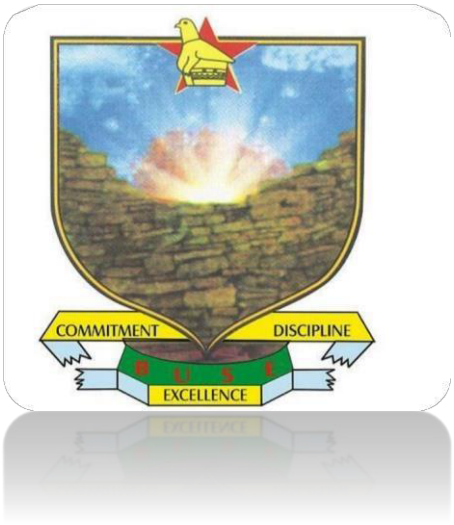
BINDURA UNIVERSITY OF SCIENCE EDUCATION

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SYNTHESIS OF A KERATIN-STARCH BIOCOMPOSITE HYDROGEL FOR SOIL HEALTH IMPROVEMENT

By

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JUNE 2026

APPROVAL FORM

The signatories named below have proved that they have taken part in the supervision, and reading of the dissertation and would like to praise the dissertation for its approval by the Bindura University of Science Education.

The research project is titled:

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DECLARATION FORM

I TINOTENDA BILLY CHITANDA do hereby declare to the Bindura University of Science

Education that the information in this research project is my unique work and all the materials and sources of data used were acknowledged as expected. This information has not been published or submitted for any educational purposes to fill any institutional requirements.

Signature of Student:  **Date: 08/06/2026**

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ABSTRACT

Excessive fertilizer use poses significant problems like increased soil salinity, biomagnification and excessive nutrient leaching. The latter has detrimental effects on water bodies, the environment and the yield of farmers. With rising concerns on sustainable agriculture peaking due to climate change which has devasted the agricultural landscapes coupled with under-utilized waste, this study used the abundant waste chicken feathers to extract keratin which was used to make a keratin-starch biocomposite hydrogel for effective nutrient management and moisture retention. The extraction process was done through alkaline hydrolysis using sodium hydroxide and several extraction parameters were optimized which showed that the process has high yields of 69 % at a high pH of 13, a concentration of 2 M, a temperature range of 80 - 95 ^{o}C and for two hours, of which exceeding the latter three results in low yields due to protein chain degradation.

The keratin and biocomposite were characterized by UV-Visible spectroscopy plus biuret test which confirmed the presence of the protein. FTIR spectroscopy showed functional groups and peaks at 1650 cm $^{-1}$(Amide I), 1540 cm^{-1} (Amide II), 1230 – 1300 cm^{-1} (Amide III) and N-H/O-H and C-H stretch vibrations, which show successful keratin extraction Analysis of the biocomposite showed that a new material had been formed through new shifts in peaks for the biocomposite and it still had active functional groups for nutrient capture. XRD spectroscopy confirmed that a hydrogel matrix had been formed since the biocomposite had a broad amorphous halo indicating flexible arrangement of polymer chains which are important for water retention and a sharp crystalline peak due to ordered crosslinked regions for mechanical strength of the biocomposite.

The synthesized biocomposite showed good nutrient retention capacities recording 78 % for Nitrate (NO_3^-) capture, 77.6 % Phosphate (PO_4^{-3}) and 92.5 % Potassium (K^+) capture, these tests were done using UV-Visible Spectroscopy and Atomic Emission Spectroscopy. It also recorded an 80 % water retention capacity. Chicken feathers proved to be a sustainable source of keratin and the biocomposite synthesized can serve as an effective moisture retaining and nutrient capture agent. This study recommends further field tests to evaluate agricultural applicability.

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LIST OF ACRONYMS

FTIR spectroscopy – Fourier Transform Infra-Red spectroscopy

UV-Visible spectroscopy- Ulta-Violet Visible spectroscopy

ppm- parts per million

XRD spectrometry- X-ray spectrometry

CHAPTER I: INTRODUCTION

1.1 Background of the study

The increasing demand for sustainable agricultural practices has led to a growing interest in the development of eco-friendly materials that can enhance soil quality and nutrient management (Khambalkar et al., 2025). Fertilizers often lead to soil degradation and fertilizer related pollution if the fertilizer leaches from the soil after application, this not only affects the environment but the economics of the farming process as most of the fertilizer is washed away and plants are left undernourished.

Nitrogen, phosphate and potassium based fertilizers leach into groundwater and increase toxicity, causing water pollution (Madjar et al., 2024). Fertilizers that leach into streams, rivers, lakes and other bodies of water disrupt aquatic ecosystems and cause algal blooms (Sagasta, 2018).

From the aforementioned research soil degradation clearly is a global challenge that threatens agricultural productivity. It can be defined as a decline in soil quality due to various reasons that include erosion, nutrient depletion and contamination it has consequences on food security and biodiversity (Food and Agriculture Organization of the United Nations, 2024). According to Smith et al., (n.d.), approximately 33% of the world's soils are highly degraded due to erosion, poor nutrient balance and poor water status.

This brings about a thought to improve soil nutrient capture and promote plant growth without harming the environment. Despite the potential benefits, current agricultural practices face challenges in nutrient retention and water capture. The recent environmental changes caused by climate change have negatively impacted the world at large (World Health Organization, 2023), Zimbabwe is currently struck with an El Niño induced drought (unicef, 2024) and effective water management for plants means prosperity or poverty for some.

With the increasing awareness of environmental issues there is a pressing need for sustainable alternatives that can enhance soil quality without adverse effects. This has sparked interest in using natural materials, particularly waste products to develop eco-

friendly products. Chicken feathers, a byproduct of the poultry industry are rich in keratin (Kamarudin et al., 2017; Sinkiewicz et al., 2017) which is a fibrous protein that has various applications in different fields. Despite the promising properties of keratin, there is limited research on the development of keratin-based hydrogels specifically designed for soil nutrient capture.

1.2 Problem statement:

There is limited understanding of the interactions between keratin-based hydrogels and soil nutrients, this gap inhibits the potential use of keratin hydrogels as effective sustainable solutions for effective soil nutrient management to improve soil health and limit fertilizer related pollution.

1.3 Research aim and objectives

Aim: This research aims to come up with an eco-friendly natural based nutrient capture hydrogel system from waste chicken feathers and starch to improve soil health through nutrient capture and water retention.

The research objectives are:

1. To optimize the extraction of keratin from waste chicken feathers.
2. To prepare a keratin-starch based biocomposite hydrogel for use as a soil nutrient capture system.
3. To investigate the effects of additives such as starch and boric acid in hydrogel formation.
4. To investigate the water retention capacity of the prepared hydrogel.
5. To investigate the nutrient capture capabilities of the hydrogel.

1.4 Research questions

1. What extraction conditions are optimum for keratin extraction through alkaline treatment for this study?
2. Can boric acid be used as a crosslinking agent for starch and keratin to form a hydrogel?
3. How do keratin-based hydrogels interact with various soil nutrients?
4. Which one, between the keratin-starch bio composite and plain soil has the best water retention and nutrient capture capacity?
5. What concentration of Keratin is optimum for effective nutrient capture?

Hypotheses

Keratin-starch bio-composite based hydrogels will significantly enhance nutrient and water retention in soils, having a positive impact on soil health, plant growth and the environment.

1.5 Significance of the study

This research aims to contribute to the knowledge regarding the use of keratin-based hydrogels in nutrient capture technology; by utilizing agricultural waste this project not only contributes to waste management but also promotes the development of biodegradable materials that enhance soil health. The findings could serve as a foundation for future innovations in sustainable agriculture benefiting farmers and the environment.

1.6 Delimitations and limitations

1.6.1 Delimitations

This study investigated keratin derived from chicken feathers as a resource for developing hydrogels, while keratin can be obtained from several sources, the decision to concentrate on chicken feathers is based on their abundance of keratin as agricultural waste. This focus allowed for a more centralized analysis of the specific properties and applications of chicken feather keratin.

The primary aim of this research was to develop and optimize keratin-based hydrogels for soil nutrient capture. The study did not explore other potential applications of keratin, such as in biomedical fields or materials science, thereby narrowed down the research scope to agricultural applications.

The research was conducted in a specific geographical area which influenced soil types, climate conditions and agricultural practices. The findings were not generalized to other regions with different environmental conditions or agricultural systems limiting the applicability of results to the studied area.

The study also focused on specific essential nutrients such as nitrates, phosphates and potassium (metal-based nutrients) commonly found in fertilizers. The research was conducted under controlled experimental conditions to isolate the effects of keratin-starch based hydrogel nutrient retention.

1.6.2 Limitations

The quality and composition of keratin extracted from chicken feathers can vary based on several factors including the age and health of the birds, processing methods, and environmental conditions. This variability may influence the performance of the keratin-based hydrogels resultantly affecting reproducibility and consistency in results.

External environmental conditions such as temperature, humidity and soil moisture levels can significantly impact the behavior of hydrogels and nutrients in the soil. These factors may introduce variability in field trial outcomes.

The interactions between keratin-based hydrogels and soil microorganisms can be complex and are influenced by variables including soil type and existing micro ecosystems. These interactions may affect nutrient availability and release.

The research focused on short to medium term impacts of keratin-based hydrogels on soil health. Long-term effects including the degradation of hydrogels in the soil and their influence on soil ecosystems over time were not captured within the study duration.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction and background of the study

Effective nutrient management is important for restoring soil health and ensuring sustainable agricultural practices. Traditional farming often relies heavily on synthetic fertilizers to boost crop yields; however, excessive use of these fertilizers can lead to nutrient runoff which contaminates water bodies and disrupts aquatic and land systems (Madjar et al., 2024). Khambalkar et al., (2025) points the necessity for alternative nutrient management techniques that minimize environmental harm while promoting safe agricultural practices.

The over application of nitrogenous and phosphate based fertilizers leads to nutrient runoff which can result in eutrophication a process that causes excessive growth of algae in water bodies(Akinnawo, 2023). Eutrophication depletes oxygen levels in the water, creating hypoxic conditions that harm aquatic life, leading to dead zones where most marine organisms cannot survive (Akinnawo, 2023).

The problems are not limited to water pollution only. Singh (2025) mentions quite a few problems that show that air and land pollution can also occur due to continuous application as nutrients are poorly managed. The fertilizers can cause “soil salinity, heavy metal accumulation, eutrophication, accumulation of nitrate, greenhouse effect and biomagnification” when excessively applied (Y. V. Singh, 2025).

2.1 Keratin as a resource

The poultry industry generates large amounts of waste primarily in the form of chicken feathers which are often discarded or incinerated; this waste not only poses environmental challenges but also presents an opportunity to develop value-added products. Research from Saha et al., (2019a) reveals that feathers make 10% of a chicken’s weight and are composed of 91% keratin, with the remaining being lipids and trace minerals.

In a comparative view, this study preferred chicken feathers over hair, wool or horns (Shah et al., 2019) as a source of keratin because they provide a sustainable source that utilizes waste to create value added biomaterials unlike hair and wool which may pose ethical issues. This makes them a perfect resource and raw material.

2.2 Structure of keratin

Keratin is a quaternary fibrous protein found in feathers, hair and hooves(Saha et al., 2019a). It is made up of multiple peptide residues forming chains consisting of intermolecular and intramolecular forces within the chain or amongst neighboring chains. The most significant intermolecular force in this study is the disulfide bond (-S-S-) between cysteine residues (Chilakamarry et al., 2021).

The intermolecular and intramolecular bonds and interactions between and within the keratin chains increases keratin's mechanical properties, insolubility in water and chemical resistance making keratin a highly durable biopolymer (Wang et al., 2016). This ultimately affects its extraction process and makes the conditions aggressive.

Keratin is an active biopolymer with diverse functional groups because of its diverse amino acid profile (Wang et al., 2016) and (Shah et al., 2019). The biomaterials made from it are usually biocompatible, biodegradable and these have active functional groups such as amino groups ($-NH_2$), carboxyl (-COOH) and hydroxyl (-OH) groups along the protein chains (Saha et al., 2019a).

These active functional groups are the areas of interest for this study as they enable interactions with other compounds and polymers through hydrogen bonding and electrostatic forces. Using this knowledge nutrient capture technologies, biocomposite synthesis and hydrogels can be made.

2.3 Extraction of keratin

Keratin extraction can be done through disruption of the disulfide bridges within the keratin structure and disruption of the intramolecular interactions. Several methods can be employed these were mentioned by Saha et al., (2019b). Chemical extraction methods are the most common methods used due to their high efficiency, simplicity and high yield. The most common is alkaline extraction method by using sodium hydroxide or potassium

hydroxide to cleave disulfide bonds and peptide linkages leading to the solubilization of keratin (Tursunova & Maksumova, 2025).

This extraction method is however subjective to many parameters such as concentration, time and temperature, these factors influence the yield and quality of keratin obtained. Another method that is employed is acid extraction method though it is less commonly used (Silva et al., 2024a). However, these methods have low yields and degrade the protein which ultimately limits its use.

Extraction by reduction has also been used, were the disulfide bonds between cysteine residues are reduced to thiol groups (Maurya & Singh, 2023a). This method is very effective as it targets the disulfide bond, this ultimately reduces polymer chain degradation by preserving the keratin's peptide back-bone. The disruption of the disulfide bridges causes the keratin to dissolve in solution.

Though these methods are effective they pose several concerns for example, reducing agents like β-mercatoethanol, thioglycolic acid, dithiothreitol and sodium sulfide (Maurya & Singh, 2023a) are toxic and expensive for large scale production. They are also environmentally hazardous and require extensive purification of the product after extraction.

Other methods utilize biological extraction methods, by utilizing organisms that can degrade keratin (keratinolytic microorganisms) (Mpaka et al., 2025) or organisms that can excrete enzymes such as keratinase. Enzymatic methods are considered environmentally friendly and energy efficient as they operate at mild conditions like temperature and neutral pH. These methods are usually time consuming and parameter sensitive especially for the microorganisms and enzymes (Sarma, 2022).

From the knowledge above, alkaline treatment was deemed cost effective and efficient for this study. It offers a balance between efficiency, cost-effectiveness and scalability while producing keratin with functional groups capable of interacting with starch. This method of extraction also offers the ability to control extraction parameters such as temperature, time and optimization of keratin yield and properties.

2.4 Development of Keratin-based hydrogels

Hydrogels are viscoelastic materials with three-dimensional networks of hydrophilic polymers that have the capacity to absorb and retain water (Ali et al., 2024). Hydrogels can be classified as synthetic, natural or hybrid depending on their source (Khansari et al., 2017). Natural hydrogels are obtained from natural sources such as biopolymers like chitin, cellulose and keratin while synthetic hydrogels are made from polymers such as polyethylene or polyacrylamide.

The development of hydrogels has emerged as a promising area of research in the context of sustainable agriculture (Khansari et al., 2017). They can play a crucial role in enhancing soil moisture retention, especially in arid and semi-arid regions. When placed in water the hydrophilic functional groups attract water molecules through hydrogen bonding (Patel et al., 2024) this causes the gel to swell till elastic pressure equals osmotic pressure.

Keratin-based hydrogels combine the advantageous chemical properties of keratin with the moisture-retaining capabilities of hydrogels, offering a sustainable solution for soil nutrient capture. Previous studies have explored various methods for hydrogel fabrication, including physical and chemical cross-linking techniques to optimize their properties for agricultural applications (Gong et al., 2025). Priya et al., (2024) presence several hydrogel fabrication methods from chemical to physical crosslinking methods.

Chemical crosslinking methods can be defined as the joining or bridging of polymer chains using small molecules with bi-functional or multi-functional groups to form three-dimensional networks of polymers (Zafar et al., 2022). Physical crosslinking can be defined as the formation of three-dimensional networks of polymers via non-covalent reversible interactions such as electrostatic interactions (Siqueira et al., 2023),by utilizing this knowledge of interactions, biocomposite or hybrid hydrogels can also be synthesized by crosslinking different polymers.

The ability of these hydrogels to retain water can significantly reduce the need for frequent irrigation and fertilization, providing economic benefits to farmers while minimizing environmental impacts. The intermolecular and intramolecular forces present in keratin as well as the functional groups were pivotal in this study as they acted as binding sites to nutrient cations and anions.

2.5 Characterization of the products

Characterization is an essential step in research as it provides assurance that the product of interest was made. It is a crucial step in evaluating the physiochemical properties, structural integrity and functional attributes of keratin and keratin-based products (Sharma et al., 2017). Characterization provides evidence on successful extraction and suitability of the produced material for its intended purpose.

2.5.1 Spectroscopic characterization

UV- Visible spectroscopy is a widely used technique for analysis and characterization, Keratin can be analyzed and quantified via this spectroscopic method because of the presence of peptide bonds as well as aromatic amino acids such as tyrosine and tryptophan (Sharma et al., 2017). The absorption bands are typically observed between 190 nm-230 nm, which correspond to the peptide backbone and an additional peak near 280 nm which identifies aromatic amino acids (Tursunova & Maksumova, 2025).

UV-Visible spectroscopy can also be used for quantitative analysis of compounds. Nitrates can be analyzed using this spectroscopic technique even though there is no physical change in color, they absorb around 200 nm-230 nm with 220 nm being their maximum absorbance (Huang et al., 2024). However, dissolved organic matter also has effective absorption in this region and it acts as an interference in the precise analysis of nitrates hence a correction factor is used on acquired results, (Huang et al., 2024)

$$A_{corrected} = A_{220} - (2 \times A_{275}) \quad (2.1)$$

Where A is absorbance at the specified wavelength.

The last analysis to be done using this technique is the analysis of phosphate using the Mehlich (III) method. This is a colorimetric method that focuses on the color producing phosphomolybdate complex which is reduced in the presence of ascorbic acid to form a blue colored complex known as molybdenum blue (Rambwawasvika, 2022). This complex has a high absorption at 885 nm but absorbs in the region 880 nm-890 nm.

The last analysis method is the analysis of potassium capture using Atomic Emission Spectroscopy, which is an analytical technique that determines the composition or rather in this study the quantity of a sample by measuring the intensity and wavelength of light emitted by excited atoms (Moreno-Rojas et al., 2016). In this method samples are vaporized

in high temperatures like plasma or flames causing electrons to move to higher energy levels, upon returning to lower energy levels they emit light at characteristic wavelengths. The emission intensity is used as it is directly proportional to concentration.

2.5.2 FTIR Analysis and XRD characterization

Fourier Transform Infrared (FTIR) spectroscopy is one of the most used characterization methods especially for organic compounds. It provides information on structure, bonding and functional groups of the compound of interest (Tursunova & Maksumova, 2025). It works by identifying compounds through measuring how they absorb specific infrared wavelengths which correspond to their unique rotational and vibrational energies (Pasieczna-Patkowska et al., 2025).

Keratin being a protein is expected to have a protein backbone which is a chain of peptide bonds. The amide (I) band (1600-1700 cm^{-1}) which is mainly due to C=O stretching vibrations is characteristic of the secondary structure of proteins (Wang et al., 2016;Tursunova & Maksumova, 2025). An amide (II) band is also expected in the region (1500-1600 cm^{-1}) this is the (-N-H) and (-C-N) stretching vibrations while the amide (III) (1200-1400 cm^{-1}) correspond to (C-N) and (N-H) deformation (Wang et al., 2016; Tursunova & Maksumova, 2025). The presence of the aforementioned peaks or bands confirm the existence of proteinaceous material.

X-ray diffraction is a non-destructive technique used to determine the crystalline and amorphous nature of polymeric materials (Speakman & Ph, 2022). It operates on Bragg's Law, where X-rays diffract off ordered atomic planes to produce characteristic peaks. Keratin and starch are semi-crystalline biopolymers, starch exhibits B-type crystallinity with peaks at $2\theta=17^0$ (Barroso et al., 2019) while keratin shows β-sheet structures with a peak around $2\theta=18^0$ (A. Singh, 2023). In crosslinked keratin keratin-starch hydrogels, XRD is used to assess the effect of crosslinkers like boric acid on polymer chain ordering.

Successful crosslinking often results in diffraction peak that are broad amorphous halo, while there are sharp diffraction peaks that can be observed due to crosslinking material creating ordered structures in the hydrogel mixture (Oluba et al., 2021). In this study, this

is expected since the chains are to be linked via borate-ester bonds and the presence of an amorphous halo will confirm the bulk hydrogel matrix which is useful in water retention.

XRD characterization provides structural evidence of crosslink formation and helps confirm that the hydrogel has the desired balance of ordered regions for mechanical strength and amorphous regions for flexibility and swelling.

2.6 Summary

This chapter focused on analyzing existing data on keratin extraction, hydrogel synthesis, qualitative and quantitative analysis of the products produced. Chicken feathers were chosen as a sustainable and abundant source for keratin compared to other sources, offering a readily available raw material that is environmentally friendly. Besides raw material analysis, the research revealed crucial information on the structure and chemistry of keratin as a biopolymer, this information is crucial as the project depends on it.

Lastly analytical techniques were also examined; these are essential from the first to the last steps of this research. The methods examined relate to this study but can also be applied outside this research. This chapter also focused on characterization research to validate the products obtained from the research. With all the information obtained, this project was completed successfully.

CHAPTER 3: MATERIALS AND METHOD

3.1.1Reagents

Ethanol (Industrial grade- supplied by Astra chemical), Sodium Hydroxide (Analytical Reagent grade- supplied by Glassworld), Hydrochloric acid (Analytical grade- supplied by UniVar Merck), Copper sulphate (Analytical Reagent grade- supplied by Merk), boric acid (Analytical Reagent grade- supplied by Merk), Starch (Food grade), Glycerol (Industrial grade supplied by Astra chemical). Ammonium Molybdate (Analytical Reagent grade- supplied by Merk), Potassium Antimony Tartrate (Analytical Reagent grade- supplied by Merk), Sulphuric acid (Analytical grade- supplied by UniVar Merck), Ascorbic Acid (Analytical Reagent grade- supplied by Glassworld), Potassium nitrate (Analytical grade- supplied by UniVar Merck). Potassium Phosphate monobasic (Analytical grade- supplied by UniVar Merck).

3.1.2 Apparatus

(500 ml, 250 ml, 100 ml) beakers, test tubes, (500 ml, 1L, 100 ml, 50 ml) volumetric flasks, hotplate, watch glass, stirring rod, weighing boats, fume hood, filter papers, funnels, vacuum filter, buchner funnel, dropper or syringe, oven, motor and pestle, spatula, cuvettes, latex gloves, 2 mm sieve.

3.1.3 Analytical instruments

UV-Vis spectrometer model number BEPYD230417001 manufactured by Bioevopeak, Atomic Emission Spectrometer model number MY22379002 manufactured by Chemetrix (Agilent Technologies), PerkinElmer benchtop research FTIR model number IRTracer-100 60,000:1 S/N ratio PerkinElmer, X-ray Diffraction Spectrometer Brucker D2 phaser 2nd generation model number Brucker D2 AXS./USA.

3.2 Sample collection and pretreatment

Chicken feathers were obtained from a local poultry farmer. The feathers were washed and soaked in warm deionized soapy water for 1 hour in a beaker to remove dirt and other impurities. They were also soaked in 70% ethanol solution over night to remove excess fat and alcohol soluble materials.

The soaked feathers were washed with deionized water to remove excess ethanol and sundried. The dried feathers were finally ground to reduce particle size.

3.3 Extraction process optimization

A stock solution of 4 M sodium hydroxide was prepared by dissolving 160 g of sodium hydroxide pellets in 1 L deionized water and dilutions of 0.5 M, 1 M, 1.5 M, 2 M, 3 M and 4 M sodium hydroxide solutions were prepared for the extraction process. The extraction was done in highly alkaline conditions from pH 10-13 according to Al-Shamaa, (2020), as this ensures the highest yield is obtained.

Four groups of 1 g of chicken feathers were accurately weighed on balance and put into 500 ml beakers. The aforementioned solutions of NaOH were then added to beakers containing the feathers, the NaOH solution was added to volumes that ensured all the feathers were fully submerged in solution. The samples were heated for 2 hours at 80^0C to determine the effect of concentration on the extraction process.

After the optimum concentration for the extraction was determined, the concentration was maintained at 2 M and the temperature varied from 20, 40, 80, 100 and 140 degrees Celsius for another batch of 1g of feathers to determine the effect of temperature on the extraction process. The samples were heated on hotplates for 2 hours with caution and careful adjusting of temperature and the thermometer readings were frequently and constantly checked during the whole procedure.

The concentration and temperature were kept constant and the time varied at 3, 4, 5, 6 and 7 hours. The effect of time on the extraction process was noted and recorded. The last parameter to be investigated was pH, the optimum extraction pH was determined by

keeping the concentration, temperature and extraction time constant while the samples were subjected to pH of 2, 5, 7, 10 and 13.

The results obtained from the above data revealed the optimum conditions for the extraction process and hence were used for this study.

3.4 Keratin extraction

The same feather sample preparation method mentioned above was used for the optimized keratin extraction, 1g of cleaned feathers were weighed on a balance and totally submerged in 2 M NaOH solution. The 250 ml beaker was then placed on a hotplate and the temperature of the mixture was monitored and maintained at 80^0C.

The dissolution step was carried out in a fume hood and the mixture was constantly stirred to ensure uniform dissolution and that all the contents in the beaker were fully submerged in solution. After 2 hours of heating at constant temperature, the feathers completely dissolved and solid residues remained. The solution was allowed to cool, and a yellow to brown liquid was obtained. The liquid was filtered through a vacuum filter as gravity filtration is slower with finer filter papers.

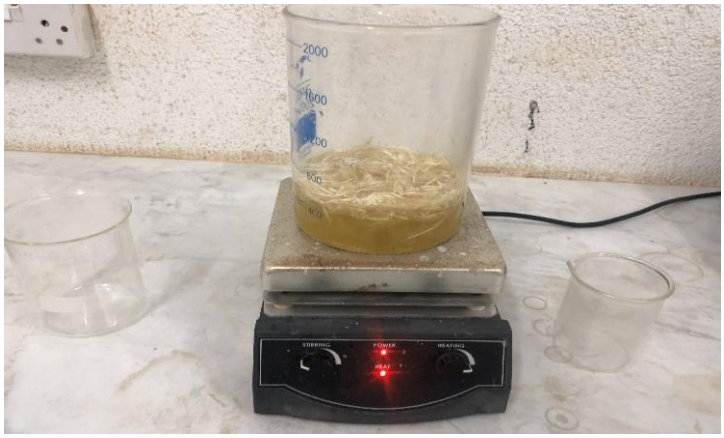


Figure: 3.1 Chicken feathers submerged in sodium hydroxide



Figure: 3.2 Dissolved feathers after heating in 2M sodium hydroxide solution.

3.3.1 Biuret test

2 mls of this obtained liquid was placed in a clean test tube and 2 mls of the prepared copper (II) sulphate solution was added (Koleva & Zheleva, 2022). The mixture was gently shaken to ensure thorough mixing and allowed to stand at room temperature for 3 minutes, the color change was observed and recorded. A water blank was also made as a control for the procedure.

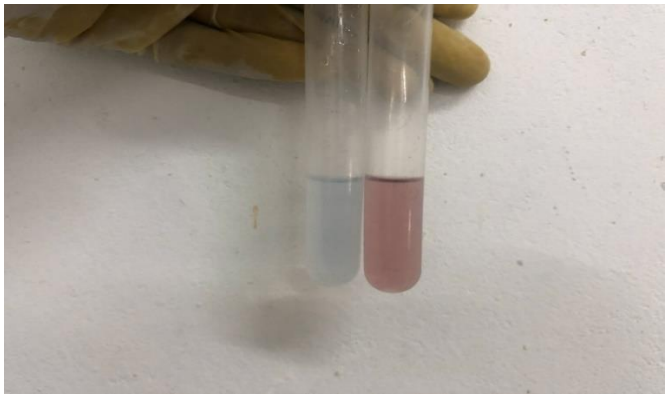


Figure: 3.3 Biuret test of the obtained solution.

3.3.2 Precipitation of the obtained keratin

Concentrated hydrochloric acid was used to precipitate the protein out of solution (Al-Shamaa, 2020). The pH was kept around 2 and 3 and the precipitate was allowed to agglomerate and settle at the bottom of the beaker. This procedure was done in a fume hood as the solution produced fumes.

The supernatant liquid was decanted and the precipitate and excess solution were filtered. The precipitate was washed with deionized water to neutralize the pH, it was then finally dried in an oven at 80 degrees, allowed to cool and ground into a powder which was stored for hydrogel synthesis. Yield analysis was done by using gravimetric methods, this involved weighing the amount of keratin powder obtained compared to the number of feathers used.



Figure: 3.4 Keratin powder

3.4 Hydrogel formation and process optimization

The extracted keratin was used in the synthesis of a starch-keratin boric acid and glycerol cross linked hydrogel by the following method. To determine the amount of starch, and boric acid that would produce a stable hydrogel multiple tests were done to investigate the effects of each reagent on the hydrogel.

3.4.1 Effect of the concentration of starch

0.5 g, 1g, 2 g, 3 g and 4 g of starch were accurately weighed and added to 20 ml of deionized water, the mixtures were stirred to mix the contents fully and heated on a hotplate until the starch dissolved and gave a slightly colorless viscous solution from a white suspension.

A 20% (w/v) solution of boric acid was prepared by dissolving boric acid in pre heated water as it does not dissolve readily in cold water, 10 mls of this solution was added to the

individual starch containing samples and heated for a few seconds to allow for thorough mixing.

10 g of extracted keratin powder was accurately weighed and dissolved in 20 ml of 2M sodium hydroxide in a 50 ml volumetric flask and it was filled to the mark with deionized water. 10 ml of this 20% solution was added to the starch-boric acid mixture and everything was reheated again for 2 minutes and then poured into a petri dish and allowed to cool. The five samples were allowed to sun dry and their drying patterns were observed.

3.4.2 The effect of boric acid on the hydrogel

2 g of starch were weighed for each of the samples and deionized water was added to all four of the samples. A stock solution of 20% (w/v) boric acid was diluted to give 5%, 10%, 15% and 20% solutions. The starch and boric acid solutions were mixed respectively and heated, the gels were allowed to cool and dry.

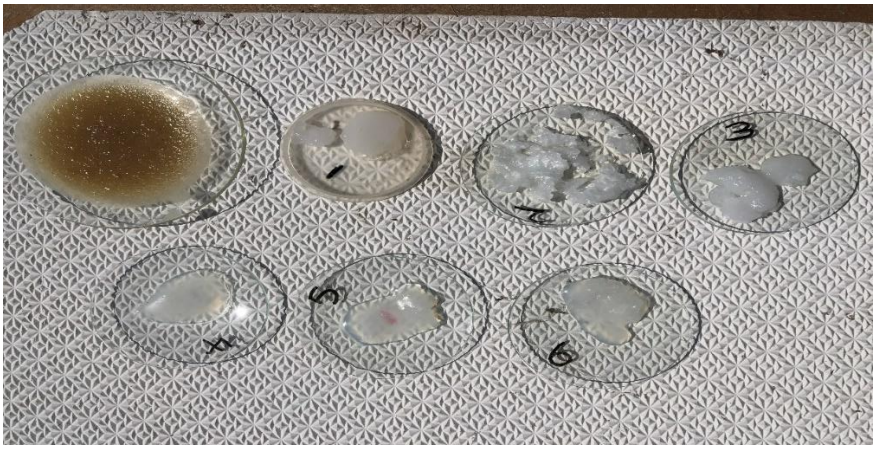


Figure: 3.5 Hydrogel samples sun-drying.

3.5 Sample preparation for characterization

15 g of optimized hydrogel sample was accurately weighed and dried at 110 ^{0}C, the dried hydrogel was crushed and powdered using a motor and pestle to reduce particle size and to increase homogeneity for analysis.

3.5.1 X-ray diffractometry analysis

The ground sample was back loaded into a rectangular sample holder to reduce orientation of irregular particles; excess material was removed with a glass slide to produce a flat and smooth surface. The loaded sample holder was then mounted and

locked in place for analysis. XRD patterns were collected using a diffractometer with Cu kα radiation $\lambda = 1.54184$, 2θ range = 0^0 to 90^0 and a step size = 0.01 for 8602 steps.

3.5.2 FTIR spectroscopy analysis

A small amount of the ground keratin and composite sample was placed directly on the diamond ATR crystal at different analysis times; uniform contact was ensured by applying constant pressure using the anvil for both samples. Background spectrum of a clean ATR crystal was collected before each sample.

The data was collected over a transmittance mode over the range 4000-400 cm^{-1}, a resolution of 4 cm^{-1}, 32 scans per spectrum were used to improve signal to noise ratio and a background scan was done before to each sample to subtract atmospheric CO_2 and H_2O vapor.

3.6 Fertilizer capture tests

The ideal hydrogel preparation based on this study was used to prepare the hydrogel for the nutrient capture and water retention tests. Soil samples were collected on NutriMaster Private Limited premises and they were washed with ultra-purified (deionized) water to remove excess organic matter and to leach out excess nutrients.

2 g of starch were accurately weighed and mixed with 20 ml of deionized water and heated on a hot plate, 5 ml of glycerol were also added and the lastly 20% (w/v) boric acid. These were the base preparations before the addition of keratin.

The soil sample was air dried and sieved through a 2 mm sieve to remove stones and other large particles and debris. A sample of NutriMaster's Cereal blend fertilizer was used for this study; this fertilizer has an NPK ratio of 6:23:28.

3.6.1 Hydrogel optimization for nutrient capture

In this section, the optimum percentage of keratin in the hydrogel bio-composite for maximum performance was determined through multiple batch tests for nitrates, phosphates and potassium. This was done to investigate the effect of the concentration of keratin on the nutrient and water retention capacity of the hydrogel.

3.6.2 Test for Nitrate retention

This analysis was performed on a UV-Vis spectrometer since nitrates can be analyzed directly at 200 nm with a correction for organic matter interference at 275 nm. A stock solution was prepared by dissolving 1.63 g of KNO_3 in 1 L deionized water to make a 1000 ppm solution of nitrate ions.

This stock solution was used to prepare working nitrate standards, from 5, 10, 20, 40 and 60 ppms by serial dilution of the stock solution using deionized water. These standards were diluted with 30 mls deionized water to account for the dilution during the leaching process and then they were finally used to construct a calibration curve for the first run at 220 nm. A 50-ppm nitrate equivalent solution was made by dissolving 0.188 g of 6:23:28 Cereal blend fertilizer in 1 L volumetric flask and then filtering to remove undissolved material.

Using the hydrogel synthesis method mentioned above, the concentration of keratin was varied from 15, 10, 5, 2.5 and 1 percent, these hydrogel samples were dried and then crushed and stored as powder for later analysis. The 15% keratin hydrogel was prepared by adding 15% keratin solution to a mixture of starch, boric acid and glycerol and then heating for 3 minutes and cooling. The resulting crust was then dried and crushed to give a powder.

To perform the nitrate capture test, 2 g of the sieved soil sample was mixed with 1 g of 15, 10, 5, 2.5 and 1 percent hydrogel powder to make five different samples. 10 ml of the 50-ppm nitrate equivalent fertilizer stock solution was then added to the five samples and the mixture was allowed to fully absorb the solution overnight. For test control purposes all the nutrient capture tests had a starch blank, soil blank and a keratin blank to also investigate the individual behavior of the standalone materials.

The samples were then leached by adding 30 ml of deionized water and subjecting them to gravity filtration. The filtrate was collected for analysis on the UV-Vis spectrometer, the cuvettes to be used were thoroughly cleaned with deionized water. The samples were first read at 220 nm to read the nitrate value, but organic matter also absorbs at this wavelength as described by Singh et al, 2019. To correct this the samples were also read at 275 nm, at this wavelength the nitrates do not absorb anything and only organic matter is read and then

a correction factor was used to correct the concentration at 220 nm to nitrate concentration only.

3.6.3 Retention test for phosphates

For this procedure the Mehlich (III) method was used without the phosphate extraction process since this study focused on nutrient retention and not liberation. The color development agent was made by dissolving 17.4 g of ammonium molybdate in 200 mls of water. 0.392 g of potassium antimony tartrate was accurately weighed and dissolved in 150 ml of water and 200 ml concentrated sulphuric acid was added to 500 ml deionized water in a 2L volumetric flask and cooled. Once cooled, the molybdate and tartrate solutions were added and toped to the mark with deionized water.

The final part for the color development agent is the murphy-riley reagent which was made by dissolving 1.056 g of ascorbic acid in 200 ml of the solution which was prepared above. This procedure was done on a UV-Vis spectrometer. The calibration standards were prepared as follows. 1000 ppm of phosphate-equivalent was prepared by dissolving 4.394 g of oven dried potassium phosphate monobasic in 1L of deionized water.

The calibration standards were prepared by serial dilution of the stock standard solution to make 35 ppm, 25 ppm, 20 ppm, 15 ppm, 10 ppm, 5 ppm and a water blank. The standards were used to make a calibration curve on the UV-Vis spectrometer.

Five batches of 2 g of the sieved and air-dried soil sample were weighed on a balance and 1 g of 15, 10, 5, 2.5 and 1 percent keratin biocomposite hydrogel powder were added into centrifugal bottles containing the soil samples. A 30-ppm phosphate-equivalent solution of the fertilizer was prepared by dissolving 0.3 g of the fertilizer and filling up to the mark in a 1L volumetric flask

20 ml of the 30-ppm phosphate-equivalent solution was added to the five centrifugal tubes containing the soil and biocomposite mixture. The mixtures were allowed to fully absorb the solution overnight. 20 ml of deionized water were then added to moist mixtures and then filtered through pre-rinsed Whatman 42 filter papers and allow to gravity filter.

1ml of each of the sample filtrates were pipetted into clean test tubes and the same was done for all the QCs and standards prepared, 9.5 ml of deionized water were added to each

test tube and 2 ml of fresh Murphy-Riley reagent was also added into each standard and a QC starting with the lowest concentration. The samples were capped, inverted to mix, and allow to react for at least 30 minutes. This procedure also included a starch blank, soil blank and keratin blank.

The cuvettes were cleaned with deionized water and the UV-Vis spectrophotometer was set at 885 nm; the standards were run first to obtain a calibration curve and then the samples followed. The samples and the standards showed a blue color that increased with concentration.

3.6.4 Retention test for potassium

For this test, atomic emission spectroscopy was used. Potassium calibration standards were prepared from a 1000 ppm potassium primary standard; standards ran from 10, 20, 40, 80 and 100 ppm with 60 ppm used as the QC. These were prepared via serial dilution of the 1000 ppm primary standard.

A s30-ppm potassium-equivalent solution of the fertilizer was prepared by dissolving 0.13 g of fertilizer in 500 ml of deionized water and then transferring to a 1 L volumetric flask and filling to the mark with distilled water. 2 g sieved soil samples were accurately weighed in batches for the five different concentrations of the hydrogel biocomposite and mixed respectively just like the other tests mentioned before. The samples were allowed to fully absorb the fertilizer solution overnight and were latter rinsed and allowed to leach and gravity filter.

The filtered samples were transferred into test tubes in a sample rake for analysis. The AES spectrometer was purged and calibrated using the above-mentioned standards and the samples were run accordingly.

3.7 Moisture retention test

The last parameter to be assessed was the water retention ability of the biocomposite with the best set of results. In this investigation plain soil without the hydrogel, a sample of the soil with the hydrogel and the hydrogel were used.

Two sets of 2 g of soil were accurately weighed in glass petri dishes. 1 g of the keratin biocomposite was weighed and added to one of the soil samples and it was gently mixed

and homogenized. 2 g of keratin biocomposite hydrogel powder were also weighed in a glass petri dish, the masses of the three samples were then weighed before adding 10 mls of deionized water and then finally weighed after adding. The samples were allowed to fully absorb the water before being allowed to sun dry and the mass of the samples was checked after every one hour.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents and discusses the results obtained from the extraction of keratin from chicken feathers and the formulation of a starch-keratin biocomposite hydrogel. The performance of the hydrogel in water retention and nutrient capture was evaluated and compared with relevant literature.

4.2 Keratin extraction process optimization

4.2.1 Effect of sodium concentration

This study utilized the extraction method from Al-Shamaa, (2020) with modifications. Sodium hydroxide was utilized to extract keratin from chicken feathers. For this study different concentrations of sodium hydroxide solution were prepared to test the effect of concentration on the extraction process (Table 4.1).

Table 4.1: Effect of different concentrations of sodium hydroxide on the yield of keratin.

Concentration (M)	**Initial mass (g)**	**Final mass (g)**	**Percentage yield (%)**
0.5	1	0.299 ±0.04	29.9 ±4
1	1	0.441 ±0.025	44.1 ±2.5
1.5	1	0.554 ±0.029	55.4 ±2.9
2	1	0.696 ±0.02	69.6 ±2
3	1	0.324 ±0.031	32.4 ±3.1
4	1	0.132 ±0.027	13.2 ±2.7

From Table 4.1 above, it can be seen that with the increase in concentration of sodium hydroxide the yield of keratin increases but there is a point that is reached where the concentration of the yield starts to fall. According to Saha et al., (2019); Chilakamarry et al., (2021) keratin is a biopolymer that is made up of chains of amino acids linked together by disulfide bonds (S-S), hydrogen bonds and hydrophobic interactions. Sodium hydroxide breaks disulfide bonds, hydrolyses peptide bonds and solubilizes keratin into peptides and amino acids (Tursunova & Maksumova, 2025).

At high concentrations (3 M and 4 M) the keratin chains are degraded extensively and hence a lower yield is obtained. Keratin extraction efficiency increased with sodium hydroxide molarity, however, concentrations above 2 M resulted in extensive keratin degradation, yielding low molecular weight peptide rather than intact keratin. For this study, 1.5-2 M sodium hydroxide provided an optimal balance between extraction yield and keratin chain preservation

Physical observations were also seen from the different reaction mediums of the samples; the highly concentrated solutions did not have any undissolved feathers by the end of the set time unlike the less concentrated solutions. There was also a significant difference in the color of the samples after the dissolution process, the highly concentrated solutions had a darker color.

A UV-Vis analysis at 280 nm was also performed to see variability in terms of protein concentration, according to Dawid, (2025) proteins can be analyzed at this wavelength to give a quantitative measure.

Table 4.2 Quantitative analysis data showing trends in keratin extracts at different sodium hydroxide concentration.

Sodium hydroxide concentration	Absorbance of extracted Keratin
0.5	0.0265
0.75	0.0113
1	0.296
1.5	0.4244
2	0.4675

Table 4.2 above shows the increasing absorbances of different keratin extracts from 0.5, 0.75, 1, 1.5 and 2 M Sodium hydroxide solutions from another run. This clearly shows that yield increases with increasing concentration. The biuret test also verified this data as the color intensified with increasing concentration.

4.2.2 Effect of temperature

Temperature was also optimized using this varying method but this time keeping concentration constant, a trend was also obtained. The temperature was varied from 20, 40, 80, 100 and 140 ^{o}C using 2 M sodium hydroxide solution for 2 hours. The yield increased gradually as the extraction temperature increased from 20 ^{o}C to 80 ^{o}C. The lowest yield was obtained at 20 ^{o}C while the maximum was recorded at 80^{o}C, However, further increase in temperature beyond 80 ^{o}C resulted in a decline in keratin yield. These results suggest that keratin extraction is favored at elevated temperatures up to an optimum point, beyond which degradation effects dominate.

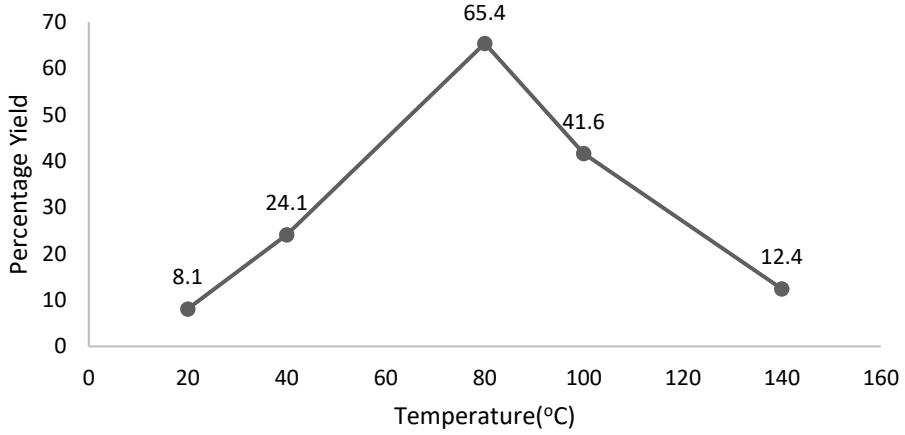


Figure: 4.1 Trend in the effect of temperature on the extraction process.

Temperature plays a critical role in alkaline extraction of keratin due to its influence on the disruption of intermolecular interactions within the feather structure. Chicken feathers are composed mainly of keratin stabilized by strong disulfide bonds, hydrogen bonding and

hydrophobic interactions which make the protein highly resistant to dissolution (Saha et al., 2019a).

As presented on figure 4.1 above, at low temperatures there was low yield because there was insufficient thermal energy to effectively disrupt the bonds and facilitate sodium hydroxide penetration into the feather matrix, as a result partial dissolution occurred as illustrated by Nwanak, (2024). With enough heating, efficient bond cleavage of the disulfide bridges occurs and the keratin is unfolded which causes it to solubilize.

At higher temperatures, a reduction in keratin yield is attributed to excessive hydrolysis of the peptide bonds under strongly alkaline conditions. This ultimately causes the protein to breakdown into amino acids which do not recover to form the peptide chain.

4.2.3 Effect of time

This parameter gave almost similar results to the ones mentioned above but have a different interpretation. The biopolymer degraded after heating for too long in 2 M sodium hydroxide solution. The time variation was as follows 3, 4, 5, 6, and 7 hours, since the 2-hour analysis was already done there was no need to repeat it. The conducted tests from the 3-hour mark showed a decrease in the amount of yield obtained and the reason was the prolonged degradation of the bonds which ravaged the polymer. The decrease was also witnessed by Sinkiewicz et al., (2017) as the concentration of alkali increased, though the author performed the extraction at very low concentrations.

4.2.4 Effect of pH

The whole extraction procedure was carried out in sodium hydroxide solutions with high pH values, to adjust the pH concentrated hydrochloric acid was used. This parameter had a significant effect on the extraction process, as pH increased the yield also increased. The extraction process should be done in highly alkaline conditions to get maximum solubility (Teklemedhin, 2018; Al-Shamaa, 2020).

Keratin is a fibrous, sulfur rich protein stabilized by extensive disulfide crosslinks (Saha et al., 2019a; Chilakamarry et al., 2021) which render it insoluble under neutral and mildly acidic conditions. High pH conditions promote the ionization of functional groups within the keratin structure and facilitates the cleavage of disulfide bonds (Gupta et al., 2017).

The maximum keratin yield was observed at strong alkaline conditions, however studies also reveal that degradation also occurs at pH 14 (Brenner & Weichold, 2021). This produces low molecular weight poly peptide chains that are often lost during precipitation and washing steps resulting in lower recoverable keratin mass.

4.3 Precipitation of the keratin

The keratin formed was precipitated according to a proposed method by Maurya & Singh, (2023b). Keratin precipitation at pH 1-3 occurred through acid induced denaturation rather than isoelectric precipitation. At such low pH extensive protonation of functional groups, charge screening by chloride ions and disruption of hydrogen bonding led to exposure of hydrophobic regions and subsequent protein aggregation.

While this resulted in rapid and effective precipitation the harsh acidic conditions likely caused partial protein denaturation and reduced accessibility of functional groups, which may have influenced subsequent hydrogel formation and nutrient adsorption performance.

4.4 Hydrogel formation

Following the optimization of keratin extraction parameters, the extracted keratin was used in the synthesis of a keratin -starch biocomposite hydrogel. The hydrogel was successfully formed through chemical crosslinking using boric acid with glycerol incorporated as a plasticizer and moisture retaining agent.

The resulting hydrogel exhibited a stable three-dimensional network structure, good cohesiveness and noticeable water absorption capacity. The inclusion of starch significantly improved gel formation and structural integrity while glycerol enhanced flexibility and moisture retention.

Boric acid acted as an effective crosslinking agent by forming reversible ester linkages with hydroxyl groups present in starch, as well as interactions with functional groups in keratin. This contributed to the development of a three-dimensional network.

4.4.1 Effect of starch concentration

As the concentration of starch increased, the brittleness the hydrogel increased at constant amount of glycerol. The hydrogels were very brittle but could hold significant amounts of water.



Figure: 4.2 High starch content hydrogel



Figure: 4.3 Low starch content hydrogel

Figure 4.2 and 4.3 show the effect of starch concentration on the hydrogel. Figure 4.3 shows a flexible hydrogel that appears almost transparent compared to Figure 4.2, though both products show good water retention with minimal differences, the addition of too much starch was concluded to cause brittle products.

4.4.2 Effect of Boric acid

Boric acid acted as an effective crosslinking agent by forming reversible ester linkages with hydroxyl groups present in starch and functional groups present in keratin this also contributed to the intended three-dimensional network of the hydrogel. The hydrogel with too much boric acid seemed to lose water faster than the ones with small quantities of boric

acid. This behavior is attributed to excessive crosslinking which resulted in a dense and rigid polymer network with reduced pore size and limited swelling capacity (Li et al., 2022).

Over crosslinking restricted polymer chain mobility and promoted syneresis, leading to the expulsion of entrapped water. Additionally, excessive boric acid reduced the availability of hydroxyl groups for hydrogen bonding with water molecules thereby decreasing water retention capacity, the wrinkle appearance shown in figure 4.4 below is due to water loss.



Figure: 4.4 Effect of boric acid concentration on the hydrogel, (**a)** 20 % boric acid, (**b)** 15 % boric acid and (**c**)10 % boric acid.

4.5 Confirmation of hydrogel formation

The synthesized keratin-starch biocomposite formed a stable hydrogel, as confirmed by its ability to absorb and retain water without dissolving. Upon immersion in water the material exhibited significant swelling while maintaining structural integrity indicating the formation of a three-dimensional polymer network. The presence of boric acid facilitated crosslinking between polymer chains while glycerol enhanced flexibility and moisture retention. The hydrogel demonstrated reversible swelling and deswelling behavior further confirming its classification as a hydrogel. An inversion test was also done to confirm if it was really a gel since gels do not flow when a container is inverted.

4.6 FTIR characterization of the extracted keratin and synthesized keratin-starch biocomposite

The FTIR spectrum of the extracted keratin exhibits several characteristic adsorption bands associated with proteinaceous materials. The broad adsorption band observed in the region 3200-3400 cm^{-1} is attributed to overlapping (-NH-) stretching vibrations of amide A and (-OH) stretching vibrations, indicating the presence of hydrogen bonding with the keratin structure, this was also reported by Silva et al., (2024b). This broadness suggests intermolecular interactions typical of fibrous proteins.

An absorption band around 1650 cm^{-1} was also observed corresponding to the amide 1 region, this was attributed to C=O stretching vibrations of the peptide backbone (Giubertoni et al., 2022). The amide 1 band is highly sensitive to protein secondary structure and its presence confirms that the polypeptide chains of keratin remained largely intact (Tursunova & Maksumova, 2025). This indicates that while sodium hydroxide disrupted disulfide linkages and hydrogen bonds to solubilize keratin, the fundamental peptide framework was preserved.

The absorption band observed in the 1230-1300 cm^{-1} region corresponds to the amide (III) vibration, which involves (C-N) stretching and (-NH-) bending (Stani et al., 2020). Amide (III) is associated with ordered protein structures and confirms the fibrous character of keratin (Saha et al., 2019a; Maurya & Singh, 2023b). Minor peaks in the 1000-1150 cm^{-1} region may be attributed to C-O stretching vibrations, possibly originating from residual polysaccharides or minor impurities, however their relatively low intensity indicates that the extracted material is keratin.

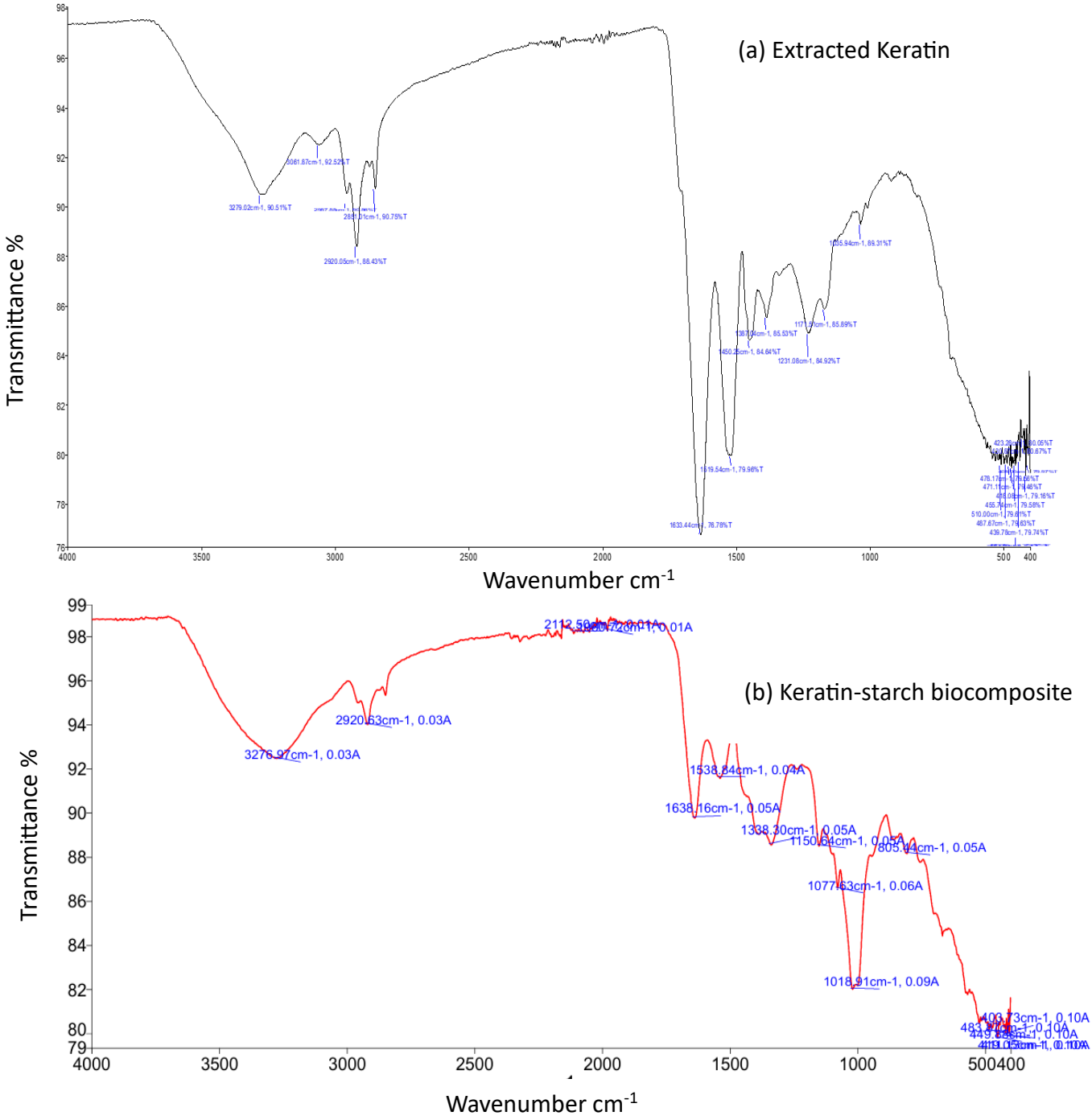


Figure 4.5 FTIR spectrum of **(a)** extracted keratin and (b)keratin-starch biocomposite.

4.6.1 Keratin-starch Biocomposite

This FTIR spectrum shows notable differences when compared to that of keratin alone, confirming successful composite formation and intermolecular interactions.

The broad absorption band in the 3200-3400cm^{-1} region becomes significantly more intense and broader in the biocomposite. This enhancement is attributed to the high density of hydroxyl (-OH) groups present in starch combined with(-NH-) groups from keratin (Teklemedhin, 2018; Saha et al., 2019a; Maurya & Singh, 2023b). The increased broadening indicates the formation of extensive intermolecular hydrogen bonding between keratin amide groups and starch hydroxyl groups, which plays a critical role in hydrogel network formation (Giubertoni et al., 2022).

The characteristic amide (I) 1650 cm^{-1} and amide (II) 1540 cm^{-1} bands of keratin are still present in the biocomposite spectrum, although slight shifts and reductions in intensity may be observed. These changes suggest that keratin is not chemically degraded during composite preparation but is instead physically embedded within the starch matrix. The slight peak shifts are indicative of altered local chemical environments due to hydrogen bonding interactions rather than covalent bond formation.

Strong absorption bands in the 1000-1150 cm^{-1} region become more pronounced in the biocomposite spectrum and are assigned to (C-O-C) and (C-O) stretching vibrations characteristic of polysaccharides. The presence of these bands confirms the successful incorporation of starch.

4.6.2 Significance of FTIR results in relation to hydrogel and nutrient capture.

The FTIR results provide strong evidence that the keratin-starch system forms a physically crosslinked hydrogel network. The extensive hydrogen bonding between keratin and starch enhances water retention capacity by trapping water molecules within the polymer matrix. Furthermore, the retention of keratin amide groups introduces functional sites capable of interacting with nutrient ions.

Amide (-CONH-), amino (-NH_2) and hydroxyl (-OH) groups present in the biocomposite can interact with nutrient species such as nitrate and phosphate through hydrogen bonding, electrostatic interactions, and ion-dipole interactions. This functional group richness

explains the observed nutrient capture performance of the hydrogel, as confirmed by subsequent adsorption and leaching experiments.

Thus, FTIR analysis not only confirms the chemical composition of the biocomposite but also supports its functional performance as a water retaining and nutrient capturing hydrogel.

4.7 X-ray Diffraction (XRD) analysis of keratin-starch biocomposite hydrogel

X-ray diffraction (XRD) analysis was conducted to investigate the structural characteristics and degree of crystallinity of the synthesized biocomposite hydrogel cross linked with boric acid. The obtained diffractogram revealed the coexistence of amorphous and semi-crystalline regions withing the hydrogel matrix.

A broad diffraction halo was observed within the approximate 2θ range of 15^0 - 25^0, indicating the amorphous nature of the synthesized material (Speakman & Ph, 2022). The broadening of this region suggests disruption of the crystalline structure of starch during gelatinization and partial denaturation of keratin during alkaline extraction (Oluba et al., 2021). Similar broad amorphous peaks have been reported in starch-based hydrogels and keratin biopolymers due to loss of molecular ordering after thermal and chemical treatment (Oluba et al., 2021).

The XRD pattern also exhibited one distinct sharp diffraction peak around 17^0 – 18^0 (2θ). This peak indicates the presence of localized ordered or semi crystalline domains within the hydrogel network (Speakman & Ph, 2022). The diffraction peak may be attributed to residual crystalline regions of gelatinized starch, retained β sheet in keratin or the formation of borate ester interactions between starch and keratin chains after crosslinking with boric acid (Barroso et al., 2019; Oluba et al., 2021).

Starch generally exhibits characteristic crystalline peaks because of the ordered arrangement of amylose and amylopectin chains (Barroso et al., 2019). However, during gelatinization heating in the presence of water causes swelling and rupture of starch granules which reduces crystallinity and generates amorphous structures, this also explains the broad halo in the diffractogram, these findings were also reported by Barroso et al., (2019). Partial recrystallization of amylose produces weak peaks around 17^0 – 23^0 (2θ), which may contribute to the small shoulder peak.

Keratin from chicken feathers often displays diffraction peaks associated with α-helical and β-sheet protein conformation which are often disrupted by alkaline treatment through interference with intermolecular bonding resulting in reduced crystallinity and broader

diffraction patterns (Singh, 2023). Despite this some beta regions remain intact which contributes to the observed sharp diffraction.

The addition of boric acid as a crosslinking agent modified the structural arrangement of the composite, it is capable of forming borate ester linkages with hydroxyl groups present in starch and functional groups in keratin (Chousidis, 2024). These intermolecular interactions can enhance structural organization within localized regions of the polymer network leading to the formation of semi crystalline regions which justifies the sharp peak at $17^0 – 18^0$ which was reported by Tiwari et al.,(2022). This strongly suggests successful crosslinking and intermolecular interaction between keratin and starch chains.

Compared to pure starch and pure keratin reported in literature (Barroso et al., 2019; . Singh, 2023), the synthesized keratin-starch hydrogel exhibited reduced crystallinity confirming the formation of a blended polymeric network. The increased sharpness of the peak in the boric acid crosslinked sample therefore indicates improved molecular organization and stabilization of the hydrogel structure.

The coexistence of amorphous and semi crystalline regions is advantageous for hydrogel applications because amorphous regions enhance water absorption and swelling capacity based on findings from Anuradha et al., (2024), while semi crystalline regions contribute to mechanical stability and structural integrity.

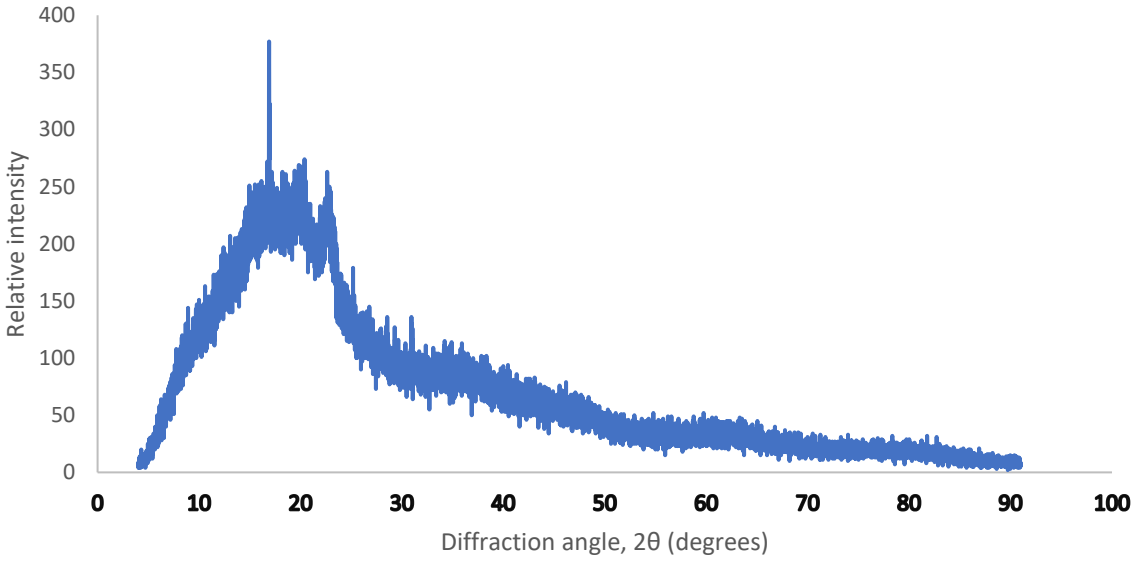


Figure 4.6 X-ray Diffractogram of the synthesized starch-keratin biocomposite hydrogel

4.8 Fertilizer capture test

The effect of keratin concentration on nutrient capture capacity of the keratin-starch biocomposite hydrogel was investigated by varying the keratin content while maintaining constant starch, boric acid and glycerol concentrations. The resulting hydrogel samples were evaluated on their ability to capture nitrates, phosphates and potassium ions which are the macro fertilizer nutrients.

The results showed that nutrient capture efficiency increased with increasing keratin concentration up to an optimum level. Hydrogels containing low keratin content exhibited limited nutrient adsorption, whereas hydrogels with moderate keratin concentrations showed highest nutrient capture for all tested ions. Further increase in keratin concentration resulted in a decline in nutrient capture efficiency.

4.8.1 Retention test for nitrate

This was investigated by varying the concentration of the keratin in the biocomposite hydrogel, while all the other ingredients were kept constant. The results showed that there was little change across the varied samples.

The test was performed in a dual method this was because according to the research done by Huang et al., (2024), nitrates absorb at 220 nm and so does dissolved organic matter, to counter this the samples were run again at 275 nm on a UV-Vis spectrophotometer. At 275 nm, only organic matter absorbs and not the nitrates. Huang et al., (2024) also suggests the use of a correction factor of A correction = A220 nm – 2A275 nm where A is the absorbance of the samples.

Nitrate capture by the hydrogel is primarily governed by electrostatic interactions between nitrate ions (NO_3^-) and positively charged functional groups within keratin. Under experimental setups, amine groups (NH_3/-NH_2-) present in keratin act as nitrate binding sites.

At low keratin concentration, the limited number of amine groups limit nitrate adsorption. As keratin concentration increased, the availability of positively charged sites increased ultimately enhancing capture. At higher concentrations the hydrogel network becomes

denser, reducing pore size and limiting nitrate diffusion into the hydrogel matrix (Li et al., 2022).

Table 4.3 Retention test results for nitrates

Keratin concentration	**Initial concentration (ppm)**	**Remaining concentration (ppm)**	**Percentage removal (%)**
1	50	33	34 ±1
2.5	50	21	58 ±0.7
5	50	11	78 ±1
10	50	24	52 ±0.8
15	50	37	26 ±1.3

One way ANOVA showed that keratin concentration significantly affected nitrate removal efficiency, $F(4, 10) = 1708$, $p<0.0001$. Nitrate removal increased with increasing keratin concentration up to 5%, at which a maximum removal of approximately 78%. Further increase in keratin concentration resulted in reduced nitrate removal, likely due to increased network density and diffusion limitations within the hydrogel matrix as presented by Table 4.3 above.

4.8.2 Effect of keratin concentration on phosphate capture

The effect of keratin concentration on phosphate capture was evaluated under identical hydrogel synthesis conditions. The results indicated a strong dependance of phosphate adsorption on keratin concentration. Phosphate capture increased with increasing keratin concentration and reached a maximum at a moderate keratin content. Beyond this optimum concentration, phosphate capture decreased.

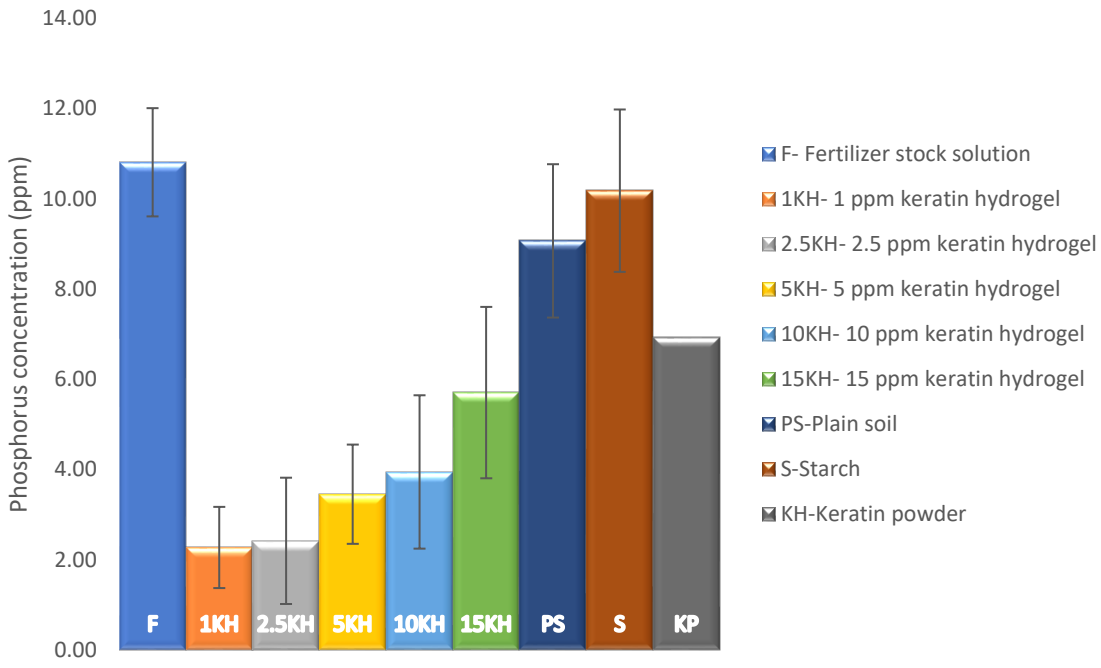


Figure :4.7 Phosphate retention by different samples.

Figure 4.7 was obtained after conducting the phosphate capture test on a UV-Visible spectrometer, this test was also performed on plain starch and keratin powder which exhibited their own retention capacity. Phosphate ions exhibit strong interactions with protonated amine groups and polar functional groups in keratin compared to nitrate, phosphate ions have a higher charge density resulting in stronger electrostatic attractions to positively charged sites within the hydrogel.

Table 4.4 Phosphate retention test

Keratin concentration	**Initial concentration (ppm)**	**Remaining concentration (ppm)**	**Percentage removal (%)**
1	10.809	2.27	79.00 ±1.2
2.5	10.809	2.4169	77.64 ±0.9
5	10.809	3.4509	68.07 ±1.4
10	10.809	3.9443	63.51 ±1.1
15	10.809	5.7038	47.23 ±1.7
Plain soil	10.809	9.0671	16.12 ±1.9

Starch	10.809	10.1804	5.82 ±1.7
Keratin powder	10.809	6.917	36.01 ±0.8

One way ANOVA revealed significant differences in phosphate removal percentage among the eight treatments, $F(7, 16) = 1423.6$, $p < 0.0001$. There was a steady decline in the adsorption of phosphate ions with the 1% solution adsorbing the most and then a gradual decline up to the 15% solution as shown in Table 4.4 above. This is attributed to the open and highly porous network formed at low keratin content, which facilitated diffusion of the bulky and highly charged phosphate ions. At higher keratin concentrations, increased network density and reduced pore size limited phosphate diffusion and accessibility of binding sites. Additionally, electrostatic repulsion between adsorbed phosphate ions and competition with borate crosslinking further reduced phosphate adsorption efficiency.

4.8.3 Potassium retention test

The influence of keratin concentration on potassium capture by the hydrogel was also investigated through UV-Visible spectrometry. The results from Table 4.5 show that the biocomposite has increased potassium uptake compared to plain starch, keratin and soil.

Table 4.5 Potassium capture test obtained from AES spectroscopy

Name of active ingredient	**ppm prepared**	**ppm retained**	**percentage removed %**
Keratin-starch biocomposite	28	2.1	92.5 ±3.7
Plain starch	28	19.6	30 ±4.1
Plain keratin	28	12.6	55 ±3.1
Plain soil	28	26.6	5 ±3

The results from Table 4.5 above are for the 5% keratin hydrogel. The comparative above shows that keratin hydrogels outperform starch, keratin powder and plain soil. Statistical analysis was performed using one way ANOVA, the results showed a significant difference in percentage removed between groups, $F(3, 8) = 628.4$, $p<0.0001$. The keratin-starch biocomposite exhibited the highest removal efficiency which was significantly greater than plain starch, plain keratin and plain soil. The large F value indicates that material type strongly influenced potassium capture.

Potassium capture occurs mainly through ion exchange and electrostatic interactions between potassium ions and negatively charged carboxylate groups ($-COO^-$) present in keratin. At extremely low concentrations there are fewer actively binding groups hence a slight decrease in capture power. At higher concentrations, the increased crosslinking density and reduced porosity of the hydrogel limited potassium ion diffusion. This reduced the accessibility of carboxylate groups leading to decreased potassium uptake despite the higher keratin content, though this arises it has little effect on the capture power.

4.9 Moisture retention test

According to Figure 4.8 the keratin biocomposite hydrogel showed an 80 % water retention capacity compared to the soil-biocomposite mixture which showed a 60 % capacity and then lastly plain soil, which retained only 17.5 % water after 8 hours at 30 ºC in an oven. This is due to the strong hydrogen bonding between the plain biocomposite and water molecules. The soil mixture showed greater retention than plain soil but less compared to the biocomposite because the soil significantly reduced water penetration and as well effective hydrogen bonding by interacting or blocking the pores of the hydrogel matrix.

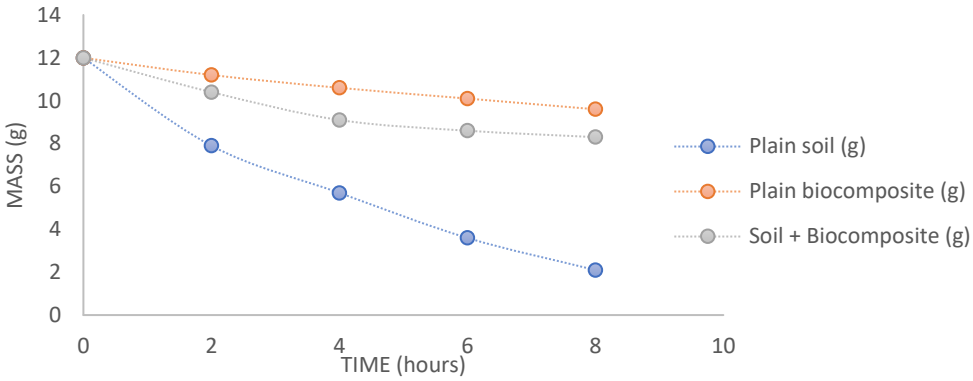


Figure 4.8 Moisture retention test.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The aim of this study was to extract keratin from chicken feathers and synthesize a starch–keratin biocomposite hydrogel for water retention and nutrient capture. The results obtained showed that keratin was successfully extracted using alkaline treatment confirming the viability of poultry feather waste as a sustainable protein source for agricultural purposes.

The study optimized the extraction process via alkaline hydrolysis method using sodium hydroxide, several extraction iterations were done to optimize keratin extraction. It was noted that effective extraction is done at high pH from 10-13, other parameters investigated included extractant concentration, which was concluded that 2 M sodium hydroxide produced higher yields and anything above this concentration degraded the protein resulting in low yields. The last parameters to be investigated were time and temperature, prolonged hydrolysis resulted in protein degradation thus this study found that the optimum extraction time and temperature are 2 hours at 80 ^{o}C respectively.

Characterization of the extracted keratin was done using Fourier Transform Infrared Spectroscopy (FTIR) which revealed that the extract had peaks similar to pure keratin. The functional groups were also confirmed and hence it was deduced that the extract was a protein. Besides instrumental analysis qualitative tests like the biuret test were also used to confirm the presents of the protein in solution and the results were positive.

After validating that the extract was keratin, the effects of the additives that were used in this research for hydrogel synthesis were investigated. Starch concentrations were varied and it was revealed that high starch content hydrogels are brittle hence an optimum concentration of 20% starch solution was used. Boric acid was used as a crosslinking agent and it was noted that higher concentrations above 10 % which was deemed optimum,

resulted in rapid water loss since the polymer chains aggressively crosslink making it difficult to trap water molecules. The biocomposite was optimally synthesized by gelatinizing 20 % starch solution, adding 5 % keratin solution and 10% boric acid.

To confirm that the obtained powder after drying and crushing was a hydrogel different methods were used. A none instrumental inversion test was done and it gave a positive result for gel formation. The synthesized hydrogel was characterized using FTIR and XRD spectroscopy, results from FTIR characterization revealed peak shifts in the new biocomposite when compared to pure extracted keratin. Though certain functional groups were preserved the shifts showed that a new compound had been formed and there were peaks indicating the presence of new bonds due to crosslinking and chain interactions.

XRD spectroscopy also showed differences between pure starch, keratin and the hydrogel composite, with peaks in starch and keratin absent in the new synthesized material which showed a new structure had been formed, the hydrogel also showed a very broad amorphous halo indicating the none ordered regions of the composite which are responsible for water retention and swelling properties and a sharp crystalline peak which is attributed to the ordered regions from crosslinking which give the biocomposite its mechanical strength. These two properties are essential for its functionality.

Nutrient capture studies were investigated after the successful characterization of the synthesized biocomposite hydrogel. Keratin concentration in the hydrogel was varied in this phase of the study, the biocomposite showed exceptional nitrate ion capture at 5 % keratin solution with a 78 % nitrate capture capacity. The hydrogel also effectively retained phosphate ions with the highest retainment rates between 2.5 – 5 % keratin and a phosphate retainment capacity of 77.6 % and 68.1 % respectively. The last nutrient ion to be investigated was potassium and the biocomposite had a retainment capacity of 92.5 % compared to plain starch, keratin and soil which had retainment capacities of 30 %, 55 % and 5 % respectively.

The last property of the biocomposite to be investigate was its ability to retain moisture, the hydrogel showed an incredible 80 % water retention capacity at 30 ºC in an oven for 8 hours compared to the soil-biocomposite mixture and plain soil which retained 60 % and 17.5 % respectively.

Overall, the findings of this study demonstrated that the synthesized biocomposite hydrogel has promising potential as an environmentally friendly soil amendment for water conservation and nutrient management.

This study contributes to sustainable development goals by promoting waste use in circular economy models, sustainable agriculture and efficient resource utilization.

5.2 Recommendations

Based on the findings of this study, it is recommended that future research focus on optimizing the keratin extraction process by investigating a wider range of alkali concentrations, temperatures and extraction durations. Further studies should evaluate the long-term nutrient release behavior of the biocomposite hydrogel under different soil types and climatic conditions.

Further research should also assess the product through other characterization techniques such as Scanning Electron Microscopy or advanced imaging techniques for pore size and internal morphology.

Mechanical and biodegradation studies are also recommended to assess the durability and environmental impact of the hydrogel in real agricultural settings. Field trials involving crop growth experiments would provide valuable insights into the effectiveness of the starch–keratin hydrogel in improving soil moisture retention and nutrient availability. Scaling up the production process and conducting economic feasibility analyses are also suggested to support potential commercialization.

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