with the detailed examination of surface morphology and elemental composition offered by SEM-EDS, yields a holistic understanding of the properties of materials. The employment of these analytical methodologies notably enhances our understanding of cellulosic materials and hydrogels, making a substantial contribution to advancements in the realms of materials science and engineering.

2.8 Conclusion

This review underscores the pivotal role of silver ions integrated within cellulose-based hydrogels in addressing antibiotic resistance challenges. It combines the historical medicinal applications of silver with cutting-edge biomedical engineering, offering a promising solution in healthcare contexts. Fourier-transform infrared spectroscopy (FTIR) is employed to delineate the chemical and molecular architecture of these hydrogels, while scanning electron microscopy (SEM) in conjunction with energy dispersive X-ray spectroscopy (EDS) elucidates the morphology and silver ion distribution.

The selection of cellulose as fundamental material justified by its biodegradability, biocompatibility, and low toxicity, making it an optimal substrate for these hydrogels. The incorporation of silver ions substantially elevates their antimicrobial effectiveness, as demonstrated by disk diffusion assays against bacterial pathogens such as E. coli. Furthermore, the ability of the hydrogels inhibit protein denaturation highlights their

anti-inflammatory properties, which are advantageous in wound management and tissue engineering contexts. The crosslinking process, especially the employment of glycine for physical crosslinking, is critical for enhancing the structural and functional integrity of hydrogels ensure that adequate mechanical strength and biocompatibility.

Future research should focus on exploring cellulose-based hydrogels doped with various metallic ions for specific applications in drug delivery and regenerative medicine. The investigation of long-term biocompatibility and in vivo degradation processes is imperative for their clinical application. Additionally, studies aimed at developing scalable production techniques to ensure efficacy and sustainability are vital for their adoption in medical settings.

In conclusion, silver ion-infused cellulose hydrogels represent a significant advancement in the field of medical materials science, especially for wound care and tissue engineering applications. By exploiting the synergistic properties of cellulose and silver ions, these hydrogels offer a sustainable, effective, and innovative approach in biomedical material research, heralding a new era of medical application and research innovation.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Apparatus

The raw materials for this study, including Napier grass, hydrogen peroxide (H2O2), sodium chlorite (NaClO2), sodium hydroxide (NaOH), silver nitrate (AgNO3), and glycine, were directly obtained from the UMK laboratory. The antibacterial assays were conducted with materials provided by the lab, including nutrient agar, distilled water, chloramphenicol, and a bacterial culture of *Escherichia coli* (E. coli). For the anti-inflammatory assays, bovine serum albumin (BSA) solution and phosphate-buffered saline (PBS) were utilized.

The study employed a range of equipment and apparatus, such as the Nicolet™ iS™ 10 FTIR Spectrometer, a laboratory drying oven, a heavy-duty blender, scissors, a reflux system as shown in Figure 3.3 and Figure 3.4, a pH meter, a timer, beakers of 250mL and 500mL capacities, measuring cylinders of 100mL and 10mL volumes, filter papers with a diameter of 110mm, a vacuum pump filter set, a 250-micron mesh sieve, a heating mantle designed to accommodate a 500mL round-bottom flask, an analytical balance with a precision of 0.0000g, a precision balance with a precision of 0.00g, a glass rod, a magnetic stirrer, and a petri dish measuring 90 x 15mm.

The antibacterial assay necessitated additional materials, including one-time use cotton swabs, forceps, sterile paper discs (6 mm), a micropipette (p-1000), micropipette tips (1000µL), a metric ruler, an autoclave (set at 121℃ for 15 minutes), a marker pen, a Bunsen burner, a wire loop, parafilm tape, an incubator, an incubator shaker, and a laminar flow chamber.

3.2 Methods

3.2.1 Preparation of Napier Grass Powder

Firstly, Napier grass was procured and meticulously cleansed before being air-dried overnight at ambient temperature to achieve optimal condition. The desiccated grass was then truncated into segments. These segments underwent a regimented desiccation in a thermostatic oven at 65°C for a duration of 24 hours to eliminate surplus moisture. After this drying phase, the grass was manually processed into a fine powder using a hand blender, a step undertaken to enhance manipulability and expedite the forthcoming extraction process. To ascertain uniformity in the powdered grass and to avert the formation of lumps or unequal particle size distribution, a rigorous sieving procedure was employed, utilizing a mesh sieve with an aperture size of 250 microns.

Figure 3. 1: (a) Napier Grass found in Taman Pinggiran, UMK Jeli Campus; (b) Napier Grass Powder After Drying and Grinding

3.2.2 Extraction Napier Grass Cellulose

The methodology employed for alkaline and bleaching treatments followed the protocols outlined by Danial et al. (2020) and Khorairi et al. (2022) with minor modifications. Comprehensive details on the chemical reagents used in the cellulose extraction are delineated in Table 3.1.

Table 3. 1: The preparation of cellulose extraction.

Process	**Alkaline Treatment**	**Bleaching Treatment**		
Chemicals	NaOH	H_2O_2	NaOH	$NaClO_2$
Concentration	4%wt	5% (v/v)	1.3% (m/v)	0.7% (w/v)
Solid / Liquid prepared	28g	30mL	7.8g	4.2g
Total volume	700mL	600mL		

Cellulose was extracted from the grass by applying an alkaline treatment, which facilitated the removal of lignin and hemicellulose. Specifically, the grass powder was dissolved in a 4 wt% NaOH solution in a 1:20 (w/w) ratio, where 35 g of grass powder was treated with 700 mL of the NaOH solution. The mixture was then subjected to a reflux for 3 hours, the details of which are illustrated in Figure 3.3.

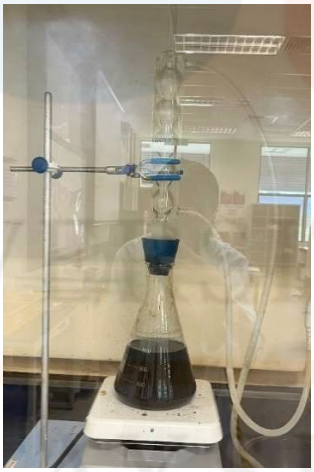

Figure 3. 2: Alkaline treatment under reflux system.

Following alkaline treatment, the mixture was filtered and repeatedly washed with distilled water until a neutral pH of 7 was attained, confirmed by a pH meter. The alkali-treated grass was subsequently dried in an oven at 70°C for 2 hours.

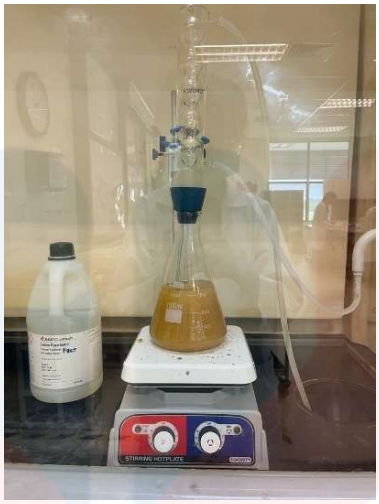

Figure 3. 3: Bleaching treatment under reflux system.

For bleaching, the dried alkali-treated sample was again refluxed at 80°C for 90 minutes as shown in Figure 3.4. The bleaching solution comprised 5% H2O2 (v/v), 1.3% NaOH (m/v), and 0.7% NaClO2 (w/v), prepared by dissolving 6.24g of NaOH in 570mL of distilled water, followed by the addition of 4.2g of NaClO2. Once fully dissolved, 30mL of H2O2 was introduced to the solution, culminating in a total volume of 600mL. The bleached grass, post-reflux, was filtered and washed until neutral pH was consistently observed. The bleaching process was reiterated until the sample's whiteness was satisfactory. The final bleached product was then oven-dried at 60°C for 2 hours to achieve the desired dryness.

3.2.1 Determination of Extraction Yield

The extraction yield of cellulose was determined using the equation described by Khorairi et al. (2022) and Akhlaq & Uroos (2023). The yield percentage was calculated using the equation 3. 1 below:

$$Extraction\ yield\ (\%) = \frac{Dry\ mass\ of\ cellulose\ powder\ obtained\ (g)}{Dry\ mass\ of\ Napier\ grass\ powder\ (g)} \times 100$$

Equation 3. 1

This method quantifies the efficiency of the cellulose extraction process by comparing the mass of the cellulose powder obtained post-extraction to the original mass of the dried Napier grass powder used.

3.2.2 Preparation of Cellulose Dissolution and Glycine solution

The preparation of the cellulose dissolution and glycine solution was conducted in accordance with the methodologies outlined by Palantöken et al. (2020), with minor modifications, following the specifications provided in Tables 3.2 and 3.3.

Table 3. 2: The preparation of 20mL of 2M NaOH.

Process	Cellulose dissolution
Weight of NaOH	1.6g
Molarity of NaOH	2M
Total Volume of NaOH	20mL
Weight of Cellulose	0.8g
Concentration of Cellulose Solution	4%

Table 3. 3: The preparation of stock glycine solution

Labelling	Gly5
Weight of Glycine	0.5g
Weight of NaOH	0.8g
Volume Of 2M NaOH	10mL
Concentration Of Glycine Solution	5%

A quantity of 0.8 g of cellulose was dissolved in 20 mL of 2 M NaOH and stirred vigorously within an ice bath (0–4 °C) for 1 hour to ensure thorough dissolution. To achieve a transparent cellulose solution devoid of native fibres, the mixture was maintained at 0 °C. Additionally, a 5% w/v alkaline glycine solution was prepared by dissolving glycine in 2 M NaOH, to be utilized in subsequent steps of the process.

3.2.3 Preparation of Hydrogels

The crosslinking method adopted in this study follows the protocol delineated by Palantöken et al. (2020) with minor modification for synthesizing cellulose hydrogel

that is physically crosslinked with glycine. Initially, cellulose was dissolved in NaOH under ice bath for one hour. Subsequently, 5% of glycine solution was incrementally added, drop by drop, while the mixture was stirred for an additional hour within an ice bath. Following an additional hour of mixing, the viscous cellulose solution was poured into moulds and left at room temperature overnight. For neutralisation, the gels were washed for one hour with a 5% acetic acid solution the following day. Furthermore, the samples undergo multiple washes until the pH value reaches 7. Finally, place the hydrogels in a freezer set to -22°C for a minimum of 4-6 hours, followed by a transfer to a freezer set to -83°C for a minimum of 12-24 hours.

3.2.4 Synthesis of silver ions incorporated cellulose hydrogels.

According to Xie et al. (2018), the method involved the impregnation of silver ion into cellulose-based hydrogel. The prepared hydrogels were immersed in 0.177M concentrations of silver nitrate solutions—specifically, 1.5g of silver nitrate dissolved in 50 ml of distilled water for a duration of 48 hours. Upon completion of the immersion period, the resulting cellulose-silver ion hydrogels were exhaustively washed with distilled water to eliminate any unreacted substances.

3.2.5 Swelling capacity in water

The swelling capacity was determined as previously described (Helmiyati et al., 2019). Dried hydrogel (0.2 g) was submerged in 50 ml of distilled water at room temperature. The swelling capacity was calculated with Equation 3. 2 as below:

$$S(\%) = \frac{m_{st} - m_0}{m_0} \times 100\%$$

Equation 3.2

Where S is water absorption, m_{st} weight swollen sample, m_0 is the weight of initial gel sample. The results swelling behaviour each sample was plotted in graph. The values were expressed as the mean triplicate standard deviation.

3.2.6 Morphological Characterization and Analysis

3.2.6.1 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The FTIR analysis method for the wavelength spectrometer, as referred by Danial et al. (2020) and Khorairi et al., (2022), was performed using the Thermo Scientific™ Nicolet™ iS™ 10 FTIR Spectrometer to capture the transmittance spectra across a wavelength range of 400 to 4000 cm^{-1}. This method facilitated the identification of functional groups within the test samples. The technique was applied to both the untreated dried Napier grass powder and the treated cellulose to reveal the molecular alterations resulting from the cellulose extraction process. Furthermore, the cellulose-based hydrogel was also subjected to FTIR analysis to determine its functional group composition.

3.2.6.2 Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Measurement (EDS)

The cross-section morphological characterization of the cross-linked cellulose-based hydrogels was examined by scanning electron microscope (SEM) (SEM, JEOL JSM-IT100) referred by El-Kemary, 2022. The current was 30A and a voltage of 20kV was utilised to conduct the measurements. The EDS detector (with an energy resolution of at least 129 eV), 20KV of voltage, and 10mm of WD were utilised to analyse elements.

3.2.6.3 In-Vitro Antibacterial Assay

The in-vitro antibacterial assay utilized the Kirby-Bauer Disk Diffusion method, which was reviewed by Hudzicki (2009) and Tendencia (2004). It involved preparing agar medium, suspending E. coli, and performing disk diffusion to assess antibacterial activity.

3.2.6.3.1 Preparation of Agar Medium

The procedure involved the preparation of nutrient agar, wherein the dehydrated medium was utilized according to the instructions provided by the manufacturer. Following the manufacturer's instructions, the medium was suspended in 500mL of

distilled water, consisting of 14g. The medium was dissolved completely by using distilled water with regular agitation. Sterilization was carried out by autoclaving at 121°C for 15 minutes. Once sterilized, the agar was poured into Petri dishes, ensuring an even spread on a flat surface, and allowed to solidify at room temperature. To prevent contamination, the agar plates were sealed with parafilm tape before being stored in the refrigerator.

3.2.6.3.2 Preparation suspension culture of E. coli

The nutrient agar broth was prepared by using a sterile flask to accurately weigh 3.25g of powder medium, which was then combined with 250mL of water. The resulting broth was subjected to autoclaving at a temperature of 121°C for 15 minutes and subsequently allowed to cool down to room temperature.

A single bacterial colony was carefully inoculated into the liquid medium by transferring a small portion of cells using a sterile wire loop that was briefly exposed to a Bunsen burner flame to ensure sterility. The wire loop was then immersed into the nutrient broth, and the mouth of the flask was securely sealed with sterile cotton plugs, ensuring they were not overly tight. The entire procedure was performed within a laminar flow chamber to minimize the risk of contamination. The flask was subsequently incubated overnight at 37°C under continuous shaking in an incubator shaker for 24 hours to promote bacterial growth and proliferation.

3.2.6.3.3 Disk Diffusion Method

Following a 24-hour incubation period at 37 °C of E. coli culture broth was evenly streaked with sterile cotton swabs to ensure bacterial attachment. The hydrogels, cut into small discs with a diameter of 6 mm, were carefully positioned on the lawns. A positive control using chloramphenicol. Then, the test sample will be variant two type of hydrogel which are with Ag+ ions and without Ag+ ions. The assay was conducted within a biological safety cabinet to minimize the risk of contamination. The plates were incubated at a temperature of 37°C for a duration of 24 hours, during which the zones of inhibition (ZOI) were observed and measured using a ruler. The measurement method for the zone of inhibition (ZOI) involved measuring the distance from the centre of the disk to the edge of the zone of inhibition, as depicted in Figure 3.2 and Figure 3.3.

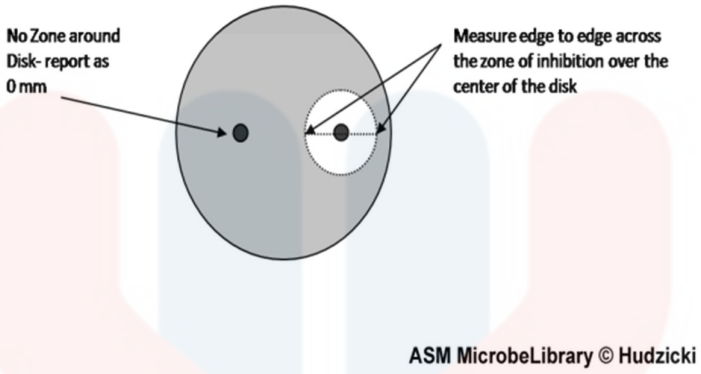

Figure 3. 4: Zones of inhibition measured: gray shading dense bacterial growth, white circle no bacterial growth.

(Source: Hudzicki, 2009)

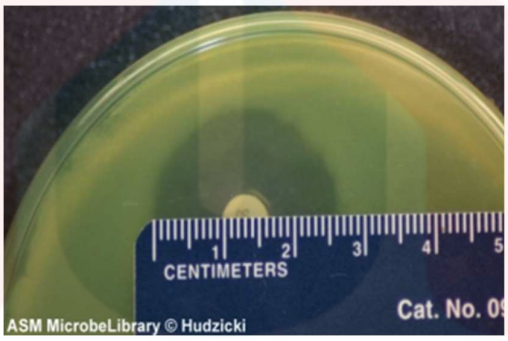

Figure 3. 5: Kirby-Bauer test protocol demonstrates zone size measurement.

(Source: Hudzicki, 2009)

3.2.6.4 In Vitro Anti-inflammatory Assay

3.2.6.4.1 Inhibition of Protein Denaturation

The anti-inflammatory estimation of silver ions-cellulose hydrogel was studied reviewed by Ruffo et al. (2022) and (M. Modi et al., 2019) evaluate the ability of tested sample to inhibit BSA denaturation, by using BSA as a model protein. 50 milligrams of tested hydrogel were suspended in 1 ml of a PBS pH 7.4 solution of BSA (1 mM). To cause the denaturation of BSA, the mixture was incubated for 30 min at 37 °C, then heated to 72°C for 3 minutes in a water bath for the denaturation process. The

absorbance of the reaction was read at 660 nm and the inhibition of BSA denaturation was estimated using Equation 3. 3 below:

$$\%\ inhibition = 100\ \times \frac{A_0 - A_1}{A_0}$$

Equation 3.3

where A_0 is the absorbance of control sample and A1 is the absorbance of silver ions-cellulose hydrogel.

Table 3. 4: Preparation of Sample for The Inhibition of Protein Denaturation Assay.

Sample	**Weight of cellulose hydrogel (g)**	**PBS buffer (mL)**	**Weight of BSA (g)**
A0 (Control)	-	2	1
A1 (with silver ions)	0.01	2	1
A2 (without silver ions)	0.01	2	1

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Extraction of Cellulose from Napier Grass

The extraction of cellulose from Napier grass, a pivotal step in this study, was meticulously conducted through a two-step chemical process. The initial phase involved an alkaline treatment, resulting in an alkaline treated sample yield of 53.05%. This was followed by a bleaching treatment, which further refined the cellulose, reducing the yield to 33.57% relative to the original dry weight of the Napier grass. These precise yields, calculated using Equation 3.1, underscore the accuracy of our methods and the effective removal of non-cellulosic components, which is essential for the subsequent hydrogel production.

The transformation in the physical appearance of Napier grass cellulose at each treatment stage, as captured in Figure 4.1, reflects the gradual elimination of impurities like lignin, pectin, and hemicellulose. Initially, the raw ground Napier grass was a dark green powder with a very large particle size (Figure 4.1(a)), indicative of the presence of various impurities. Post-alkaline treatment, the sample exhibited a slightly brighter colour (Figure 4.1(b)), suggesting the partial elimination of these impurities. The bleaching treatment, performed in two cycles, resulted in a desirable white hue of cellulose (Figure 4.1(c) and (d)), further indicating increased purity crucial for hydrogel formation.

Moreover, the bleaching process also led to cellulose becoming more brittle. For effective hydrogel formation, a smaller cellulose particle size is preferable, as it offers a larger surface area relative to volume, thus enhancing the reactivity of cellulose. Consequently, the cellulose was further processed into finer particles using a blender and sieved to a size of 250 microns.

The role of hydrogen peroxide and sodium chlorite in the bleaching treatment was significant in removing a considerable quantity of residual hemicelluloses and lignin from the alkaline treated sample. This was evidenced by the production of highly reactive superoxide radicals ($O2^{\cdot -}$) that facilitated the oxidation of these components into carboxylic acids stated by Hafemann et al. (2019). Morphological studies of the extracted cellulose, conducted using an optical microscope (Figure 4.2), revealed thin and transparent-like cellulose fibres, characteristic of pure cellulose. This is indicative of a successful extraction and purification process.

In conclusion, the study conducted by the methods from Danial et al. (2020) and Khorairi et al. (2022) with minor modifications, have effectively demonstrated the extraction and purification of cellulose from Napier grass through alkaline and bleaching treatments. The observed changes in colour and morphology throughout the process are indicative of the successful removal of non-cellulosic impurities and the achievement of a purified cellulose product, suitable for further applications such as hydrogel preparation.

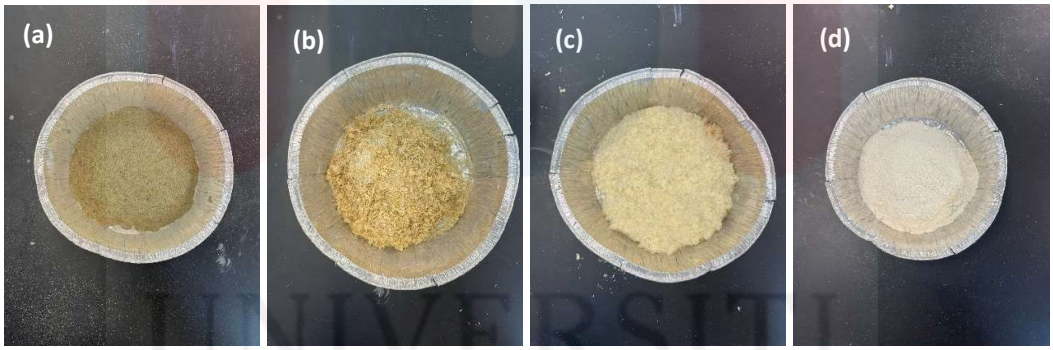

Figure 4. 1: Photographs image: (a) raw Napier grass (b) alkaline-treated sample (c, d) Bleached sample and (d) extracted cellulose sample.

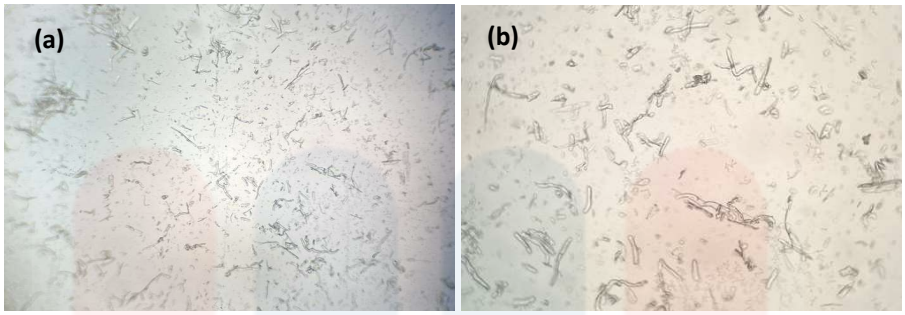

Figure 4. 2: Cellulose under light optical microscope within magnification of x4 (a) and x10 (b).

4.2 Fabrication of Cellulose-Glycine Hydrogels

Building upon the purified cellulose obtained from the extraction process in Section 4.1, we embarked on the fabrication of cellulose-glycine hydrogels, following the procedure adapted from Palantöken et al. (2020). The initial phase of this process was focused on the dissolution of cellulose in 2 M NaOH to form a 4% concentration. This crucial step, conducted with vigorous stirring in an ice bath at 0–4 °C for one hour, ensured the preservation of cellulose's structural integrity and prevented its degradation due to temperature sensitivity.

During this dissolution phase, the cellulose transitioned from a white to a colourless and viscous solution, highlighting the cellulose's amphiphilic nature and enhanced solubility under these conditions. This change is attributed to the highly solvated and coordinated ionic structures formed by cellulose, disrupting hydrogen bonding. The interactions and hydrogen-bond disruption facilitated by sodium and hydroxyl hydrated ions in the NaOH solution played a key role in cellulose dissolution, as the low-temperature conditions favoured more polar conformations around the C–C bonds of cellulose, promoting enhanced solubility (Kihlman, 2012; Lindman et al., 2010; Reyes et al., 2022).

Following the successful dissolution of cellulose, the hydrogel preparation phase commenced. This involved a modified crosslinking method where a 5% glycine solution was gradually added to the cellulose solution while stirring continued in the ice bath. The use of the NaOH/glycine aqueous systems weakened the hydrogen bonding in

cellulose, leading to more activated hydroxyl groups and facilitating bonding interactions between cellulose chains.

The introduction of glycine as a physical crosslinking agent marked a pivotal step in the process. Glycine, being a simple amino acid, potentially interacts with the cellulose chains in the alkaline solution, facilitating cross-linking at various points. This crosslinking is believed to enhance the mechanical properties of hydrogel, such as elasticity and tensile strength, making them suitable for applications that require durability and flexibility.

The composition of the hydrogels, as listed in Table 4.1, followed the formulation CL4Gly5, where the cellulose concentration was 4%, and glycine concentration was 5% with respect to the total NaOH solution. The meticulous addition of glycine and the controlled temperature during the process were instrumental in ensuring the homogeneity of the mixture. After stirring, the viscous cellulose solution was poured into molds and left at room temperature overnight to form gelation. The appearance of gelation form in yellowish colour.

Table 4. 1: Composition Name, Glycine Solution Amount and NaOH In 20ml.

Composition Name	NaOH Solution (mL)	Glycine Solution (mL)
CL4Gly5	18mL	2mL

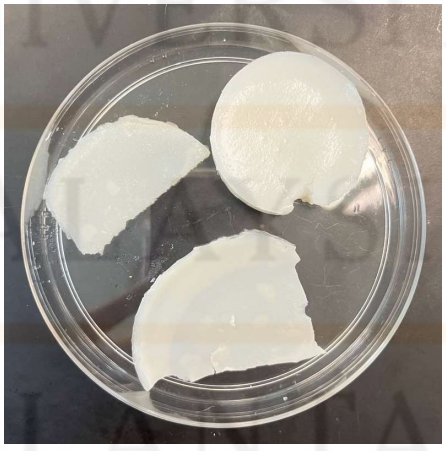

Figure 4. 3: Formation of Cellulose-glycine hydrogel

The subsequent washing process with a 5% acetic acid solution was crucial for neutralizing the hydrogels. This process, repeated until a pH of 7 was reached, was essential for removing residual NaOH and acetic acid, ensuring the safety and neutrality of the hydrogels for applications, particularly in biomedical fields. Besides, the colour of the hydrogels turns from yellowish into white colour during neutralisation process. As a result, the hydrogels will show as Figure 4.3.

The self-assembled characteristics of the cellulose-glycine hydrogels depend on the glycine amount in the solution. The hydrogel bonds form when the carboxylic acid groups in glycine are protonated, leading to pH-dependent swelling of the gels. Ionic interactions also play a significant role, as the dissolution of cellulose in 2M NaOH in an ice bath facilitates the extension of cellulose chains, forming a multidimensional structure (Palantöken et al., 2020).

However, the texture of hydrogel was found to be harder than anticipated, deviating from the softness reported in the literature for cellulose hydrogels physically crosslinked by glycine (Palantöken et al., 2020). This unexpected result might be due to the concentration ratio of cellulose and glycine, which, in the research study, was significantly different from the 5% cellulose and 10%-50% glycine ratio typically used. Furthermore, placing the hydrogel in a freezer, as per the standard protocol, led to cracking, suggesting a potential issue with the freeze tolerance of hydrogel.

These findings contrast with the existing literature, where hydrogels with higher glycine concentrations exhibit more flexible properties. The discrepancies observed in our study could be attributed to several factors, including the specific characteristics of Napier grass cellulose, variations in chemical interactions within the hydrogel matrix, or the freezing method employed.

The observed textural hardness and freeze-induced cracking raise important considerations. It suggests that the formulation and processing conditions, including freezing protocols, play a pivotal role in determining the final properties of cellulose-glycine hydrogels. These limitations highlight the need for further optimization of the hydrogel composition and processing parameters, with a specific focus on optimizing the ratios of cellulose to glycine and enhancing freeze-thaw resilience.

Moreover, the unexpected textural properties and behaviour under freezing conditions unveil the complex dynamics of factors that influence hydrogel characteristics. This encompasses the molecular interactions within the hydrogel matrix and the impact of freezing protocols on the structural integrity of hydrogels. Such initial findings, though unforeseen, offer critical insights for the advancement of hydrogels possessing customized features.

In summary, the production of cellulose-glycine hydrogels, following the methods outlined by Palantöken et al. (2020), revealed the possibility of developing biocompatible and structurally sound materials. However, our investigation encountered unforeseen obstacles, highlighting the need for continued research to decode the complex chemistry of hydrogel formation and to refine processing conditions for specific end-use applications.

4.3 Silver Ions Deposition onto Cellulose-Glycine Hydrogel

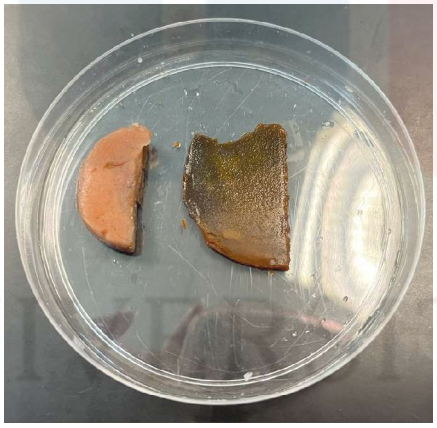

Figure 4. 4: Formation of Cellulose/silver-glycine hydrogel

Following the fabrication of cellulose-glycine hydrogels, the focus shifted to the deposition of silver ions onto hydrogels to form cellulose/silver-glycine hydrogel. The cellulose-glycine hydrogels were immersed in a 0.177M (3%) silver nitrate solution for 48 hours, resulting in a significant colour transformation from white to amber tone (Figure 4.4). This colour change is indicative of the reduction of silver ions (Ag+) to elemental silver (Ag) (Lalsangpuii et al., 2022). This colour serves as a visual confirmation of the successful incorporation of silver ions into the hydrogel matrix. The

choice of a 3% silver nitrate concentration was informed by strong antibacterial and accelerated wound healing by Xie et al. (2018).

However, this study encountered certain limitations. One challenge was ensuring uniform deposition of silver ions throughout the hydrogel matrix, a factor critical for consistent antimicrobial activity. Additionally, the long-term stability and release kinetics of silver ions within the hydrogel are areas that require further investigation to fully understand their implications in medical applications.

In summary, the incorporation of silver ions into the cellulose-glycine hydrogel matrix represents a significant step forward in the development of functionalized hydrogels with robust antimicrobial properties. The chosen concentration of silver nitrate proved effective for ion deposition, as evidenced by the colour change and anticipated antimicrobial properties. This advancement opens up new avenues for the application of these hydrogels in medical and therapeutic fields, particularly in scenarios where antimicrobial action is crucial.

4.4 FTIR for Napier Grass, Cellulose and Cellulose-Glycine Hydrogel.

The application of Fourier Transform Infrared (FTIR) Spectroscopy has been instrumental in analysing the chemical composition changes from Napier grass to cellulose, and further to the cellulose-glycine hydrogel. This section presents a detailed comparative study based on FTIR spectra, which are depicted in Figures 4.5, 4.6, and 4.7. The comprehensive analysis underscores the correlation between these changes and the structural and functional aspects of the hydrogels.

Napier grass stems, primarily composed of cellulose, hemicellulose, and lignin, exhibit characteristic FTIR peaks corresponding to various functional groups like esters, ketones, alcohols, alkanes, and aromatics (Somseemee et al., 2021). In both the Napier Grass and the cellulose sample, certain similarities are observed in their FTIR spectra. Notably, both spectra display peaks indicative of the stretching vibrations of hydroxyl (–OH) groups and C–H groups, essential components in cellulose and hemicellulose. In Napier Grass (Figure 4.5), the peak at 3337.63 cm^{-1} aligns closely with the broad peak typically observed between 3200-3500 cm^{-1}, suggesting a significant presence of –OH groups (Khenblouche et al., 2019). This is mirrored in the cellulose sample (Figure 4.6)

by the peak at 3329.30 cm^{-1}. Additionally, the peak at 2917.16 cm^{-1} in Napier Grass and 2896.29 cm^{-1} in the cellulose sample both indicate C–H stretching vibrations, further corroborating the presence of cellulose and hemicellulose structures.

Another similarity lies in the peaks associated with the interaction of water within the plant's structure. In Napier Grass (Figure 4.5), the peak observed at 1602.33 cm^{-1} closely correlates with the peak at 1638 cm^{-1}, commonly assigned to the bending vibration of absorbed water and carboxylate groups (Somseemee et al., 2021). This correlation is also seen in the cellulose sample with a peak at 1647.02 cm^{-1}, highlighting the significance of water's interaction with the plant's structural components.

However, there are notable differences in the FTIR spectra of these two samples. In Napier Grass (Figure 4.5), the peaks at 1240.27 cm^{-1}, 1373.61 cm^{-1}, and 1507.56 cm^{-1} suggest the presence of hemicellulose and lignin, with their transformation following alkali treatment and bleaching indicating the successful removal of these components (Lorwanishpaisarn et al., 2023). In contrast, the cellulose sample primarily focuses on the cellulose content, with less emphasis on lignin and hemicellulose.

Furthermore, the peak at 1732.74 cm^{-1} in Napier Grass, similar to the absorption peak at ~1734 cm^{-1}, is attributed to the C=O stretching in acetyl and ester groups of polysaccharides, including p-coumeric acids in lignin and hemicellulose. The alteration or absence of this peak post chemical treatments is indicative of the removal of most lignin and hemicelluloses (Lorwanishpaisarn et al., 2023). Conversely, in the cellulose sample, this aspect is less pronounced, focusing more on the cellulose content.

The cellulose (Figure 4.6) specimen demonstrates distinctive peaks at 1160.38 cm^{-1} and 896.60 cm^{-1} aligning with the canonical bands of cellulose attributed to C – O – C stretching in β-(1→4)-glycosidic linkages, as identified by Khenblouche et al. (2019). These peaks signify an enhancement in the cellulosic component ratio following chemical treatment, an aspect that is not extensively highlighted in the analysis of Napier Grass.

In the Fourier Transform Infrared (FTIR) spectroscopic examination of the cellulose-glycine hydrogel, depicted in Figure 4.7, a sequence of pronounced peaks emerges, each denoting particular functional groups and structural constituents of the hydrogel. Generally, significant functional groups of hydrogels include hydroxyl (OH),

carbonyl (C=O), amine (NH2 or NH), ether (C-O-C) and Methylene groups (CH2) (Soni et al., 2019). In this research study, the significance of functional group identifiable the composition of hydrogel.

The peak observed at 3328.96 cm^{-1} associated with OH stretching vibrations, a hallmark of cellulose, suggesting the prevalence of hydroxyl groups. These groups are crucial for hydrogen bonding and the formation of the hydrogel. This peak exhibits a degree of similarity to the OH stretching vibrations seen at 3443 cm^{-1} in other hydrogel samples, as mentioned in the study by Palantöken et al. (2020). Additionally, the peak at 2903.74 cm^{-1} in the cellulose-glycine hydrogel could be attributed to C–H stretching vibrations in cellulose, resonating with the findings at 2895 cm^{-1} for cellulose bands in the literature.

Furthermore, the peaks observed at 1058.21 cm^{-1} and 997.05 cm^{-1} may be associated with C–O stretching vibrations in cellulose, aligning with the C–O stretching vibration reported at 1053 cm−1 for cellulose (Palantöken et al., 2020). In hydrogels containing glycine, the presence of peaks related to the twisting modes of NH_2 (around 898cm-1) and CH_2 (around 1372cm-1) exhibit an incremental increase in intensity. This variation in intensity may be attributed to a higher concentration of glycine within the cellulose hydrogels. A critical observation from the FTIR analysis suggests that the interaction between cellulose and glycine predominantly involves physical crosslinking rather than chemical bonding (Palantöken et al., 2020). The literature review highlights that while carbonyl (C=O) groups are present in the hydrogel, this study reveals a diminished intensity of C=O peaks, implying a possible reduction in crosslinking density. The possible reduction in crosslinking density can observed through the scanning electron microscopy (SEM) analysis for further clarification.

The additional peaks at lower wavenumbers, such as 401.83 cm^{-1}, 409.85 cm^{-1}, 417.85 cm^{-1}, 421.64 cm^{-1}, 440.83 cm^{-1}, and 460.01 cm^{-1}, while not explicitly detailed in the referenced content, might indicate specific molecular interactions or structural configurations unique to the cellulose-glycine hydrogel. These peaks could be integral to understanding the hydrogel's complex molecular architecture.

In summary, the FTIR analysis provided a detailed comparative study of the chemical composition changes from Napier grass to cellulose and then to the cellulose-glycine hydrogel. These findings correlate with the structural and functional aspects of

the hydrogel, emphasizing the importance of specific functional groups in its formation and stability. The integration of Figures 4.5, 4.6, and 4.7 ensures a clear visual representation of these spectroscopic differences and similarities, enhancing the understanding of the molecular structure of hydrogel.

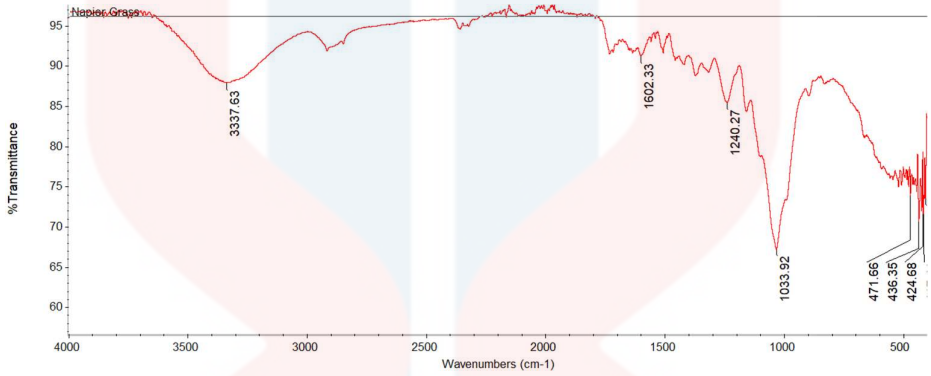

Figure 4. 5: Fourier-transform infrared spectra of Napier Grass.

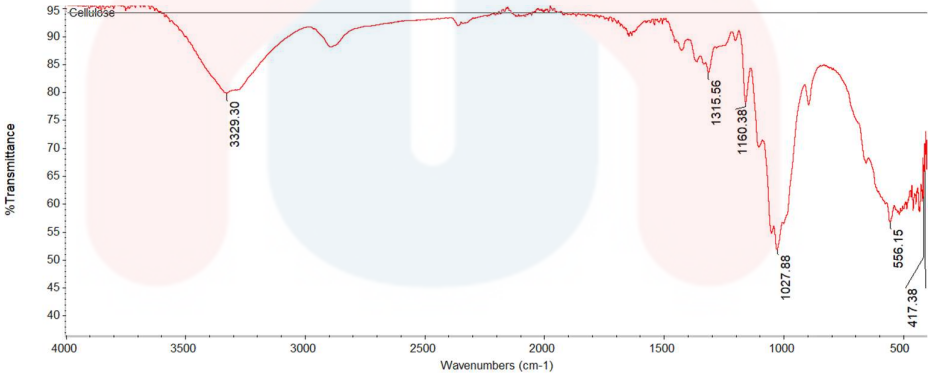

Figure 4. 6: Fourier-transform infrared spectra of Cellulose.

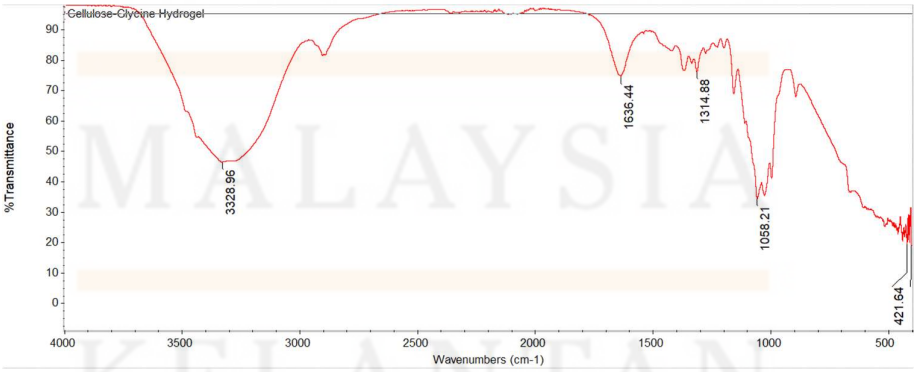

Figure 4. 7: Fourier-transform infrared spectra of Cellulose-Glycine Hydrogel.

4.5 Studies on the swelling ratio of Hydrogel

The swelling ratio of cellulose-glycine hydrogels has been studied with particular focus on the incorporation of silver ions (Ag+) and their potential antimicrobial applications. The swelling ratio is indicative of the hydrogel's capacity to absorb water relative to its dry weight, a critical property for biomedical applications where interaction with pathogens in aqueous environments is common (Kurniati et al., 2021; T. Wang et al., 2020). Swelling is governed by the absorption of solvent and reaches equilibrium when the hydrogel is fully saturated (Islam et al., 2021).

Both the cellulose-glycine hydrogels with and without Ag+ exhibited lower swelling ratios than anticipated. In our study, the maximum swelling ratio observed was 9.5% for cellulose-glycine hydrogels without Ag+ after 48 hours, and a slightly lower ratio of 7.5% for cellulose/silver-glycine hydrogels indicate on Figure 4.8. The result of swelling ratio of hydrogel calculated using Equation 3.2. However, there is any standard value for the swelling ratio of hydrogels, as it varies greatly depending on specific type of hydrogel and its intended application.

The lower swelling ratio in the cellulose/silver-glycine hydrogels could be attributed to the increased crosslink density due to the incorporation of Ag+ ions. This enhanced crosslinking potentially restricts the hydrogel's expansion and reduces its fluid uptake capacity, contrary to our initial hypothesis. In contrast, hydrogels with high swelling capacity are preferred for applications like wound healing and tissue regeneration due to their ability to absorb bodily fluids and facilitate nutrient and drug transport (Feng & Wang, 2023).

Conversely, the moderated swelling behaviour and earlier plateau of the cellulose/silver-glycine hydrogels may offer distinct advantages in certain biomedical applications. In tissue engineering, for example, a more predictable and reduced variability in swelling behaviour is essential. Controlled swelling is crucial to maintain the mechanical integrity of the hydrogel and to prevent the structural integrity of hydrogels and avoiding excessive pressure on adjacent tissues, a consideration of paramount importance in applications such as implants or scaffolds. Fan et al., (2020), emphasize the necessity of fine-tuning the swelling characteristics of hydrogels to ensure their operational efficacy and biocompatibility in tissue engineering contexts.

Future studies should aim to fine-tune the equilibrium between antimicrobial effectiveness and the swelling capacity of hydrogels. This might entail experimentation with varying levels of Ag+ ion concentrations or the investigation of alternative crosslinking techniques. Moreover, it is critical to explore the sustained release dynamics of Ag+ ions from the hydrogel framework and evaluate their influence on both the antimicrobial activity and compatibility with tissues.

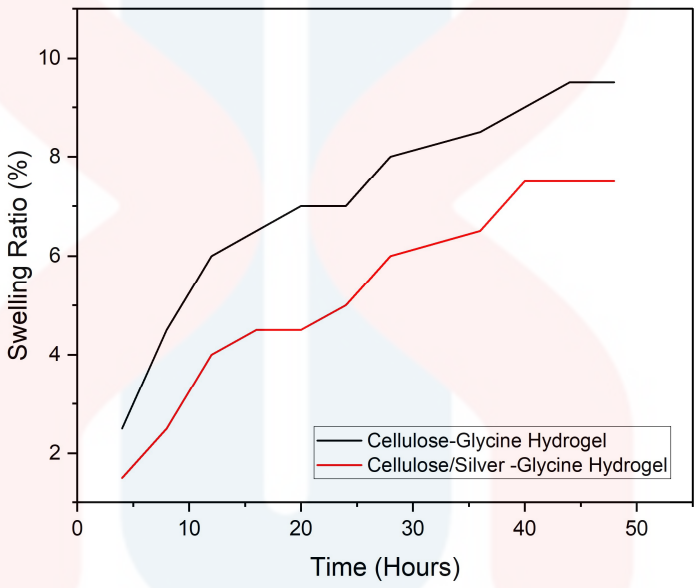

Figure 4. 8: Comparison of swelling ratio of cellulose-glycine hydrogel and cellulose/silver-glycine hydrogel.

4.6 SEM Structural Analysis and FTIR Correlation

The SEM analysis, as depicted in Figures 4.9 (left and right), provided an in-depth examination of the microstructural features of both cellulose-glycine and cellulose/silver-glycine hydrogels, vital for their applications in the biomedical field. Captured at different magnifications, the images unveiled the inherent fibrous architecture that is critical for the hydrogels' mechanical robustness and functional performance. The hydrogels displayed a heterogeneous topography with a diverse fibre network and variable density areas, contributing significantly to their porosity, a key factor in fluid dynamics (Chai et al., 2017).

Further analysis indicated an entangled arrangement of fibres, suggesting physical crosslinking, possibly due to the interaction with glycine. This structural complexity is likely to affect the hydrogel's resilience under various mechanical stresses (Lin et al., 2022). The porosity and pore size distribution, observable at the microscale, indicated a matrix designed for high absorption capabilities. According to (Pinthong et al., 2023) stated that pore size in hydrogels plays a critical role in determining their surface absorption capacity.

Despite the general trend that larger pores typically offer greater fluid absorption, this study found that both hydrogels demonstrated lower swelling ratios of 9.5% and 10%, respectively, as indicated in Figure 4.8. This suggests that smaller pores lead to lower swelling ratios, beneficial for tissue engineering applications where controlled swelling and nutrient flux are essential (Caliari & Burdick, 2016).

The cellulose/silver-glycine hydrogel (Figure 4.9 right) exhibited similar structural features to the cellulose-glycine hydrogel (Figure 4.9 left), including network structure, porosity, pore size, and visibility. However, a notable difference in the cellulose/silver-glycine hydrogel was a coarser surface texture observed post silver ion infusion, marked by bright, dispersed spots that potentially indicate silver inclusions, warranting EDS analysis to confirm the presence of silver ions within the hydrogel.

On the other sides, there is a clear correlation between the SEM structural observations and the FTIR findings. The strong hydroxyl (–OH) and C–H stretching vibrations identified in the cellulose and cellulose-glycine samples (Figures 4.6 and 4.7) underscore a dense hydrogen-bonding network within the cellulose matrix. Hydrogels characterized by Fourier-transformed infrared (FTIR) spectroscopy confirmed the formation of structure (Bukhari et al., 2015), as evidenced by the SEM images showing a well-defined fibre arrangement and consistent pore structure. The FTIR analysis of the cellulose-glycine hydrogel (Figure 4.7), with distinct peaks at 3328.96 cm^{-1} and 2903.74 cm^{-1} corresponding to O–H and C–H groups, suggests that glycine primarily functions as a physical crosslinker. This aligns with the physical crosslinking inferred from the SEM images.

Unfortunately, cellulose/silver-glycine hydrogel, especially post silver ion infusion, while not directly correlated with specific FTIR data, still provides valuable insights. The coarser texture could indicate surface modification due to silver ion incorporation,

which may affect its physical properties and interaction with biological entities, despite not significantly altering the cellulose's chemical structure.

In summary, SEM and FTIR analyses collectively provide a detailed picture of the hydrogels' microstructure and molecular composition. SEM analysis offers physical evidence of the hydrogels' structural integrity and complexity, while FTIR spectroscopy gives insights into the chemical stability and integrity of the cellulose structure. These techniques together illustrate the intricate relationship between the hydrogels' physical structure and molecular composition, enhancing our understanding of their properties and potential biomedical applications.

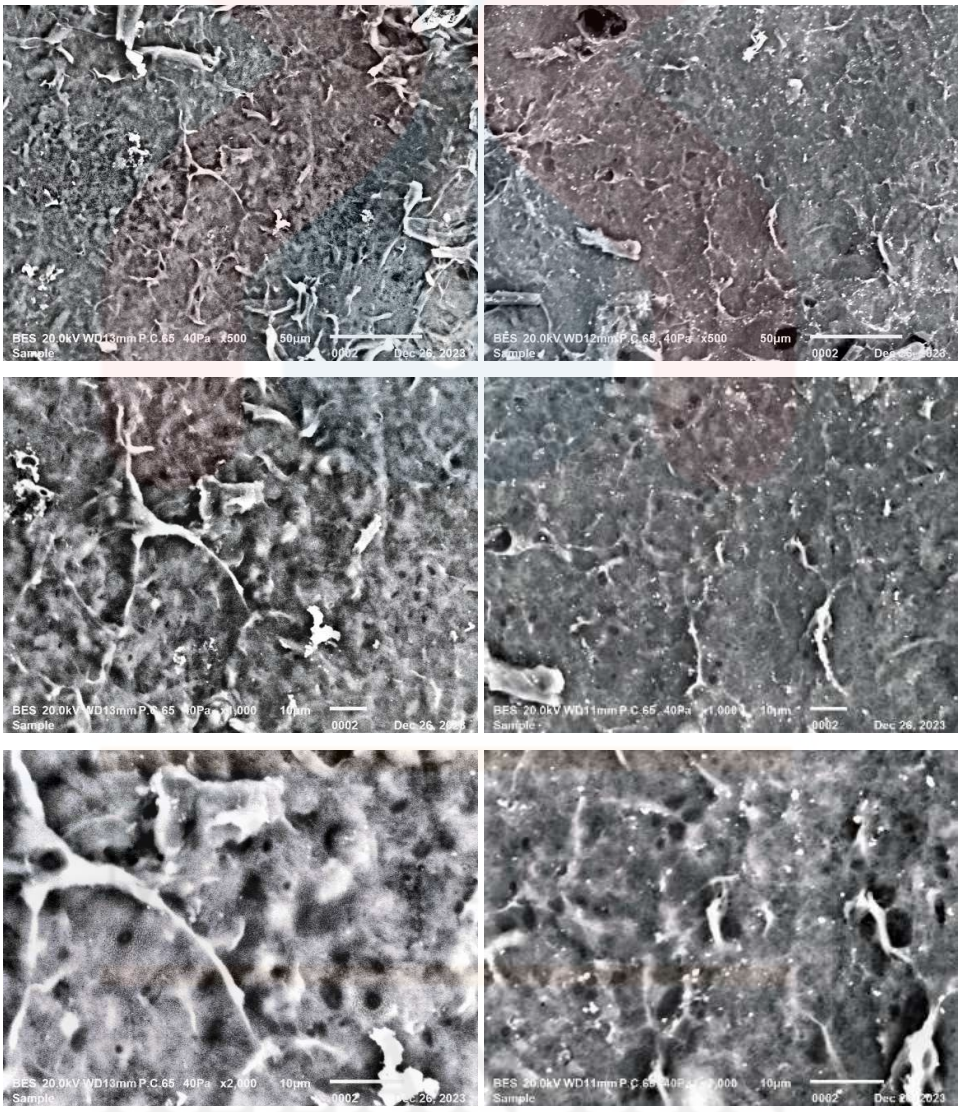

Figure 4. 9: SEM image of cellulose-glycine hydrogel (left) and cellulose/silver glycine (right) with different magnification.

4.7 In Vitro Antibacterial Assay

The assessment of the antibacterial properties of cellulose-glycine hydrogels, particularly the variant incorporated with silver ions, was conducted using the disk-diffusion method. This assay, a standard in evaluating antibacterial activity, provides a visual representation of the efficacy of the tested materials against bacterial growth, in this case, *Escherichia coli*. The results, illustrated in Figure 4.10, showcase the presence of an inhibition zone around the disk embedded with the silver ion-incorporated hydrogel, a clear indication of its antibacterial potency.

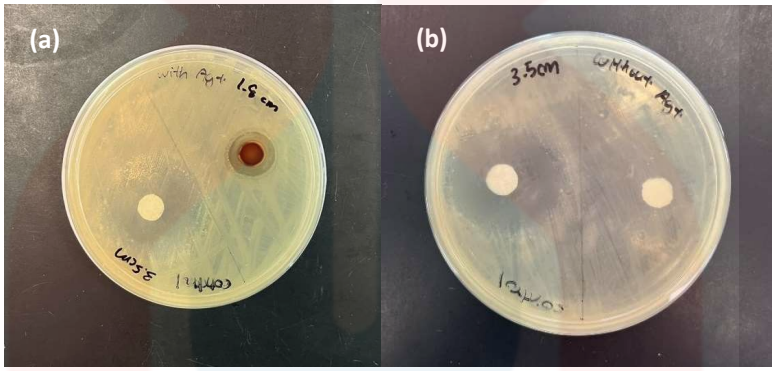

Figure 4. 10: Disk diffusion test against *Escherichia coli* using hydrogel disc incorporated with silver ions (a) and without silver (b) ions compared with paper disc for positive control.

The disk-diffusion test is crucial in determining the efficacy of antimicrobial agents. In this assay, the size of the inhibition zone is directly proportional to the material's antibacterial strength. Chloramphenicol, a well-known antibiotic, was used as a positive control. The results showed a significant inhibition zone of 35mm, surpassing the expected range of 21-27mm for chloramphenicol 30mcg, according to standard interpretive criteria. This discrepancy suggests an elevated concentration of chloramphenicol compared to standard commercial discs, a factor that should be considered in the interpretation of the results.

In the case of the cellulose/silver-glycine hydrogel (Fig. 4.10 (a)), an inhibition zone measuring 18mm was recorded, signifying a modest but effective diffusion of silver ions from the hydrogel matrix that successfully hinders bacterial growth. The antimicrobial efficacy of silver ions, as stated by Rai et al. (2019), is highlighted by

their contribution to the formation of this inhibitory zone. The migration of silver ions from the hydrogel into its surroundings establishes a zone adverse to bacterial growth, thereby affirming the material's antimicrobial capabilities.

Conversely, the cellulose-glycine hydrogel (Fig. 4.10 (b)) displayed no discernible inhibition zones, signifying the lack of inherent antibacterial activity within the hydrogel's composition. This finding accentuates the indispensable role of silver ions in bestowing antibacterial characteristics upon the hydrogel. Absent these ions, the cellulose-glycine matrix fails to demonstrate comparable antibacterial efficacy, highlighting the necessity of silver ion integration in applications requiring antibacterial attributes.

In summary, the disk-diffusion assay effectively demonstrates the enhanced antibacterial properties of the cellulose-glycine hydrogel through the incorporation of silver ions. The presence of these ions significantly improves the hydrogel's ability to inhibit bacterial growth, a feature that could be highly beneficial in numerous biomedical applications, particularly in wound dressings and other contexts where infection prevention is critical. However, it is also essential to consider the potential cytotoxic effects of silver ions, thus necessitating a careful balance between antibacterial efficacy and biocompatibility in the design of such hydrogels.

4.8 In Vitro Anti-Inflammatory Assay

The evaluation of the anti-inflammatory properties of the cellulose-glycine hydrogels was conducted through an in vitro assay focusing on protein denaturation. This method, a well-established paradigm in assessing anti-inflammatory activity (Salve et al., 2022), allows for the quantification of the capacity of hydrogels to inhibit the denaturation of proteins. Table 4.2 shows the percentage of inhibition protein denaturation for both variants of the hydrogel: one infused with Ag+ ions and the other devoid of these ions. Notably, the variant containing silver ions demonstrated enhanced anti-inflammatory properties compared to its counterpart without silver ions.

Table 4. 2: Anti-inflammatory activity of cellulose-glycine with silver ions and without silver ions using BSA denaturation method.

Sample	Absorbance (660nm)			% of Inhibition of Protein Denaturation			% inhibition (mean±SD)
	a	b	c	a	b	c	
Control	1.112	1.112	1.115	-	-	-	-
Cellulose/Silver-Glycine Hydrogel	0.724	0.724	0.723	34.892	34.892	35.157	34.980±0.153
Cellulose-Glycine Hydrogel	0.998	0.996	0.997	10.252	10.432	10.583	10.422±0.173

Protein denaturation is a destabilizing process where proteins lose their functional conformation due to stressors such as heat or pH changes, leading to an inflammatory response (Salve et al., 2022). This denaturation often leads to protein aggregation, which in turn can activate inflammatory pathways. Consequently, the prevention of protein denaturation is pivotal in mitigating inflammatory responses, making it a critical parameter in the evaluation of anti-inflammatory agents.

In this investigation, the anti-inflammatory efficacy of the hydrogel was determined through its capacity to inhibit the denaturation of bovine serum albumin (BSA), employed as a standard protein model. The results of inhibition of protein denaturation calculated using Equation 3.3 indicated that the cellulose/silver-glycine hydrogel exhibited a moderate percentage of inhibition rate (34.980±0.153%), in contrast to the cellulose-glycine hydrogel, which showed a significantly lower rate of protein denaturation inhibition (10.422±0.173%).

The reason that cellulose/silver-glycine hydrogel shown higher inhibition rate of protein denaturation due the protective effect against BSA denaturation and explained by several mechanisms. Firstly, the hydrogel that contains silver ions can help prevent proteins from breaking down due to heat at 72°C. This is because the silver ions can chemically interact with the proteins, helping to keep them stable and reducing inflammation (Sidhu et al., 2022).

These outcomes highlight the critical role of silver ions in augmenting the anti-inflammatory attributes of the cellulose-glycine hydrogel matrix. The presence of silver ions likely contributes to the hydrogel's ability to stabilize protein structures, thereby preventing their denaturation and subsequent inflammatory response. This attribute of the hydrogel, augmented by the presence of silver ions, suggests its potential applicability in biomedical contexts where anti-inflammatory properties are requisite. However, it's essential to balance these properties with potential cytotoxicity concerns associated with silver ions, warranting further investigation into the optimal concentration and application methods to maximize therapeutic efficacy while minimizing risks.

4.9 EDS Silver Ion Analysis and Antimicrobial Correlation

In the preceding SEM analysis (Section 4.6), the detection of bright spots within the cellulose/silver-glycine hydrogel was noted, potentially indicative of silver inclusions. To verify the composition of these inclusions, Energy Dispersive X-ray Spectroscopy (EDS) analysis was conducted, primarily focusing on confirming the presence of silver ions within the hydrogel. This analysis was essential for substantiating the SEM structural findings and understanding the elemental composition contributing to the hydrogel's antimicrobial properties.

Confirming the initial hypothesis, the EDS results, as depicted in Figure 4.11, demonstrated the presence of silver ions. Meanwhile, Figure 4.10 corroborated the incorporation of silver ions for antimicrobial functionality. The pronounced silver signal in the EDS spectrum aligned with the SEM-observed bright spots, reinforcing the successful integration of silver ions into the hydrogel matrix. This elemental confirmation is pivotal in establishing a direct link between the hydrogel's structural characteristics and its antimicrobial efficacy. The EDS results, as depicted in Figure 4.11, conclusively demonstrated that silver ions are present within the hydrogel, with the silver constituting 44.69% by weight and 17.38% by atomic percentage.

However, the EDS analysis also unexpectedly revealed a substantial presence of silicon within the hydrogel. The sample composition, primarily silicon (55.31% by weight and 82.62% by atomic percentage), presented in Figure 4.11, was surprising

since silicon is not a typical component of hydrogel formulations. Generally, hydrogel formulations are expected to contain oxygen (O) and carbon (C) (Chen et al., 2022), and literature reviews have highlighted C and O as common elements in hydrogels (Khaleghi et al., 2020). The absence of peaks for these elements in the EDS graph analysis suggests the possibility of silicon being a contaminant or residue from the sample preparation process. This finding prompts further investigation into its impact on the hydrogel's properties, particularly in biomedical applications.

Correlating these elemental findings with the hydrogel's observed antimicrobial properties, as demonstrated in the in vitro antibacterial assay (Section 4.7), a clear connection emerges between the presence of silver ions and the hydrogel's ability to inhibit bacterial growth. The significant amount of silver, as verified by EDS, supports the hypothesis that silver ions are crucial in the hydrogel's antimicrobial activity. This understanding is vital in discerning how the elemental composition of the hydrogel influences its functionality, especially in biomedical contexts where antimicrobial properties are paramount.

In conclusion, the EDS analysis not only corroborated the SEM findings by confirming the presence of silver ions but also revealed the unexpected element of silicon. This discovery adds a new dimension to our understanding of the hydrogel's composition and necessitates further research to fully comprehend its implications. The strong correlation between the silver ion content and the hydrogel's antimicrobial properties underscores the potential of these hydrogels in biomedical applications, while highlighting the importance of comprehensive material characterization in developing functional biomaterials.

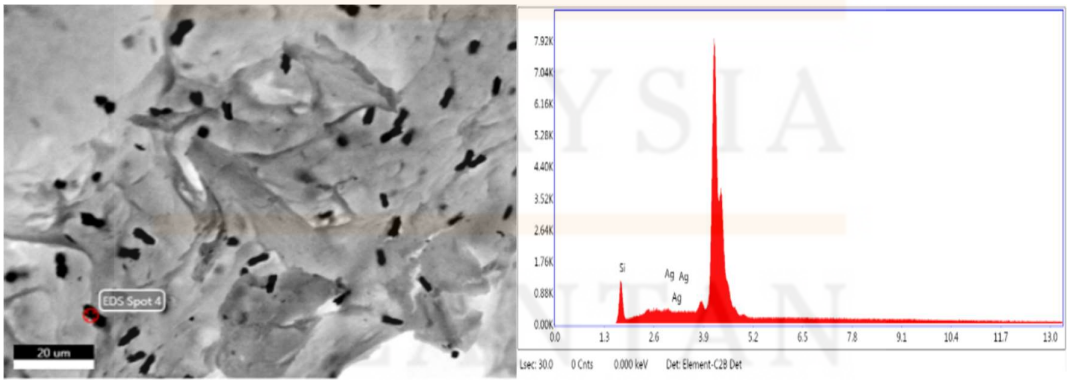

Figure 4. 11: EDS spectra of cellulose/silver-glycine hydrogels.

CHAPTER 5

CONCLUSION & RECOMMENDATIONS

5.1 Conclusion

In conclusion, the results highlight the potential for cellulose-based hydrogels infused with silver ions in biomedical applications. Achieving the objectives to create hydrogels with advanced structure and improved functionality through efficient extraction and purification of cellulose from Napier grass, with meticulous Fourier-transform infrared spectroscopy (FTIR) analysis confirming integrity and purity. Morphological analysis through Scanning Electron Microscopy (SEM) showed a unique porous and fibrous design crucial for the efficient transport of nutrients and integration with cellular structures, positioning these hydrogels as highly potential for applications in tissue engineering and wound healing.

Effective integration of silver ions manifested in subtle modifications to surface texture, directly linking to the antimicrobial capabilities confirmed through Energy-Dispersive X-ray Spectroscopy (EDS) analysis. Hydrogels enhanced with silver ions showed significant antimicrobial action, particularly against Escherichia coli, evident in a marked inhibition zone. The discovery of silicon within the hydrogel matrix has further investigation into its source and implications on hydrogel properties.

Functional tests emphasized the hydrogels' outstanding antibacterial and anti-inflammatory performance, with cellulose/silver-glycine hydrogels displaying a considerable ability to inhibit protein denaturation. This function indicates a strong potential in applications requiring anti-inflammatory action, such as treatment of chronic wounds and fabrication of tissue engineering scaffolds.

This study provides significant insights into biomedical materials science, underlining the appropriateness of developed hydrogels for a range of medical

applications. Results create a strong foundation for future research aimed at refining and broadening the application spectrum of sustainable, effective, and biocompatible hydrogels in healthcare. Structural robustness, controlled swelling behaviour, and antimicrobial effectiveness of hydrogels, especially those enriched with silver ions, demonstrate capability in addressing modern healthcare challenges while adhering to principles of sustainability and efficiency.

5.2 Recommendations

Recommendations for advancing the development of cellulose-based hydrogels infused with silver ions include optimizing their antimicrobial efficacy and biocompatibility. Determining the optimal concentration of silver ions becomes paramount, with Atomic Absorption Spectrometry (AAS) serving as a precise measurement tool. This ensures a balance between maximizing antimicrobial properties while maintaining safety for human cells. Meanwhile, cytotoxicity assays, such as the MTT assay, should assess compatibility with human cells by measuring cell viability to gauge potential cytotoxic effects of silver ions.

Furthermore, exploration of innovative crosslinking techniques can enhance mechanical properties and biodegradability of these hydrogels. Investigation of various crosslinking agents and methods aims to improve structural integrity and functionality, vital for application in tissue engineering and drug delivery. Rheological testing and tensile strength measurements become crucial for evaluating mechanical behaviour, while biodegradation studies ensure safe decomposition under physiological conditions.

Implementing these approaches will unlock the full capabilities of cellulose-based hydrogels enhanced with silver ions for medical use. Such strategies will aid in developing efficient and secure solutions to meet modern healthcare needs, capitalizing on the distinct qualities of these materials to better patient care results.

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APPENDIX A

Calculation of extraction yield of cellulose after alkaline treatment:

$$Extraction\ yield(\%)$$

$$= \frac{18.56}{35g} \times 100\%$$

$$= 0.5302 \times 100\%$$

$$= 53.03\%$$

Calculation of extraction yield of cellulose after bleaching treatment:

$$Extraction\ yield(\%)$$

$$= \frac{6.23}{18.56g} \times 100\%$$

$$= 0.3356 \times 100\%$$

$$= 33.57\%$$

Calculation of the swelling ratio for cellulose-glycine hydrogel:

$$S(\%) = \frac{final\ weight - initial\ weight}{intial\ weight} \times 100\%$$

$$= \frac{0.205 - 0.2}{0.2} \times 100\%$$

$$= 0.025 \times 100\%$$

$$= 2.50\%$$

Calculation of inhibition of protein denaturation:

$$\%\ inhibition\ =\ 100 \times \frac{A_0 - A_1}{A_0}$$

$$= 100 \times \frac{1.112 - 0.724}{1.112}$$

$$= 100 \times 0.349$$

$$= 34.9\%$$

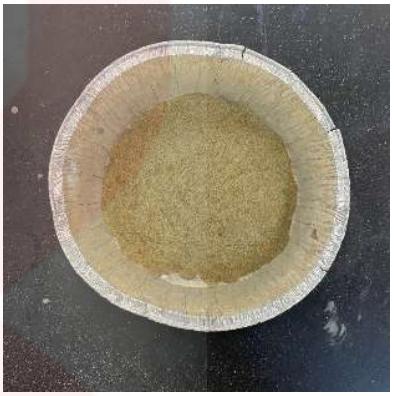
APPENDIX B

The data result for the swelling ratio to both hydrogels:

Time (Hours)	Swelling Ratio (%)	
	Cellulose-Glycine Hydrogel	Cellulose/Silver -Glycine Hydrogel
4	2.50	1.50
8	4.50	2.50
12	6.00	4.00
16	6.50	4.50
20	7.00	4.50
24	7.00	5.00
28	8.00	6.00
36	8.50	6.50
40	9.00	7.50
44	9.50	7.50
48	9.50	7.50

APPENDIX C

Napier Grass collected from Taman Pinggiran, UMK Jeli Campus.



The grass was dried at 65°C for 24 hours and ground into a fine powder.

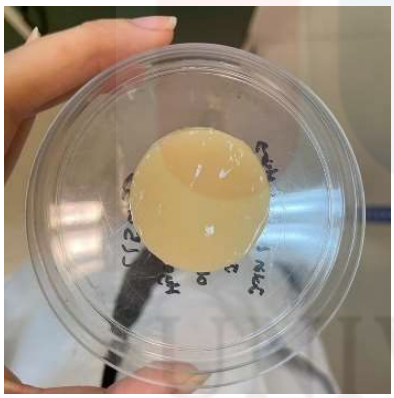
The grass powder underwent alkaline treatment with reflux for 3 hours.

Post-alkaline treatment, the sample colour turned slightly brighter yellowish.

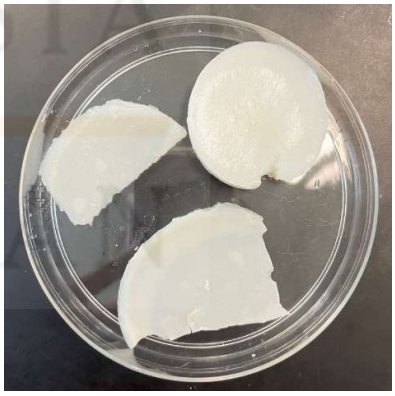
Bleaching treatment was conducted in two cycles, each with a 3-hour reflux.

After two bleaching cycles, the desired white hue of cellulose was achieved.

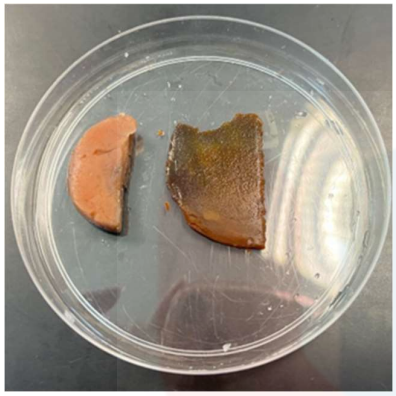
Cellulose was dissolved, and a glycine solution was added under an ice bath.



The mixture was stirred, poured into molds, and left at room temperature overnight, forming a yellowish gelation.



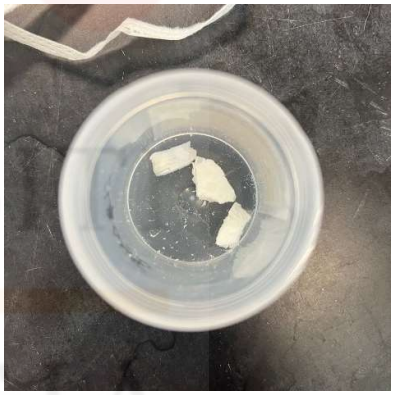
Hydrogels were neutralized with a 5% acetic acid solution until they turned white.



Prepared cellulose hydrogels were immersed in a 3% silver nitrate solution for 48 hours to form amber tone colour of cellulose/silver-glycine hydrogel.

Conducted an in vitro antibacterial test using the disk diffusion method.

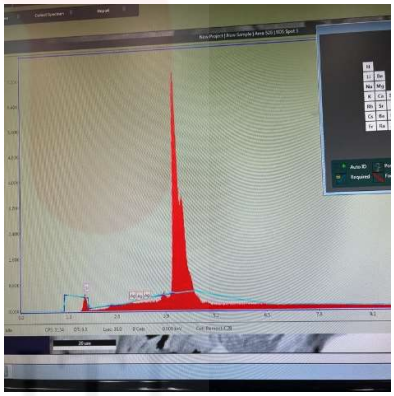
Performed an in vitro anti-inflammatory assay to evaluate the inhibition of BSA denaturation.



Measured the swelling ratio capacity in water by submerging dried hydrogel samples for 48 hours.

Analysed untreated grass, treated cellulose, and cellulose-based hydrogel using FTIR Spectroscopy.

Examined the cross-section of both hydrogels using SEM.



Performed EDX analysis to determine the elemental composition.