Fabricating zero-valent Cu NPs on Oxidized Alginate-Chitosan ZnO polymer biomatrix for 4-nitrophenol reduction and dyes discoloration

By

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This thesis is submitted as partial fulfillment of requirements for the degree of

MASTER OF SCIENCE IN

CHEMISTRY

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Department of Chemistry, School of Natural Sciences,

National University of Sciences and Technology, (NUST),

Islamabad, Pakistan 2023

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Dedication

Dedicated To My Unwavering Pillars of Support, My Beloved Parents, “Muhammad Akhtar and Farzana Sadiq and My Teacher Dr. Saifullah”.

Statement of Original Authorship

I Momina Islam hereby state that my MS thesis titled "Fabricating zero-valent Cu NPs on Oxidized Alginate-Chitosan polymer biomatrix for 4-nitrophenol reduction and dyes discoloration" is my work and has not been submitted previously by me for taking any degree from this National University of Science and Technology or anywhere else in the country/ world.

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ACKNOWLEDGMENTS

All praise to Allah Azawajal for giving me the information, resources, and power needed to do this endeavor. I am immensely grateful to Dr. Shahid Ali Khan for his kind guidance, unwavering support, and invaluable expertise throughout my research. His insightful feedback, constructive criticism, and constant encouragement have been instrumental in shaping the direction and quality of my research.

I would like to extend my heartfelt thanks to my GEC members, Dr. Azhar Mahmood, and Dr Mudassir Iqbal. Their expertise, insightful comments, and suggestions have greatly contributed to the development and improvement of my research. I also acknowledge NUST (National University of Science and Technology) and all its departments (SNS and SCME) for the facilities and technical support. I'd also like to thank the faculty and support staff for their assistance in the lab.

I would like to express my utmost gratitude to my family and my friends and my seniors for their support, understanding, and encouragement throughout this arduous journey.

Lastly, I would like to express my sincere appreciation to all those who have supported me in any way during the research. Your contributions, whether big or small, have had a profound impact on my academic journey, and I am deeply grateful for your support.

Momina Islam

ABSTRACT

Various types of dyes and phenolic compounds are being extensively used in industries. The frequent use of these colors contaminates water. Humans and other living things can get sick from this tainted water. Therefore, treating the contaminated water is important. The biodegradation process can be used to remove these colors from water, although it isn't very effective. The use of metallic nanoparticles for dye degradation offers the environment some very promising advantages. In this thesis alginate was oxidized to aldehyde and then reacted with chitosan to make the imine bond later ZnO nanoparticles were added .The matrix serves as supporting material for the synthesis of Cu nanoparticles . To create zero valent copper nanoparticles, copper ions from an aqueous solution were adsorbed on films and then treated with sodium borohydride solution. A sudden change in color shows the synthesis of nanoparticles, which XRD analysis also verified.Synthesized films of OAlg-CS/ZnO were characterized using FTIR. Oxidized alginate and its polymer nanocomposite showed exceptional degradation efficiency i.e more than 80% in all the cases . Reason behind is functionalization provides more adsorption sites for metal ions ultimately leads to the formation of more nanoparticles.The synthesized films also showed exceptional anti-bacterial activity against less resistant strain of s.aureus.

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LIST OF ABBREVIATIONS

FTIR	Fourier transform infrared spectroscopy
EDS	Energy Dispersive Spectroscopy
SEM	Scanning electron microscopy
TGA	Thermogravimetric analysis
XRD	X-ray diffraction analysis
DSC	Differential Scanning Calorimetry
OAlg	Oxidized Alginate
Alg	Alginate
ZnO	Zinc Oxide
CS	Chitosan

CHAPTER 1

INTRODUCTION

Our environment is always changing, but problems associated with it are also changing. It is crucial to be aware of all these issues and their effects due to unpredictable weather patterns, an increase in natural disasters, and many other factors. One of the biggest issues our planet is currently facing is pollution.

1.1 Pollution

Pollution is the presence of any material or its introduction into the environment that has poisonous or negative effects.

Chemical substances and their byproducts damage the air, the water, and the soil. Waste products from industry and agriculture contaminate the water, harming people, plants, and animals. Land pollution results from many human activities such as deforestation, mining, littering, and construction that damage the earth's surface. The air is directly contaminated by hazardous smoke that comes from factories, vehicles, the burning of fossil fuels, and tanneries.

1.2 Environmental Pollution

The three main elements of the environment—air, water, and soil—are together referred to as environmental pollution. They could be to blame for either recent or ongoing environmental damage. Chemical pollutants may also produce damaging physical events, such as greenhouse gases that cause global warming.[1] When an element or substance cannot be broken down by the environment without causing harm to it, pollution is the result. The destruction process can take a few days to thousands of years (as it does with radioactive pollution, for example), because the elements involved are not generated by nature. In other words, pollution happens when nature cannot naturally degrade an element that has been introduced to it.[2]

Environmental pollution, which has been around for centuries but only became major after the industrial revolution in the 19th century, must be treated seriously since it negatively affects natural resources like water and air, which are essential for life to exist on earth. Indeed,

Animals, including people and plants, could not survive without it or if it existed in different quantities. The irresponsible use of fossil fuels and improper disposal of industrial waste are the main causes of pollution.

1.3 Types of Pollution

Following are the main forms of pollution:

- Soil pollution
- Air pollution
- Water pollution
- Noise pollution

Figure 1. 1 Types of Pollution

1.3.1 Water Pollution

The fact that no living thing on earth can survive without water helps us to grasp the significance of water in our life. The vast majority of water on Earth is covered by marine water, which, without proper processing, is unfit for human consumption. Fresh water from surface and subsurface water sources is the only source of drinkable water. Since water is a major component of biological cells' physiological processes, water quality is crucial to our survival. Our bodies are two-thirds water, and we cannot go even one day without it. 95% of the human brain is made up of water.[3] Dehydration can occur when the body's water content falls by merely 2%.

For the upkeep of their physiology and morphology, all living species' cells and organs need water.When dangerous substances are added to water, it becomes contaminated and is no longer suitable for human consumption or the survival of aquatic and marine species. Water contamination poses a serious threat to the entire ecosystem in addition to marine life.

Given that it keeps getting worse and more people are dying from it every day, water pollution has now turned into a significant issue for the entire world system. In addition to industrial waste, other natural occurrences like earthquakes, storms, land slides, etc. have contributed to the degradation of water quality. There are numerous reasons why toxins are added to water, which creates pollution and harms aquatic life.[4] Water contamination is an issue that is becoming more and more serious on a global scale.Water contamination was also a result of quakes, hurricanes, and ground slides, among other natural disasters. Lives are at danger due to various toxicant additions and water pollution reasons.According to recent studies, streams and rivers provide a significant amount of phosphorous (P) and nitrogen (N) to agricultural regions. Pakistan is ranked 80th out of 122 nations for its water challenges. In Pakistan, almost 16 million people lack access to clean water. Every year, 250,000 kids die from water-borne diseases.[5]

1.3.2 Sources of water pollution

According to a report issued by the Environment Protection Agency (EPA) in 1990, chemical leaching from landfills where they are utilized to boost crop yield is responsible for more than 50% of water body contamination. There are two primary categories of water contamination sources. Point sources, such as residential sewage, industrial effluents, and mining, are those that originate from a single, recognizable source. Another is non-point sources, which are sometimes referred to as diffuse pollution because no single source can be identified. Consider agricultural area where non-point sources include dirt washed into streams by rainfall, animal dung, fertilizers, and pesticides. Sources of water contamination can be physical or chemical pollutants, such as temperature changes, huge, obvious objects, dyes that alter the color of water, or colorless pesticides that have dangerous effects on both humans and marine life. According to published research, the number of fertilizers in the USA that contain nitrogen and phosphorous grew 20-fold between 1945 and 1993 and nitrogen is the primary source of contamination. Organic matter, heavy metals, deforestation, eutrophication, and infections are the main causes of water pollution in Asia.[6]

Following are some direct and indirect ways that cause water pollution.

- Dumping hazardous garbage.
- Wastewater from industry containing Pb2+, Ar+3, or +5.
- Field water contaminated with poisonous substances including pesticides, fungicides, and fertilizers.
- Lab chemicals and other untreated substances can be hazardous and pollute the environment.
- Waste-burying landfills have the potential to contaminate groundwater over time.
- Construction debris directly contaminates water sources.
- Batteries are thrown into potentially dangerous bodies of water at junk yards or out of ignorance.
- Rivers are contaminated by diseases, non-biodegradable chemicals, detergents, cleansers, and other garbage when sewage and drain water are thrown in them.
- Fossil fuel spills and leaks endanger marine life.
- When materials are exposed to the environment or water during mining, they break down and pollute the ecosystem when it rains.

1.3.3 Pollutants and their effects on Human Health

The threat that pollution poses to human health is serious. Numerous illneses, including diarrhea, cholera, malaria, dengue[10], typhoid, and HIV/AIDS, are emerging as a result of water pollution. Because many dangerous chemicals, including carbon dioxide, carbon monoxide, sulfur dioxide, and CFCs, are released into the environment unchecke, air pollution also has many detrimental consequences on human health. Air pollution causes asthma, lung cancer, TB, and other breathing issues. High fertilizer, pesticide, and herbicide use as well as the improper disposal of non-biodegradable pollutants like radioactive elements all contribute to land contamination. When industrial waste comprising paints, dyes, and other detergents is discharged into the environment unprocessed, it can result in numerous major health problems, including cancer, renal, liver, and skin illnesses.

1.3.4 Heavy metals

Elements having specific gravity five times greater than that of water like lead, arsenic and mercury. One of the most delicate ecological challenges in the entire world is the release of wastewater from companies that use heavy metals. Lead (Pb), chromium (Cr), cadmium (Cd), copper (Cu), and arsenic (As) are industrial wastes that carry heavy metal pollutants typically present in water. Heavy metals are considered as priority pollutants as a result of more stringent protocols and rules. The presence of such toxic metals in wastewater poses a serious threat to both the environment and people.As a result, their removal from wastewater should be done using inexpensive and environmentally friendly adsorbents.

Over the past few decades, the release of heavy metals into aquatic habitats has intensified into a major problem. These pollutants enter the marine ecosystems as a result of numerous industrial operations. The ecosystem of surface and groundwater has been badly harmed by chemical contamination, raising serious questions about the viability of life in the polluted area. The most prominent sources of heavy metal contamination in the environment include electroplating, textile production, tanning, metallurgical waste, and mining. There are 670 textile businesses in Pakistan that discharge waste materials into water streams without waste management. Copper is a significant hazardous heavy metal that is present in waste output, is not biodegradable, and moves up the food chain. Although copper (Cu) is present in humans only in tiny amounts, it is essential for good health. However, its absorption above the nominal dose could be dangerous. Electrical factories may be the source of water contamination with copper. It could result in diarrhea, cramping muscles, convulsions, vomiting, and atrophy. According to the USEPA and WHO, the MCL for Cu is 1.3 mg/L and 1.0 mg/L, respectively.[7]

1.3.5 Dyes

Dyes, which resemble pigments, are used to color a variety of materials, primarily textiles and goods made of leather. Dyes are organic molecules with an aromatic structure and a specific material affinity. The majority of colors are applied as aqueous solutions. Due to auxochromes like -OH, -Cl, -Br, -NO2, -COOH, -NHR, -NH2, etc., dyes are soluble in water. Auxochromes may ionize in water, which makes dyes soluble. These groups are also in charge of enhancing dye colors.Based on their charge and nature, or whether they are acidic or basic, auxochromes can be categorized.

i. Color of dyes

Dyes are organic aromatic compounds but they pose colors because:

➢ They absorb light in visible region.

➢ They have color bearing groups (chromophores).

- They exhibit resonance of electrons.

- They have a conjugated system.

ii. Types of dyes

Dyes can be of following types

(a) Synthetic dyes

(b) Natural dyes

Natural colors are derived from both plants and animals. Like Tyrin purple, madder is a natural dye made from the root of the madder plant. There are two types of synthetic dyes: azo dyes and non-azo dyes. Azo dyes can also be classed as dispersion, sulfur, acidic, basic, and reactive dyes. Based on their makeup, synthetic dyes can also be categorized as acidic or basic.[8] The majority of dyes that are readily available are azo dyes. The pharmaceutical, textile, and cosmetic sectors all employ these colors. Because dyes are very toxic, inappropriate disposal of waste water containing dyes can cause major skin and respiratory issues.

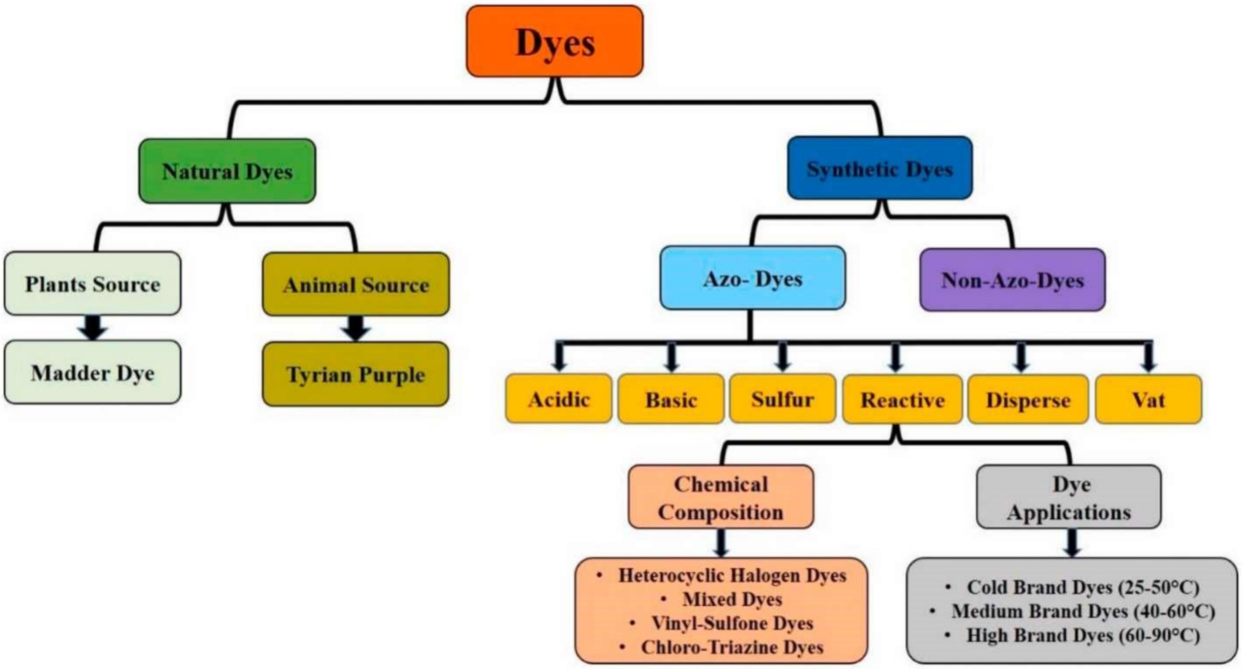


Figure 1. 2 Types of Dyes

iii. Uses of Dyes

Dyes are mostly used as follow

- In laboratories
- As an indicator
- In textile industries

iv. Textile Industry:

Only natural dyes were employed as textile dyes up until the 19th century; however, the textile industry has since started using synthetic colors. Since then, synthetic dyes have become widely utilized due to their benefits, including simple manufacture and the availability of dyes in a variety of colors. When these colours are dumped into bodies of water untreated, they cause pollution. Due to the presence of dangerous organic substances like naphthalene and benzidine, these synthetic colours in waste water are highly carcinogenic.

v. Strategies for dye degradation

The color and quality of water are significantly impacted by industrial effluents containing dye compounds.It has an impact on aquatic life as well as human health. So it is imperative that you to avoid catastrophic problems, remove these dyes and pesticides from the waste water. The following techniques are being used to degrade water pollutants.

1. Using physical means.

2. Bio-based techniques

3. Chemical processes.

vi. Need for dye degradation

While dyes have many advantages, there are also numerous ways in which they endanger human life. The various dye risks are shown below

- Since dyes are frequently soluble in water, they pollute the water.
- Dyes affect the absorption and reflection of sunlight from water, which affects the underwater photosynthetic activity.
- Many dyes are carcinogenic, and some can irritate skin. Small amounts of dyes, some of which are even undetectable by the human eye, have a significant impact on the transparency of water bodies.[9]

Therefore, these hazardous colors must be eliminated or, better yet, degraded in order for living things to survive.

In order to eliminate or at the at least reduce the amount of harmful compounds in waste water, effective solutions for the treatment of organic waste reduction are needed.[10]

Colored chemicals and hazardous metals are being removed using a variety of methods, including electrochemical treatment, filtration, precipitation, osmosis, flotation, coagulation, flocculation, and adsorption. Physical methods' main drawbacks, such as cost, the potential for secondary pollution formation, and partial treatment, prevent them from being successful; as a result, attention should be paid to developing techniques that completely transform organic pollutants, especially with the help of a powerful catalyst.[11, 12]

1.3.6 Nitrophenols

The effect of pollution on human health is currently a hotly debated subject. Chemical production helps the economy thrive and plays a crucial role in modern living.

However, the widespread use and production of reagents and chemicals results in environmental deterioration because many organic reagents are hazardous and their harmful byproducts. Chemicals from the phenolic group are among the most prevalent and dangerous environmental pollutants due to their extensive use in industry. These phenolic compounds come in 3 isomeric forms. Depending on where the -NO2 group was located, these isomers had various physical and chemical characteristics.

o-nitrophenol m-nitrophenol p-nitrophenol

Figure 1. 3 Isomeric form of Nitrophenol

Above are the three isomeric forms of nitrophenols. The properties of nitrophenols highly depends on the position of –NO_2 group.

i. Nitrophenol Poisoning

If 4-NP is released into the environment, it poses a major threat to public health and the ecosystem due to its tremendous toxicity. The hazardous consequences of this substance may include liver and kidney damage, blood problems, skin and eye irritation, effects on the central nervous system, headaches, nausea, sleepiness, cyanosis, and weariness.

4-NP has been associated with numerous harmful adverse consequences in people, including teratogenic, mutagenic, and carcinogenic potential.[13]

ii. Commonly used Removal Methods of Nitrophenol

Due to the extensive environmental harm that 4-nitrophenol has caused as a pesticide and industrial chemical, there has been a recent increase in interest in the detection, quantification, and removal of this substance. 4-NP is notoriously difficult to get rid of due to its high solubility and stability in water. 4-NP cannot be removed from water using standard water cleaning techniques. High levels of phenolic compounds make wastewater resistant to some additional biological treatments that are normally employed to clean domestic and industrial wastewater. As a result, numerous physio-chemical methods have been required to remove 4-NP, including adsorption, microwaveassisted catalytic oxidation, the electro-Fenton approach, photocatalytic degradation, electrocoagulation, microbial degradation, and electrochemical treatment.[14]

1.3.7 Metallic Nanoparticles

Due to their applications in various areas, metallic nanoparticles have drawn growing attention in recent years. Some of the various applications are as follow

- Sensing
- Drug delivery
- Bio-imaging
- Antibacterial coating

Metallic nanoparticles have a large surface area and can attract dye molecules to their surfaces for adsorption and catalytic breakdown. The dye molecules can be catalytically broken down after being adsorbed through a variety of methods, such as oxidation, reduction, or interaction with reactive species produced by the nanoparticles.[15]

Metallic nanoparticles can improve the stability of catalysts employed in dye degradation processes, increasing their reusability and stability. By preventing catalyst aggregation or deactivation, they can lengthen their useful lives and enable numerous cycles of dye degradation.

Because of their enormous surface area, high catalytic efficiency, simple manufacture, and selectivity for specific types of reactions, metallic nanoparticles are thought to be an extensively used catalyst for the breakdown of organic contaminants.[16, 17] They were utilized in a variety of processes, including electrochemical processes, coupling processes, and oxidation and reduction processes. However, in contrast to noble metals, which have more stable nanoparticles, the majority of the readily available metal nanoparticles limit the practical environmental applications. Agglomeration and separation are additional issues related to the utilization of nanoparticles. It is highly challenging to remove nps from the reaction mixture due to their incredibly small size. Agglomeration, which is brought on by van der Waals interactions and reduces surface area, lowers catalytic effectiveness.

1.4 Why Sodium Alginate?

Due to a number of factors, sodium alginate alginate nanocomposites have attracted interest as potential catalysts for the breakdown of dyes and paranitrophenols.

- Abundant and renewable supply: Brown algae, which are used to make the biopolymer sodium alginate, is a plentiful and sustainable source. It is a desirable material to use for the synthesis of nanocomposites due to its abundance and renewability.
- High surface area: The sodium alginate nanocomposite form has a high surface area-tovolume ratio when it is produced using nanoparticles or other nanomaterials. With more active sites for catalytic reactions due to the increased surface area, the catalytic efficiency is improved.
- Enhanced stability: The sodium alginate is more durable and stable because to the nanocomposite structure, making it appropriate for long-term catalytic applications. The mechanical, thermal, and chemical stability of the composite can be improved by adding nanoparticles.
- Ion exchange abilities: Sodium alginate has the ability to exchange ions and molecules with other substances. This property allows the nanocomposite to efficiently adsorb and trap dye molecules or paranitrophenols.

- Catalytic activity: Metal or metal oxide nanoparticles, such as silver or iron oxide, can demonstrate excellent catalytic activity when integrated into the sodium alginate matrix. Through a variety of methods, including adsorption, oxidation, reduction, and photocatalysis, these nanoparticles can speed up the breakdown of dyes and paranitrophenols.
- Low toxicity and biocompatibility: Sodium alginate is thought to be biocompatible, which is helpful for applications including wastewater treatment and environmental cleanup.[17]

CHAPTER 2

LITERATURE REVIEW

Aldehyde groups are commonly used in hydrogel synthesis because they enable cross-linking reactions, which result in the formation of a three-dimensional network structure. By combining the unique properties of aldehydes made from sodium alginate and chitosan, this synthesis opens new avenues in biomedical and environmental applications. This innovative process paves the way for enhanced drug delivery systems, antibacterial activity , and food packaging.

Kong, Chen et al. present method for the synthesis of dialdehyde from sodium alginate by using NaIO4 in the dark condition and keep it stirring for 6 hours enough time to break the carbon-carbon bond and two aldehyde groups finally obtained. To remove the unreacted materials and salts, the solution was dialyzed using water, and the final photo cross-linkable product was lyophilized for utilization in the fabrication of hydrogels for bone regeneration application. [18]

Sánchez-Morán, Ahmadi et al.In a beaker 8g of sodium alginate was dissolved in 400ml od distilled water.After it is completely dissolved add certain amount of NaIO4 in a shady condition keeping the temperature at 25 °C .The reaction was performed for the period of 24h and after that quenching agent was added.Excess ethanol was added to make gel which then can be washed by centrifugation and then dried by the help of freeze drying.After that hydrogels were made with N2-hydroxypropyl trimethyl ammonium chloride chitosan (HACC) for the biomedical and antibacterial applications. [19]

Yang, Dan et al.In this method alkoxy amine functional group was introduced to the sodium alginate (NaAlg) backbone to make alginate alkoxy amine (NaAlg-AA) and then the resulting functionalized polymer was cross linked with the oxidized alginate which was prepared prior to this from the method reported earlier i.e by using sodium periodate . Both the separately prepared polymers were mixed together at 37 °C by varying ratios and pH.And found its applications in the formation of hydrogels for biomedical applications.[20]

Yu, Niu et al. In this method collagen-chitosan based membranes were modified because without the modification they have very poor mechanical properties and for that sodium alginate was oxidized to make dialdehyde (ADA) by the use of oxidizing agent.The films were prepared by 1:1 ratio by mass of chitosan and collagen.And then membranes with enhanced mechanical properties were made by the addition of different degree of oxidized alginate like COL-CS-10%, COL-CS50%.This new membrane with enhanced mechanical properties finds its applications in wound healing. [21]

Gao, Li et al. In this experiment, polyhedral oligomeric silsesquioxane (POSS) grafted polymer were prepared by grafting methacrylic acid , sodium alginate and polyhedral oligomeric silsesquioxane (POSS-SAG-MAA). In which sodium alginate was used as crosslinking agent and oxidizing agent that was used was NaIO4. Different ratio of NaIO4 were used to oxidize (POSS-SAG-MAA) for varying amount of time as well like 4h, 8h, 12h,16h,20h and 24h to obtain POSS grafted oxidized sodium alginate composite. This biosustainable POSS grafted oxidized sodium alginate composite then found its application in the modification of collagen instead of cadmium in the tanning industries.This is one of the environmental aspects.[22]

Ponzio, Le Houerou et al. In this study sodium alginate grafted with catechol membranes were produced. The resulting membranes were very strong , free standing with asymmetric composition and self healing.Sodium alginate and and Alg-CAT were first dissolved in 50mM buffer solution an then stirred for 3 hours. Due to the slow oxidation of catechol on the polymer the solution becomes slightly brown. Dopamine hydrochloric acid was added and left it unstirred for a day. When membrane was formed at the water-air interface it is detached with the help of a scalpel and let it dry. These Alginate-Catechol @polydopamine membranes found its applications in various fields.[23]

Lu, Zhang et al. During this study partial oxidation of sodium alginate was done and KMnO4 was used as an oxidizing agent. 2% w/v solution of sodium alginate was prepared in enough DI water. 3.2g of KMnO4 was dissolved in 1000ml of distilled water and boil it for 1 hour and let it stand overnight. MnO2 thus formed can be filter out. And then the oxidation of sodium alginate was happened in the dark conditions until or unless the color of the solution changes from violet to colorless. During that process H2SO4 was used and as catalyst and pH was maintained in 3-4 range.After that the solution was dialyzed and lyophilized for further applications. This method was used to over the problem of lower degradability that occur in natural sodium alginate.[24]

Li, Xu et al.In this study, low molecular weight sodium alginate was prepared using Hydrogen peroxide as an oxidizing agent. Dissolve sodium alginate make 1.5% w/v solution of it. When it is completely dissolved, add 30% hydrogen peroxide. All this is happening in a water bath for the period of 5h at 50 C . With the help of HCl and NaOH pH is maintained. In order to stop the reaction, flask is taken out of water bath and cooled to chill the reaction.And then the product was dialyzed and freeze dried for further use and characterizations. This low molecular oxidized sodium alginate was then used for tissue engineering.[25]

Wang, Huang et al. During this study sodium alginate sulphates were prepared from sodium alginate through the reaction with cholosulphonic acid in formamide. And then acrylamide groups were introduced on the surface of polymer to make SAS-AAm and after that transferred into primary amines to make –NH2 through Hoffman rearrangement. Then free available hydroxyl groups on the surface of sodium alginate sulphates (SAS) coupled with –NH2 in the presence of glutaraldehyde. The newly developed membranes were then subjected to different characterization techniques and is used for selective adsorption of low-density lipoproteins.[26]

Uysal, Emil-Kaya et al.In this study, first iron (Fe) nanoparticles were synthesized and their surface was covered with oxide layer in order to give them more stability.2g of sodium alginate completely dissolved in 100 ml of distilled water. In this homogenized solution now add 1g of Fe nanoparticles and sonicate it for 30 minutes. From this solution droplets were added into CaCl2 solution under the magnetic stirring. The filtered gels were then added into 2% acetic solution containing 2g of chitosan. These chitosan-coated gels are now then washed several times and then washed to neutralize the ph. Now these chitosan coated Fe based sodium alginate gels used for Nd which is a rare earth metal from waste water.[27]

Zhao, Yao et al.In this study sodium alginate nanocomposite was formed with TiO2 and Temozolomide (TMZ) for biomedical application. Titanium oxide plays important role in the inhibition of brain cancer by affecting protein expression.0.5 mg/ml and 0.10 mg/ml nanoparticles were dissolved in double distilled water of TiO2 and TMZ respectively. Both the solutions were mixed together in 3:1 at 20 C and continue to stir for 24h.Centrifugation was done in order to remove any impurities. Different concentration of sodium alginate solution was then sprayed on TiO2-TMZ to make TiO2-TMZ-alginate nanoparticles for improving neuroblastoma treatment.[28]

Xiang, Ma et al. In this study oxidized sodium alginate was prepared from sodium alginate by adding 1 ml of 8% HCL , 50 ml of ethanol and 50 ml of 10% solution of sodium periodate was added. The reaction was stirred in the dark for 18h.After the reaction was stopped the product was dialyzed and freeze dried.Silver nanoparticles were prepared from silver nitrate (AgNO3) and ammonia solution.0.02g of oxidized alginate was dissolved in 5ml of distilled water and then mixed into the ammonia-silver solution at different temperatures to get different sized nanoparticles that showed enhanced antifungal activity by penetrating into the cell membrane.[29]

Dodero, Scarfi et al.In this study sol-gel method was used for the synthesis of ZnO nanoparticles. 1ml of 1 molar NaOH and 2ml of 0.25molar zinc acetate were mixed strongly before adding SA solution at 30-80 C. The white precipitate thus formed undergo washing and drying.SA and PEO were taken in 70/30% ratio to make the final concentration of a polymer. First sodium alginate was dissolved in DI water and then 0.25% by weight of ZnO nanoparticle was added in the solution PEO solution was added and system was kept under stirring for 24h and 4 C to prevent the degradation of the polymer and then membranes were generated through electrospinning and washing cross-linking process. The resulted membranes found its applications in wound dressing.[30]

Li, Mei et al. During that study chitosan-alginate bioactive coating containing polylysine combined with modified atmospheric packaging was done .2% w/v of chitosan solution was dissolved in 1% acetic acid solution and 0.1% w/v solution of sodium alginate was dissolved in calcium chloride solution. Solutions were continue to stir at 40 C for 4h. After that sodium alginate and chitosan in 1:4 were mixed together along with glycerol.After that different amount of polylysine was added into already prepared homogenized solution to get different concentrations.

This coating was then utilized for the inhibition of myofibril oxidation and degradation of farmed putterfish during cold storage for 18 days.[31]

Wu, Qi et al. During this study sodium alginate based adsorbent were made in combination with graphene oxide and cyclodextrin. Graphene oxide was prepared by already reported method.100mL distilled water was used for the dilution of graphene oxide through ultrasonic suspension. Sodium alginate and cyclodetrin in 1:1 was added into the above mentioned suspension and let it stir until the solution completely homogenized.The solution was now made to rest to remove air bubbles.With the help of a syringe add this solution into the 5% $CaCl_2$ solution.These beads after 12 hours were washed with distilled water and used for the adsorption of methylene blue.[32]

Thakur, Pandey et al. During this study sodium alginate based adsorbent was prepared by incorporating it with TiO2 nanoparticles and cross-linked it with acrylic acid as co-polymer. For the preparation of organic-inorganic hydrogel synthesis, various amount of TiO2 was dispersed in distilled water and then stirred for 12h after that sonicate them for 4h. Now 1 g of sodium alginate was dissolved in this nanoparticle solution. On complete dissolution, add acrylic acid in different concentration to this solution in the presence of a cross-linker and let it stir for 2 hours. Now add excess amount of acetone was added to remove uncross-linked polymer from co-polymer. This organic-inorganic composite was then used for methylene blue adsorption.[33]

Bendtsen and Wei et al. The purpose of this study was to develop and characterize acrylate alginate for use in tissue engineering applications. Acrylic anhydride and pyridine were utilized as catalysts in the acrylation process. The acrylate alginate was characterized using Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and gel permeation chromatography (GPC). The results showed that acrylated alginate had improved mechanical properties and higher thermal stability than untreated alginate. The researchers also observed that acrylated alginate increases cell development and proliferation, making it a good material for tissue engineering.[34]

Hua and Wang et al. By grafting acrylic acid onto sodium alginate with potassium persulfate as an initiator and N, N'-methylene bisacrylamide as a crosslinker, sodium alginate-g-poly(acrylic acid) was created. The reaction took place in an aqueous solution containing a constant ratio of sodium alginate to acrylic acid. After that, the solution was nitrogen-purged to remove any dissolved oxygen. [35]

Al-Ghamdi & Khan,et al. During this study chitosan and CMC based sheets were made for the stabilization of Au based nanoparticles for reduction of paranitrophenol and dyes.Chitosan membrane was made by dissolving 1% w/v chitosan in 3% v/v solution.After the chitosan membrane gets dried CMC coating was done on the surface of chitosan.In the next step sheets were dipped in 0.5mmol HAuCl4.3H2O for 24 h so adsorption sites gets saturated. The sheets were then treated with sodium borohydride solution to prepare zero valent catalyst that can be used for the degradation of methylene blue , methyl orange and paranitrophenol.[36]

Lin, Bruzzese et al. During this study alginate oxidized nanocellulose were prepared by the crosslinking with calcium chloride by lyophillization of alginate and oxidized cellulose.The newly synthesized hydroxal group on the surface of cellulose provides the mechanical stability. During that study TEMPO mediated oxidation of cellulose take place then oxidized cellulose and alginate mixed together to form membranes which then later lyophilized and cross-linked with $CaCl_2$ and lyophilized again to make sponges.[37]

CHAPTER 3

EXPERIMENTAL SECTION

Experimental

3.1. Material

Sodium alginate (NaAlg) (CAS no.) Company name, Chitosan (MWt), viscosity, degree of polymerization, degree of acetylation, PVA, Ethylene glycol, Sodium periodate ($NaIO_4$) , Acetic acid (CH_3COOH) , , Sodium Hydroxide (NaOH) , NaCl.

3.1.1. Apparatus and Instruments

Hot plate stirrer, magnetic bar, , pH strips, centrifuge machine, weighing balance, FTIR, SEM, XRD, Tensiometer and TGA analyzer for analysis and characterization.

3.1.2. Oxidation of Alginate

5 g powder of NaAlg was dissolved in 80 mL of distilled water. After that 7.5 g of $NaIO_4$ was added into the solution in the dark conditions with continuous stirring at ambient temperature. After the addition of oxidizing agent the reaction was carried out for 6 h at room temperature. After 6 h, the reaction was quenched by the addition of ethylene glycol (8 mL) under stirring for half an hour. The oxidized alginate was purified by precipitation with the addition of 3 g NaCl and excess ethanol. The product was then centrifuged at 7000 rpm for 5 min (2-3 times) with water and ethanol to remove side products like excess iodine. The gel then formed was then dried and stored for further modification.

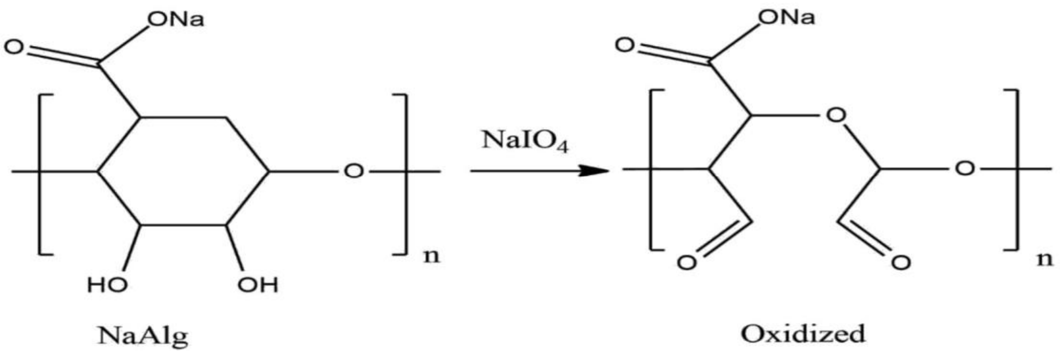


Figure 3. 1 Oxidation of Alginate

3.1.3. Reaction of CS with OAlg

Different amount of oxidized alginate (0.3 ,0.4, 0.5) were mixed with fixed amount of chitosan i.e 0.5g in 3% acetic acid solution to prepare different weight ratio of chitosan-DA membrane. After the addition of chitosan , the solution was stirred overnight to maximize the chances of crosslinking.When the solution is homogenized , PVA equivalent to 30% weight of membrane was added into that solution. PVA will act as binder. The solution was stirred for 1 more hour. The homogenized solution was then transferred to a petri dish. Then the hydrogel films were air-dried and stored for further characterization and applications

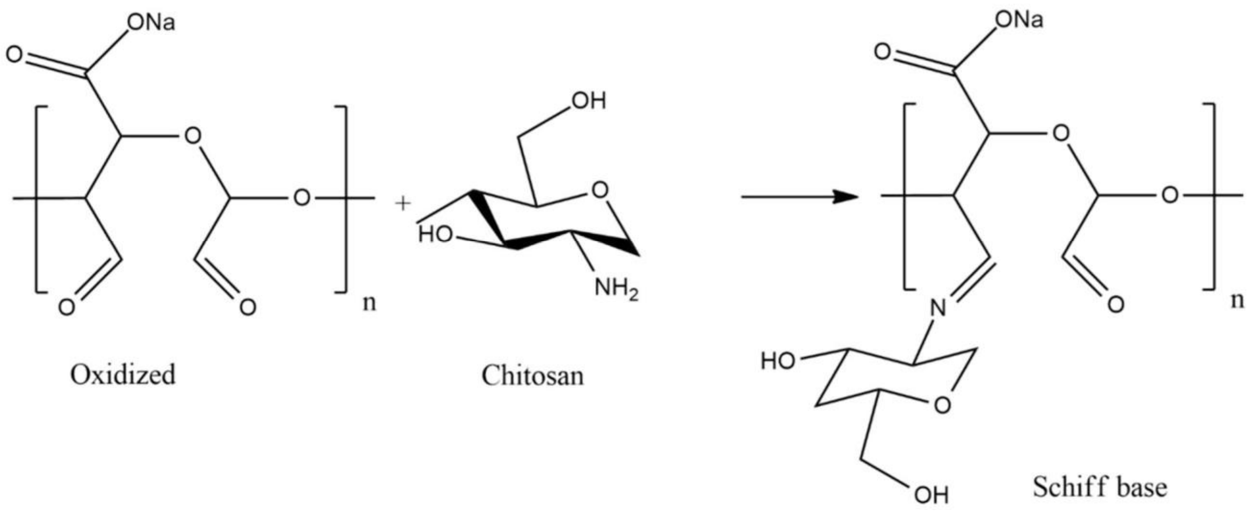


Figure 3. 2 Reaction of Alginate and Chitosan

3.2. Preparation of composite Films

3.2.1. Preparation of Alginate Films

700 mg of pure alginate was dissolved in distilled water to prepare alginate membrane and 30% w/v PVA is added into it as a binder. The homogenous mixture was casted into round plastic petri dish. The membrane was subsequently allowed to dry in air.

3.2.2. Preparation of Alg-ZnO Films

700 mg of pure alginate was dissolved in distilled water to prepare alginate membrane and 30% w/v PVA is added into it as a binder.10,15 and 20% by weight of ZnO nanoparticles were sonicated for 20 minutes before adding into the homogenous solution. The homogenous mixture was then casted into round plastic petri dish. The membrane was subsequently allowed to dry in air.

3.2.3. Preparation of Chitosan Films

700 mg of pure chitosan was dissolved in 1% Acetic acid solution to prepare chitosan membrane. The homogenous mixture was casted into round plastic petri dish. The membrane was subsequently allowed to dry in air. After drying, the membrane was washed with solution of NaOH to neutralize the effect of acetic acid. After washing several times with distilled water, the membrane was allowed to dry on the same petri dish at room temperature.

i. Preparation of CS-ZnO Films

700 mg of pure chitosan was dissolved in 1% Acetic acid solution to prepare chitosan membrane.

10,15 and 20% by weight of ZnO nanoparticles were sonicated for 20 minutes before adding into the homogenous solution. The homogenous mixture was then casted into round plastic petri dish. The membrane was subsequently allowed to dry in air. After drying, the membrane was washed with solution of NaOH to neutralize the effect of acetic acid. After washing several times with distilled water, the membrane was allowed to dry on the same petri dish at room temperature.

3.2.4. Preparation of OAlg-CS-ZnO Films

Nanoparticles were weighed (10%, 15% , 20%) according to the weight of membranes. Different amounts of nanoparticles were sonicated for 20-30 mins before adding to the solution. Into the DA-Chitosan-PVA crosslinked solution add different ratios (10% , 15% ,20%)of ZnO nanoparticles were added.The solution was stirred for 1 more hour until the nanoparticles completely dissolved. After drying, the membrane was washed with solution of NaOH, and then with distilled water to reduce non-cross-linked aldehyde groups and imine bonds between OAl and chitosan and to neutralize the effect of acetic acid. After washing several times with distilled water, the membrane was allowed to dry on the same petri dish at room temperature. The homogenized mixture was then poured into the petri dishes.The films were air dried and stored for further characterization and applications.

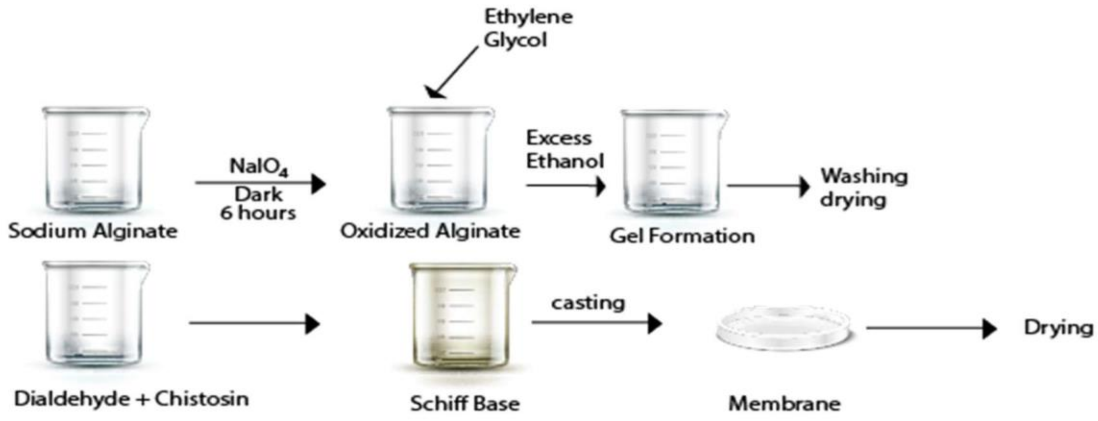


Figure 3. 3 Schematic diagram for film casting

3.3. Synthesis of ZnO nanoparticles

ZnO nanoparticles were synthesized from co-precipitation $ZnSO_4$ and NaOH in 1:1.From 1molar solution of each , solutions of different molarity were prepared and mixed together. ZnO nanoparticles precipitates out , which after filtration undergo drying.

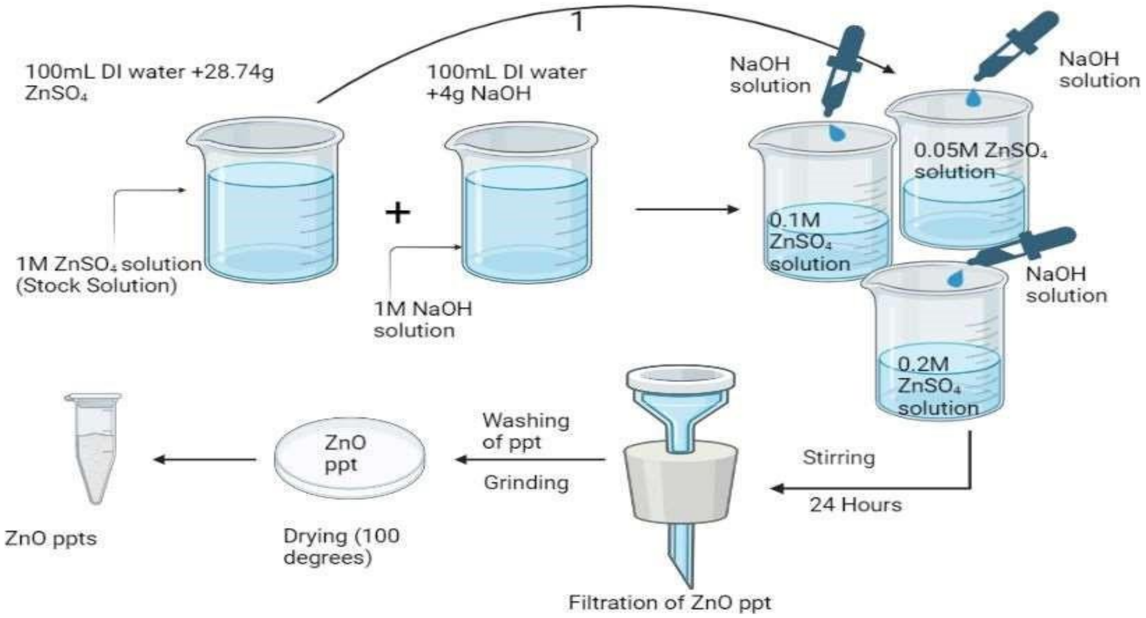


Figure 3. 4 Synthesis of ZnO nanoparticles

3.4. Synthesis of Cu nanoparticles

For the synthesis of Cu nanoparticles , films are dipped in $CuSO_4$ overnight to properly adsorb Cu^{2+} on the film. The film then treated with $NaBH_4$ to reduce Cu^{2+} . The schematic diagram is shown below.

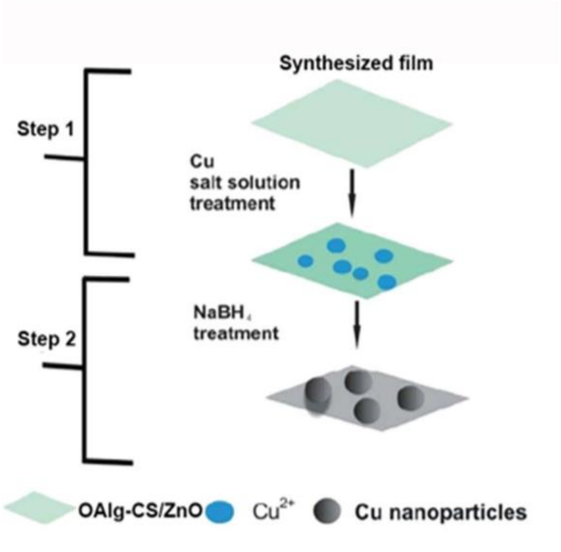


Figure 3. 5 Synthesis of Cu nanoparticles

CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, the characterization of starting material Sodium alginate, Oxidized Alginate (OAlg), and coupled products are explained in detail. The main characterization techniques employed are:

- FTIR
- SEM
- XRD

The synthesized materials were further tested for:

- Thermogravimetric Analysis
- Conductivity
- Mechanical Properties

Furthermore, the biomedical applications of these novel coupled products were studied in comparison to the starting materials (O-CMCh and MA-CMCh). The biomedical applications that were studied till now are:

- Anti-bacterial Activity
- Catalytic degradation of Dyes
- Catalytic Reduction of Paranitrophenol (4-NP)

4.1. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is used to attain information about functional groups present in substances. Organic compounds and some inorganic compounds absorb electromagnetic radiations of IR region 4000 cm-1 to 400 cm-1 (2.5μm to 25μm) and produce stretching and bending vibrations by the transition of electrons in the same electronic shell from a lower vibrational level to a higher vibrational level. The variable dipole moment of bonds is responsible for vibrations that appear in the form of IR spectra.[38]

4.2. FTIR of Alginate and Oxidized Alginate (OAlg)

FTIR spectra of alginate and oxidized alginate are illustrated in Figure 4.1. The comparison of the two spectra confirmed the fabrication of OAlg and the of the carbonyl group to the polymer structure. The absorption band 3416 cm^{-1} depicts the O-H stretching vibrations which are common to both Alg and OAlg. C-H stretching vibrations can be seen at 2925 cm^{-1} in the spectra of OAlg and Alg represents C-H stretch. The antisymmetric C=O stretch of the -COO group is clearly indicated at 1640 cm^{-1} and the band at 1070 cm^{-1} corresponds to the C-O stretching vibrations of the -C-O-C- group which is common to both spectra. The indicative band at1730cm^{-1} represents the formation of aldehyde group.

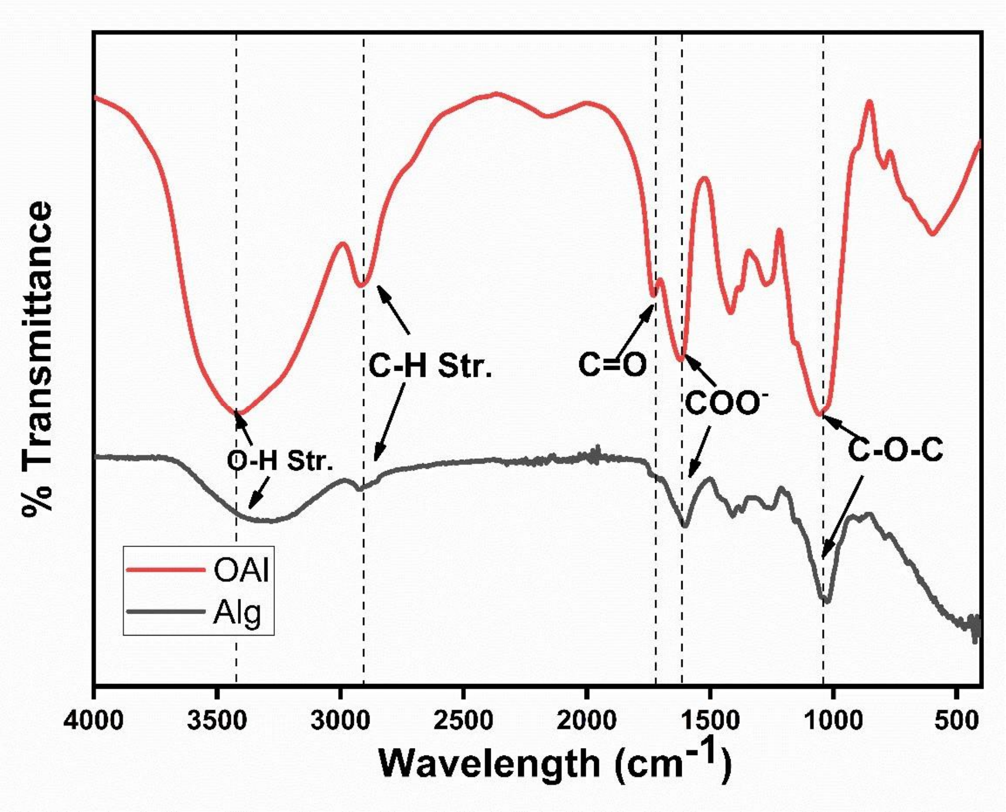


Figure 4. 1 Oxidation of Alginate

4.2.1. FTIR of Crosslinked Product

FTIR of cross-linked product was analyzed. The new peak at 1590 cm^{-1} was observed because of the reaction between chitosan and OAlg to make C=N to make Schiff base. The new peak appears 1310 cm^{-1} which represents C-N stretch.

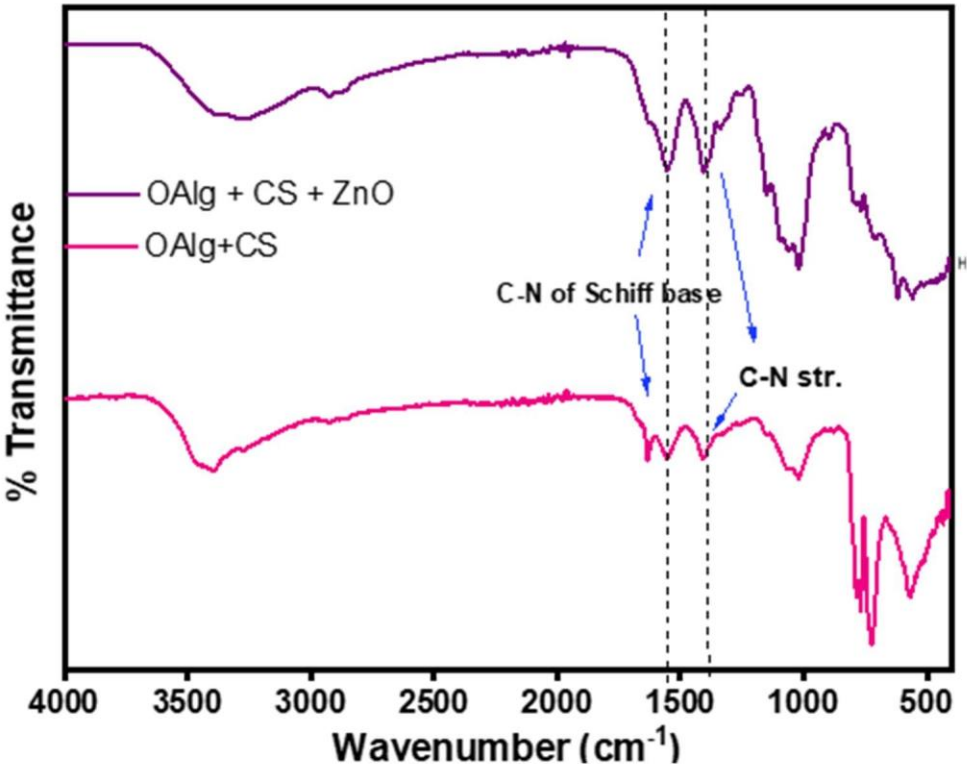


Figure 4. 2 Scigg base formation

4.3. XRD Analysis

XRD is an analytical technique which is versatile and non-destructive and used for the analysis of crystalline materials. This technique allows the study of structure of the material which includes crystallite size, d-spacing and arrangement of atoms. In XRD analysis, monochromatic x-rays interact with crystalline samples. X-ray beam is generated in a cathode ray tube by heating the filament. These x-rays are then passed through monochromator to get monochromatic x-rays which are then focused on the material to be analyzed. X-rays reflect after being targeted on the material and are detected by the detector which is then processed and converted into signals. XRD data can be used to determine the crystallite size by using the Scherer equation

The XRD analysis of the samples was done in the range of 5° to 80° using a diffractometer with Cu-Kα (K = 1.5406 A°) radiation at room temperature. The XRD spectra of OAlg-CS and its nanocomposites with different ratios of ZnO were analyzed. The cross-linked product showed little crystallinity which then disappears by the addition of inorganic fillers.

All the spectra clearly depict that the starting material and all the synthesized products are amorphous in nature showing peaks of 2θ at 20° – 25° and there is no crystallinity in the structure before and after coupling.

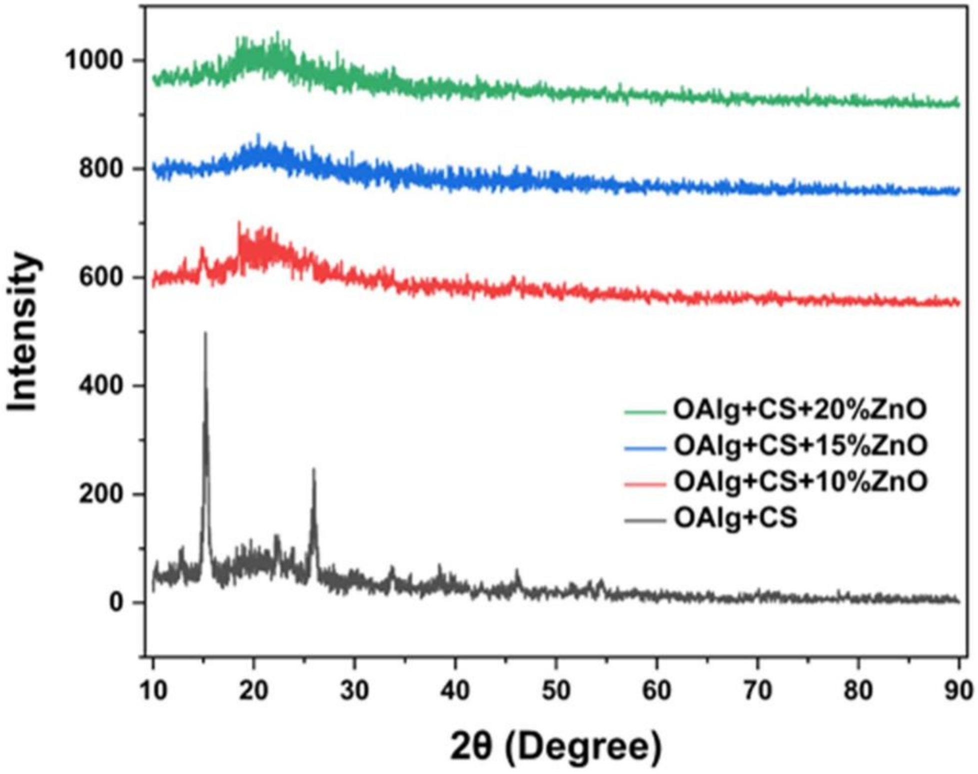


Figure 4. 3 XRD of synthesized Polymer nanocomposite

4.3.1. XRD Analysis OF Fabricated Cu nanoparticles

Graph (a, b, c, d) displays the XRD analysis of copper nanoparticles embedded films of OAlg-CS, OAlg-CS/ZnO-2, OAlg-CS/ZnO-15 , OAlg-CS/ZnO-10 , OAlg-CS respectively. All spectra shows additional peaks at 2θ 41.4° (111), that are the distinctive diffraction peaks belongs to copper nanoparticles.

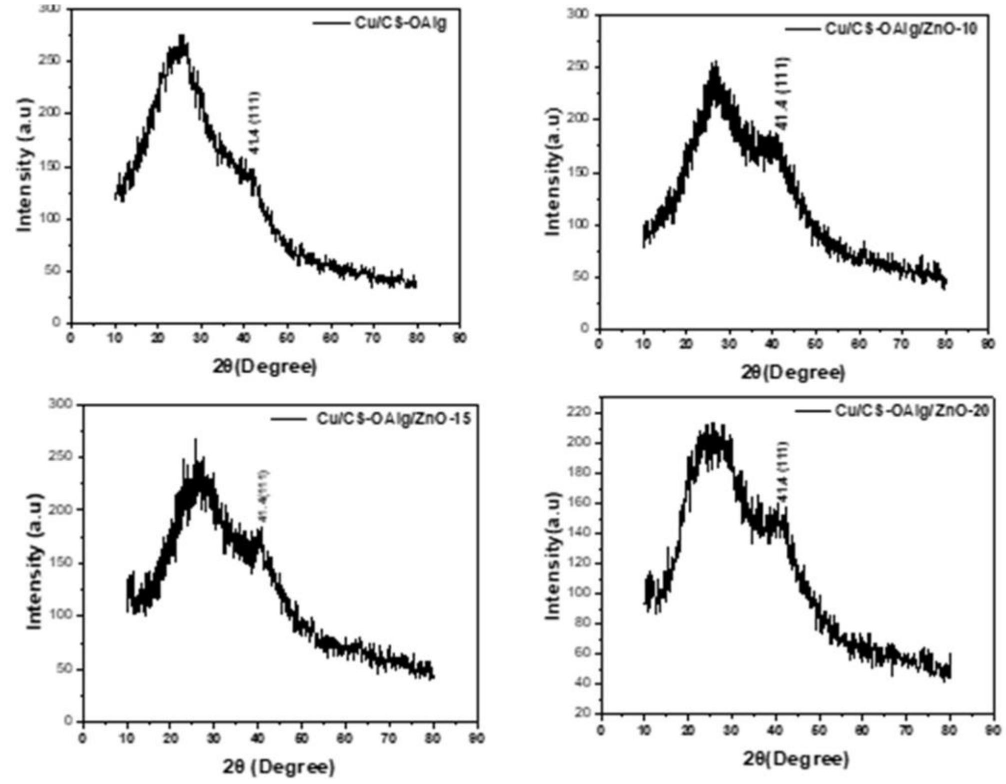


Figure 4. 4 XRD of Synthesized Cu nanoparticles

4.3.2. XRD Analysis OF ZnO nanoparticles

All the detectable peaks could be indexed as the ZnO wurtzite structure found in the standard reference data (JCPDS: 36–1451). The main peaks are 31.7 , 34.5 , 36.2, 47.4 , 56.5 , 62.8 which according to literature confirms the formation of ZnO nanoparticles.

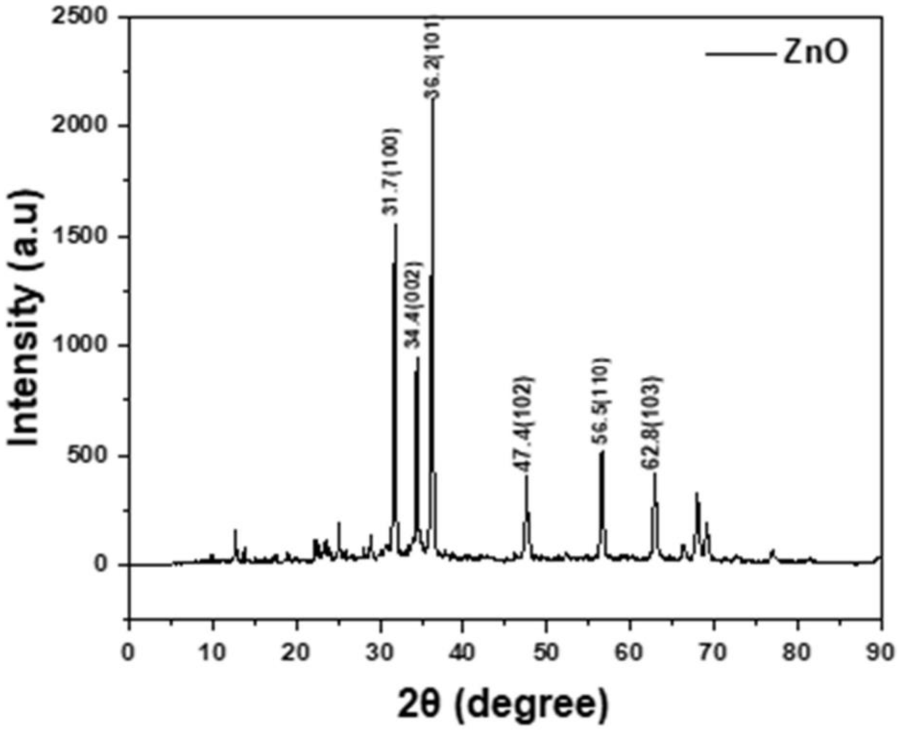


Figure 4. 5XRD of ZnO nanoparticles

4.4. SEM and EDS Analysis

The morphologies of the prepared materials were determined by using SEM (Nova-Nano) at different resolutions with 10-50kV working voltage. The morphological analysis of all synthesized products after the formation of copper nanoparticles were analyzed.

4.4.1. SEM And EDS Analysis of Cu/OAlg-CS

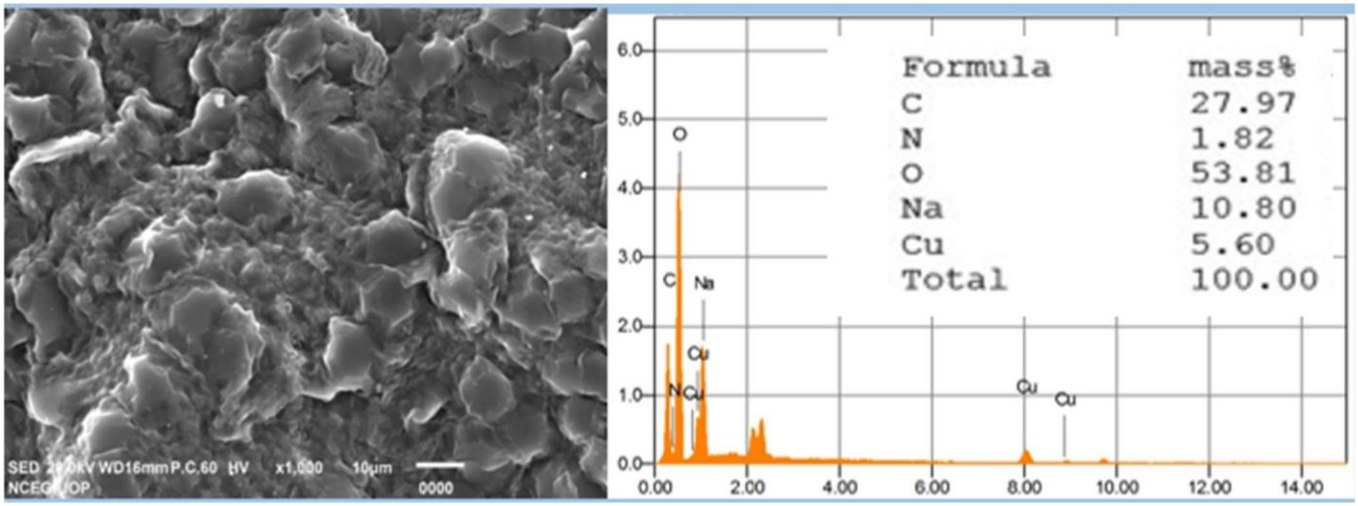


Figure 4. 6 SEM and EDS Analysis of Cu/OAlg-CS

The white particles on the surface represents Cu nanoparticles and the EDS also confirms that the mass percent of Cu nanoparticles is 5.60

4.4.2. SEM And EDS Analysis of Cu/OAlg-CS/ZnO-10

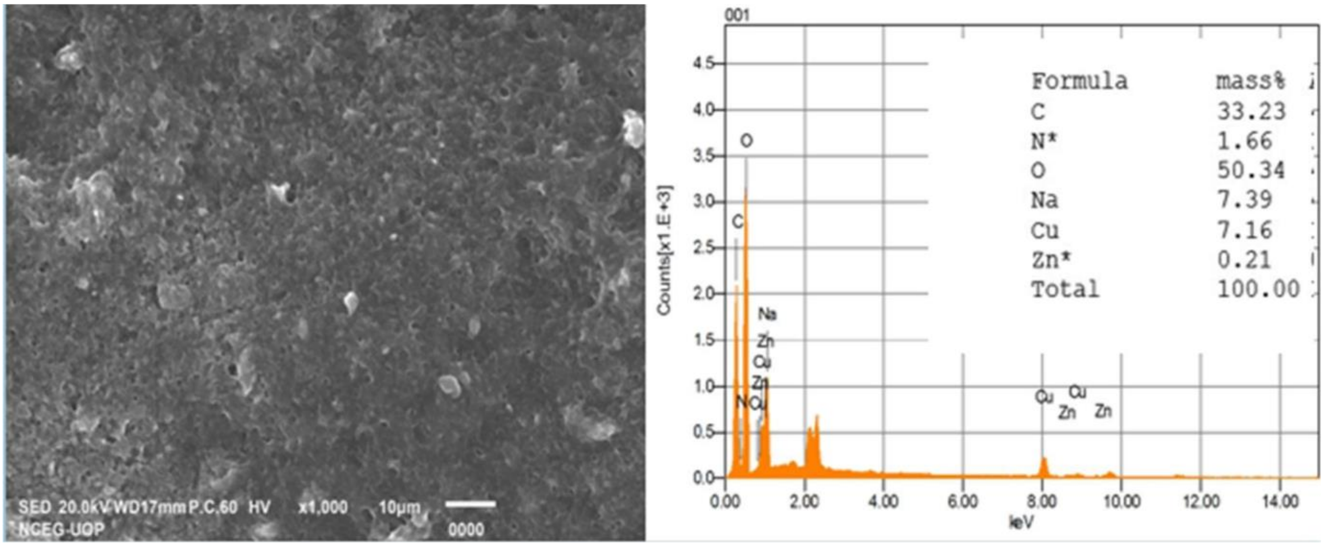
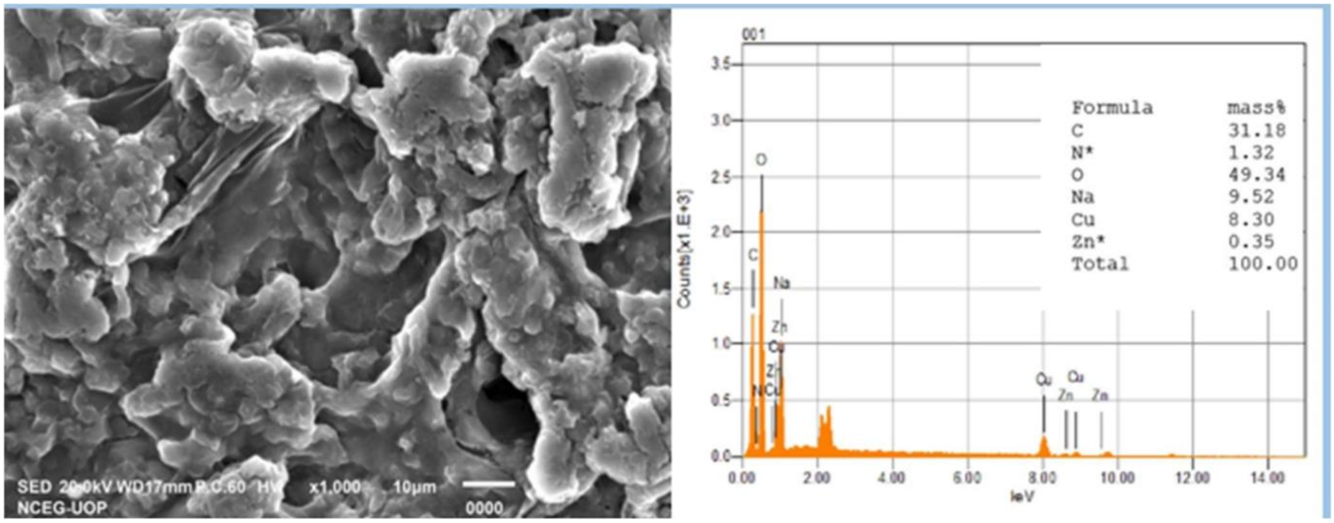


Figure 4. 7 SEM and EDS Analysis of Cu/OAlg/ZnO-10

The white particles on the surface represents Cu nanoparticles and the EDS also confirms that the mass percent of Cu nanoparticles is 7.16.

4.4.3. SEM And EDS Analysis of Cu/OAlg-CS/ZnO-15

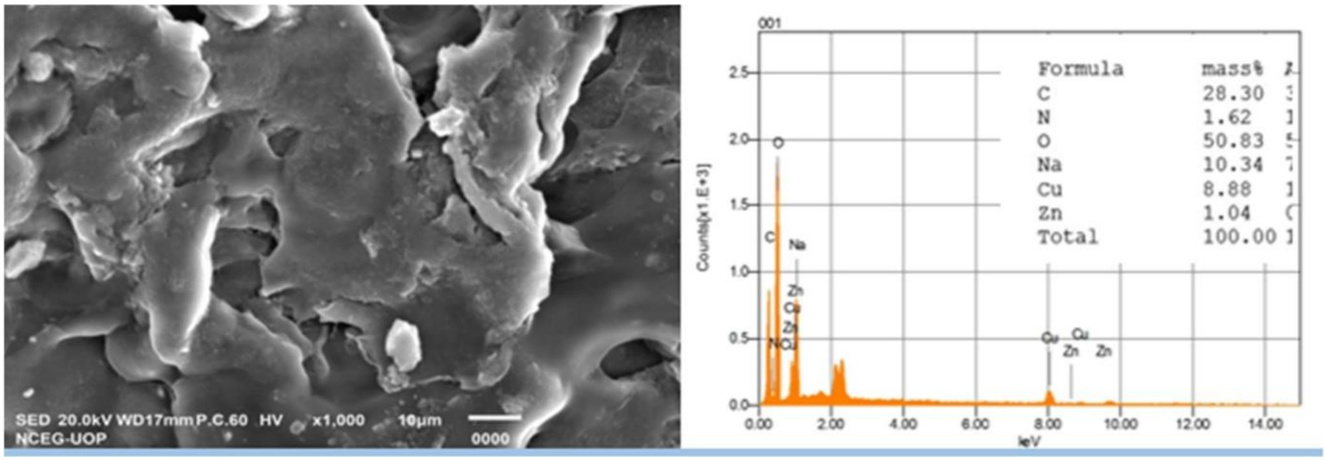


Formula	mass%
C	31.18
N*	1.32
O	49.34
Na	9.52
Cu	8.30
Zn*	0.35
Total	100.00

Figure 4. 8 SEM and Analysis of Cu/OAlg-CS/ZnO-15

The white particles on the surface represents Cu nanoparticles and the EDS also confirms that the mass percent of Cu nanoparticles is 8.30.

4.4.4. SEM And EDS Analysis of Cu/OAlg-CS/ZnO-20



Formula	mass%
C	28.30
N	1.62
O	50.83
Na	10.34
Cu	8.88
Zn	1.04
Total	100.00

Figure 4. 9 SEM and EDS Cu/OAlg-CS/ZnO-20

The white particles on the surface represents Cu nanoparticles and the EDS also confirms that the mass percent of Cu nanoparticles is 8.88%.

It is clearly evident from the SEM analysis that the prepared films are highly porous and the mass percent of Cu nanoparticles increases increases as we increase the concentration of ZnO nanoparticles. Greater the concentration of ZnO nanoparticles greater the adsorption of Cu which ultimately increases the efficiencyof the film. That’s why Cu/OAlg-CS/ZnO-20 gave the best results.

4.4.5. SEM Analysis of ZnO nanoparticles

The morphologies of the prepared materials were determined by using SEM (Nova-Nano) at different resolutions with 20kV working voltage. The morphological analysis of synthesized ZnO nanoparticles were analyzed which showed spherical or slightly elongated shape of ZnO

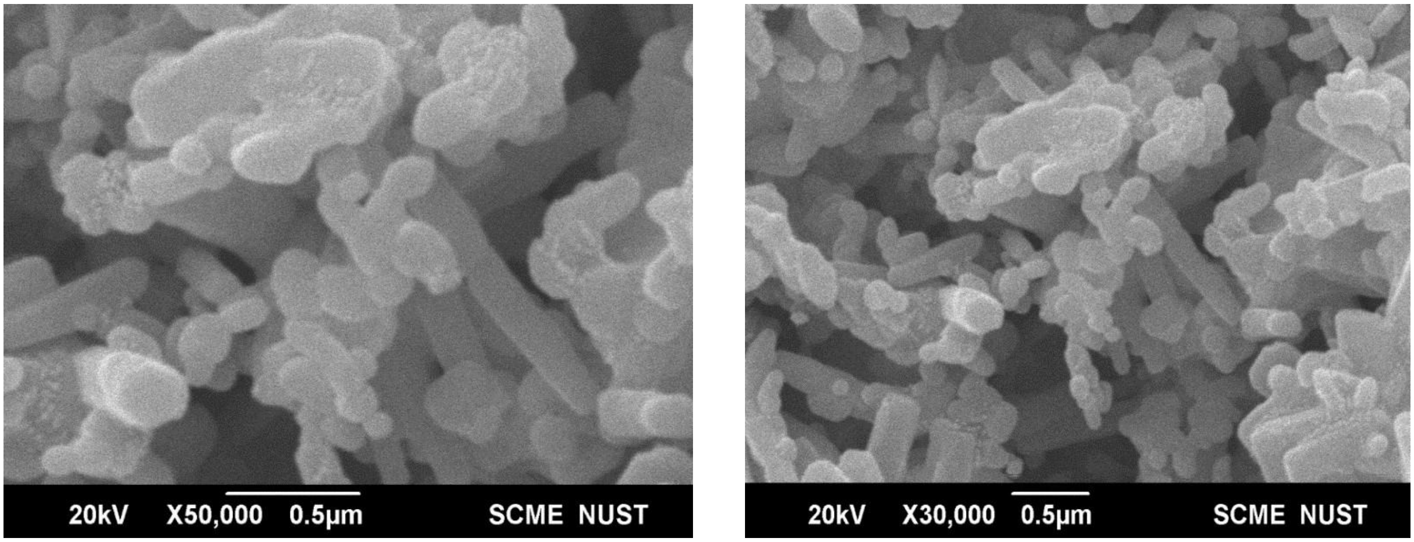


Figure 4. 10 SEM Analysis of ZnO nanoparticles

4.5. Thermogravimetric Analysis:

4.5.1. Sodium Alginate

TGA analysis of Sodium Alginate indicates that 10 % of total mass loss takes place between 80-120 ºC and at 250 ºC 39.462 % mass loss takes place due to the glycosidic bond breakage that further leads to more 12.53 % loss to final degradation and almost 38 % residue mass left. The

DTA graph shows 3 peaks. The first is below the 100 ºC indicates water loss and the second between 200-250 ºC, the major peak indicates the glycosidic bond breakage and the third is broader than the other two, indicating final degradation.

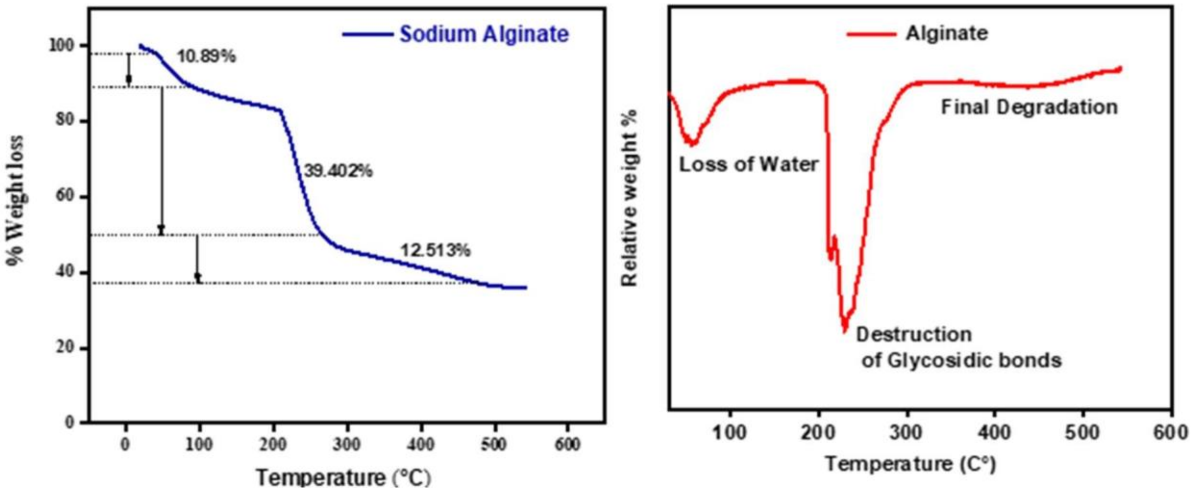


Figure 4. 11 TGA and DSC curve of Alginate

4.5.2. Oxidized Alginate-Chitosan

The modified product of alginate when reacted with chitosan showed better stability than the pure sodium alginate with the residual mass of 39%.And the breakdown starts at 282 ºC which way more stable than sodium alginate.

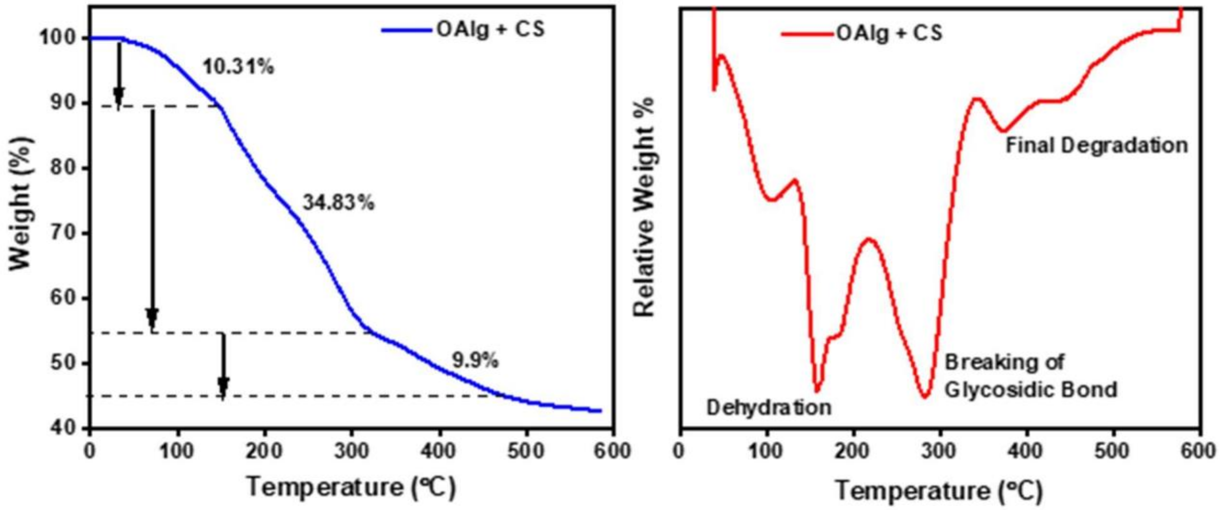


Figure 4. 12 TGA and DSC curve of OAlg-CS

4.4.3 Polymer nanocomposite with different percentage of ZnO

The TGA and DSC curves of all the polymer nanocomposite are compared with the starting material and the all the synthesized products are thermally more stable than the parent polymer. This detailed thermal analysis can be helpful for further studies and applications.

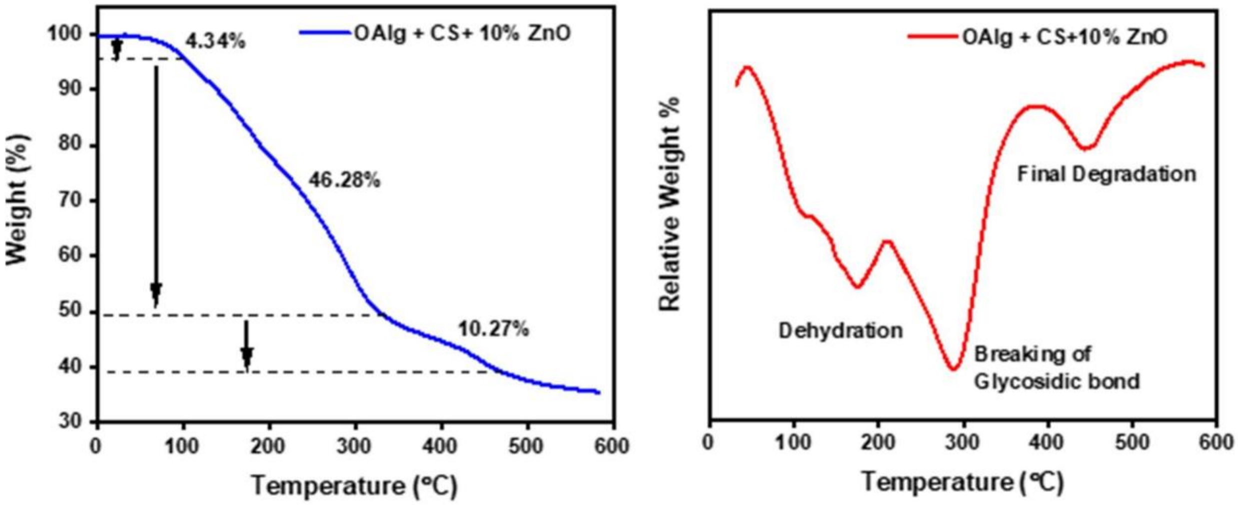


Figure 4. 13 TGA and DSC curve of OAlg-CS/ZnO-10

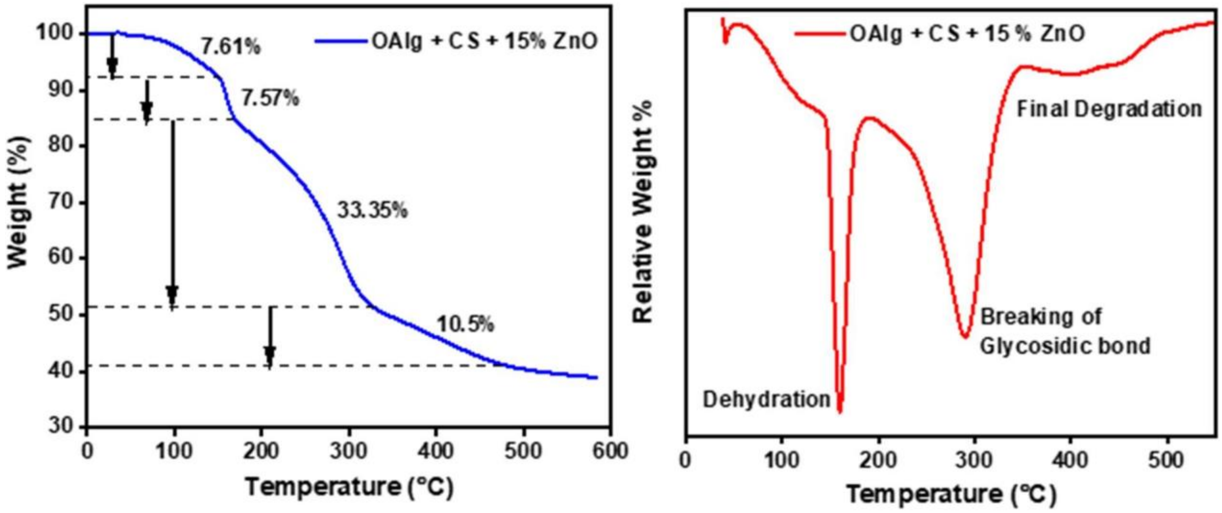


Figure 4. 14 TGA and DSC curve of OAlg-CS/ZnO-15

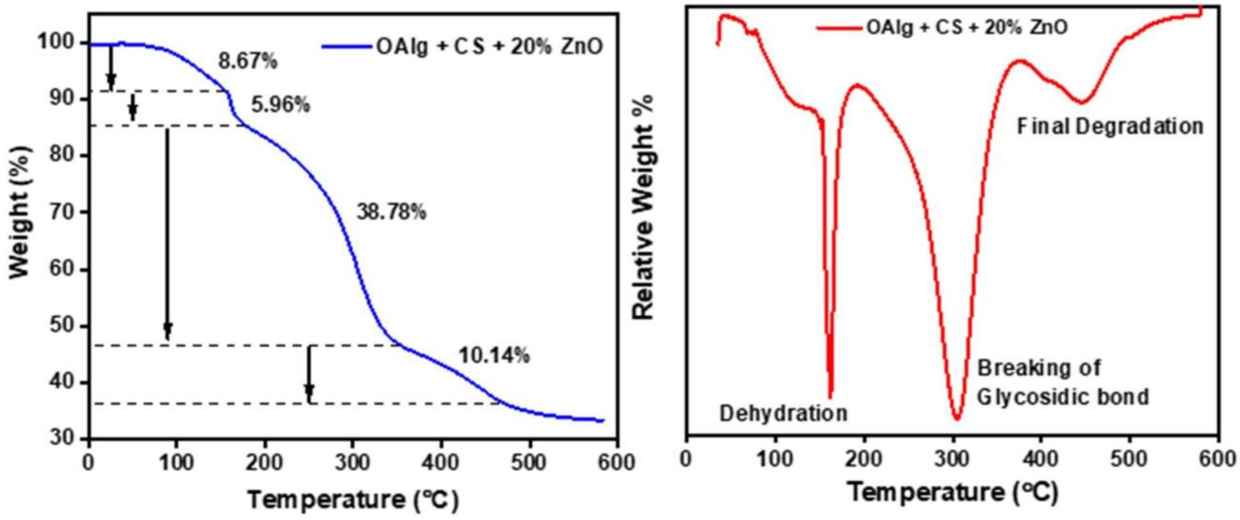
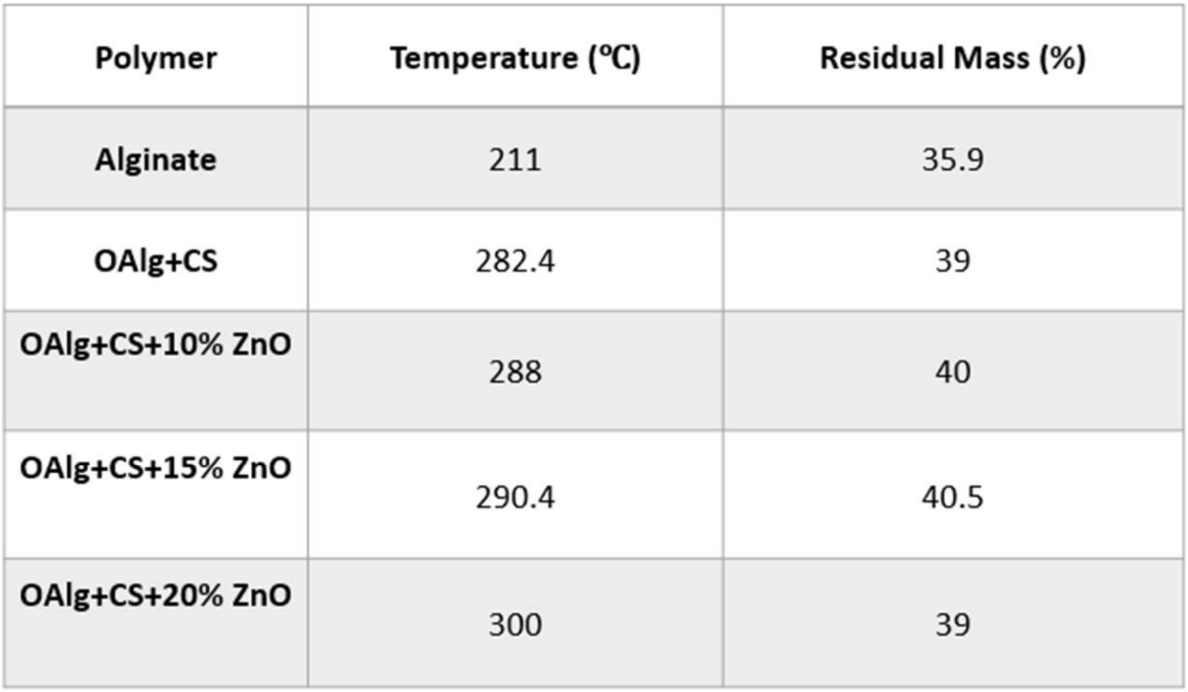


Figure 4. 15 TGA and DSC curve of OAlg-CS/ZnO-20



Polymer	Temperature (℃)	Residual Mass (%)
Alginate	211	35.9
OAlg+CS	282.4	39
OAlg+CS+10% ZnO	288	40
OAlg+CS+15% ZnO	290.4	40.5
OAlg+CS+20% ZnO	300	39

Table 4.1 TGA data of all polymer nanocomposites

4.6. Contact Angle

Contact angle tells us about the hydrophilicity and hydrophobicity of the prepared films. If the angle is less than 90 ° then the films are hydrophilic and if the angle is more than 90 ° then the films are hydrophobic. Table below showed that all the synthesized films have angle less than 90° which confirms the surface hydrophilicity of the films.

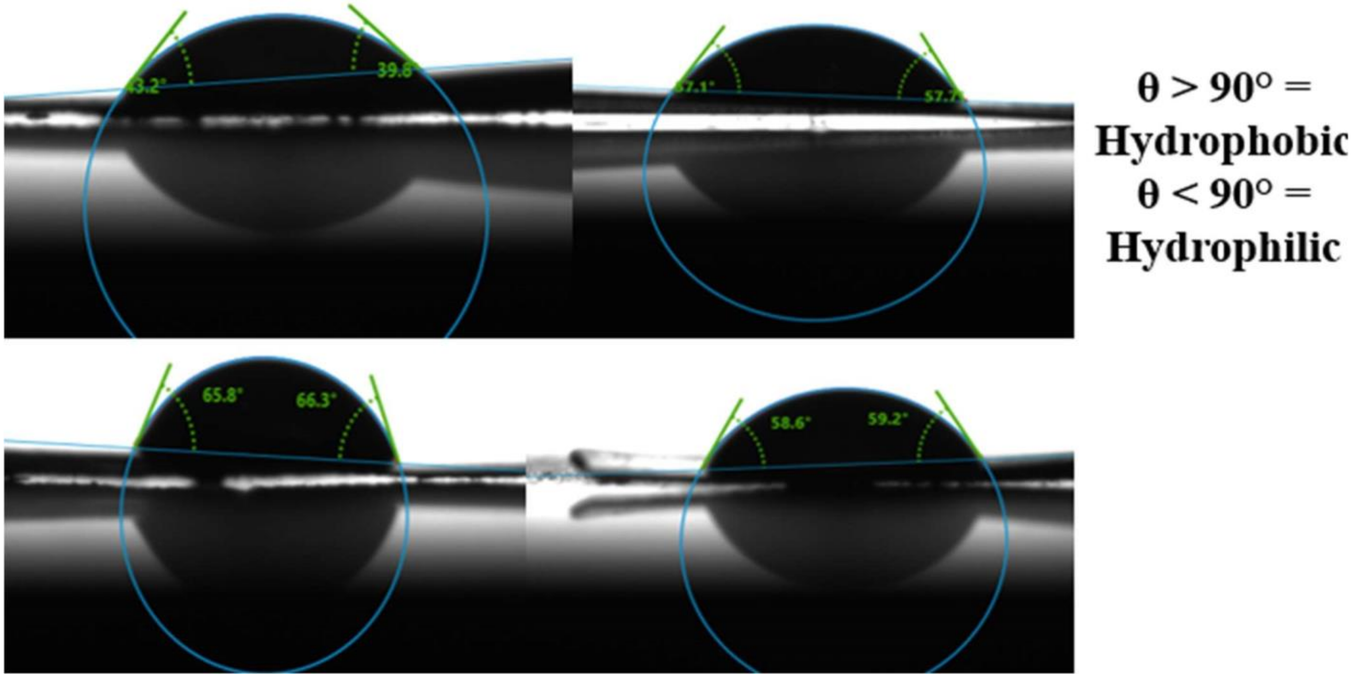
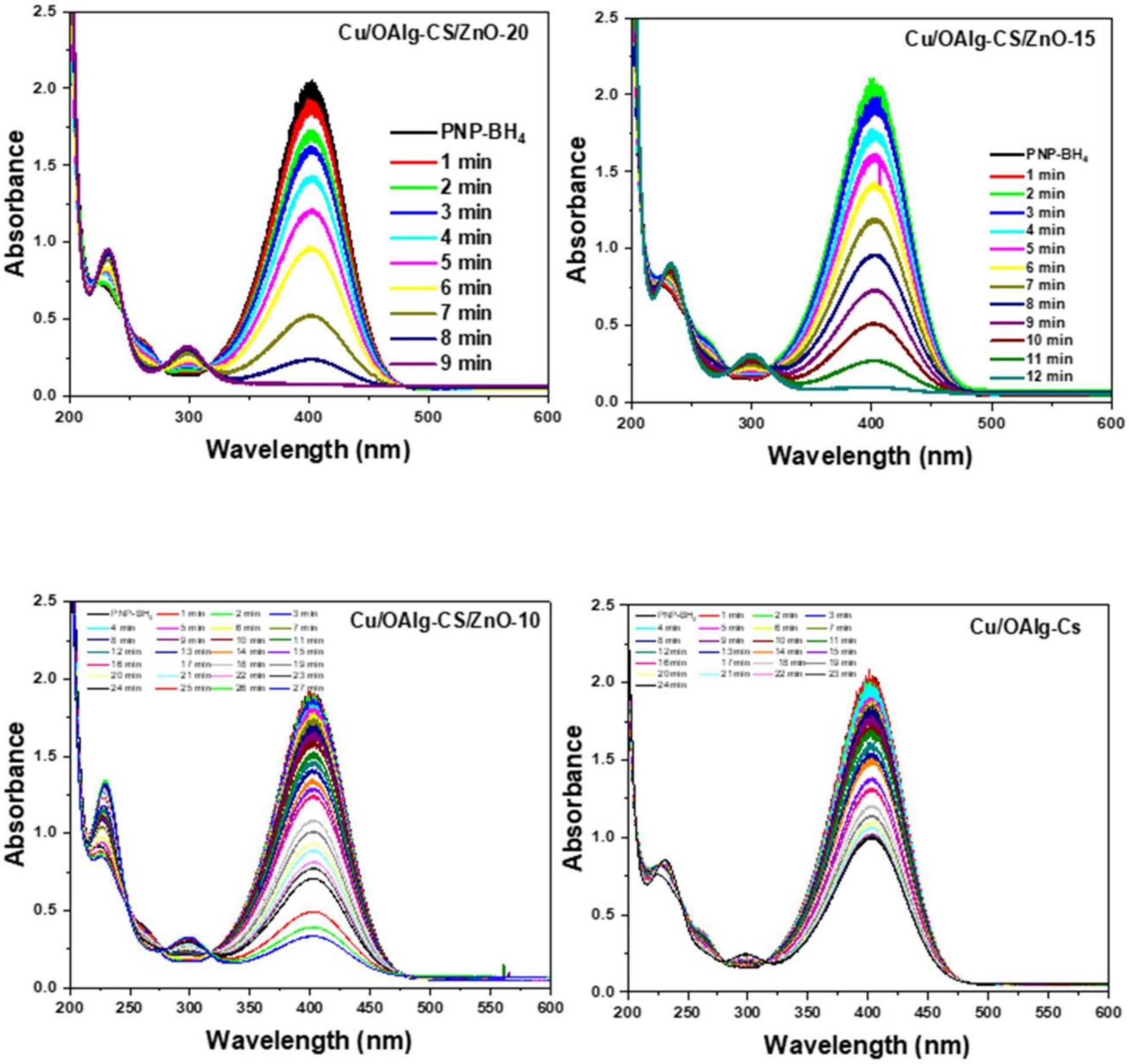


Figure 4. 16 Contact angle of all synthesized films

4.7. Reduction Analysis of 4-Nitrophenol

First UV cuvette containing 2.5ml of aqueous solution of 4NP and 0.5ml of NaBH4 and its spectra was recorded after every one minute till half an hour. shows that no change in absorbance intensity at 400nm was observed. Which shows the poor catalytic performance of only $NaBH_4$ towards the reduction of 4NP. After that under similar conditions Cu^0 loaded strip was introduced to the cuvette containing reaction mixture. Change in color of 4NP was observed, it starts disappearing as soon as the Cu^0 strip was introduced. Shows that absorbance intensity of peak at 400nm progressively decreases and completely disappeared after 9mints, show excellent catalytic reduction of 4NP by Cu^0 loaded alginate film.



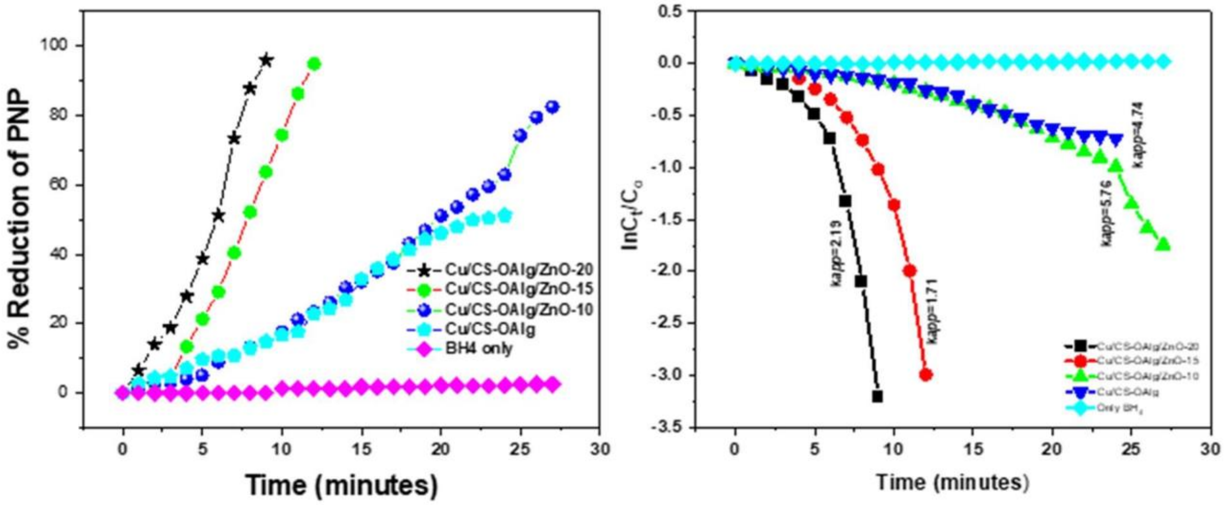


Figure 4. 17 Reduction Analysis of 4-NP

Above mentioned graphs shows the catalytic activity of four different polymer nanocomposites for the reduction of 4-NP.

Figure A shows the catalytic activity of 20-ZnO/OAlg-CS for the reduction of 4-NP.

Figure B shows the catalytic activity of 15-ZnO/OAlg-CS for the reduction of 4-NP.

Figure C shows the catalytic activity of 10-ZnO/OAlg-CS for the reduction of 4-NP.

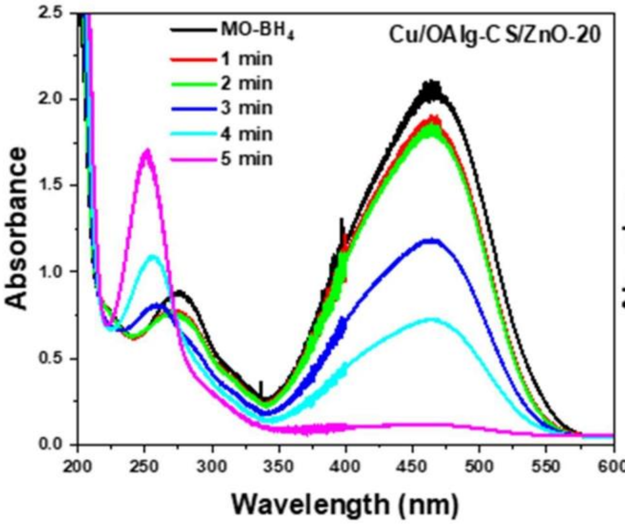
Figure D shows the catalytic activity of ZnO/OAlg-CS for the reduction of 4-NP.

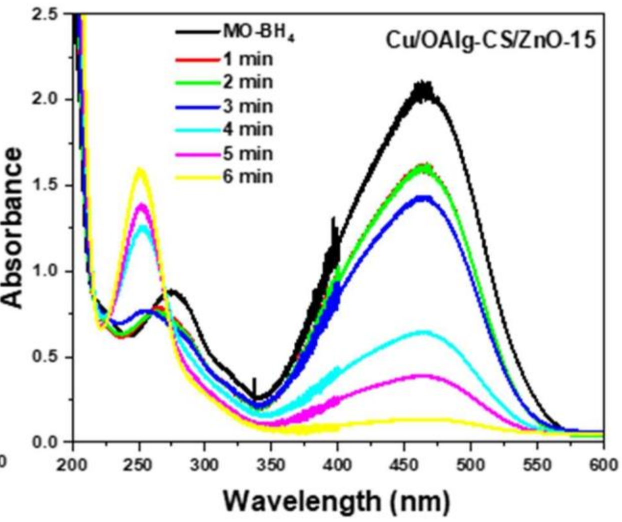
Figure E shows the comparison by comparing their catalytic activity and calculating percentage reduction.

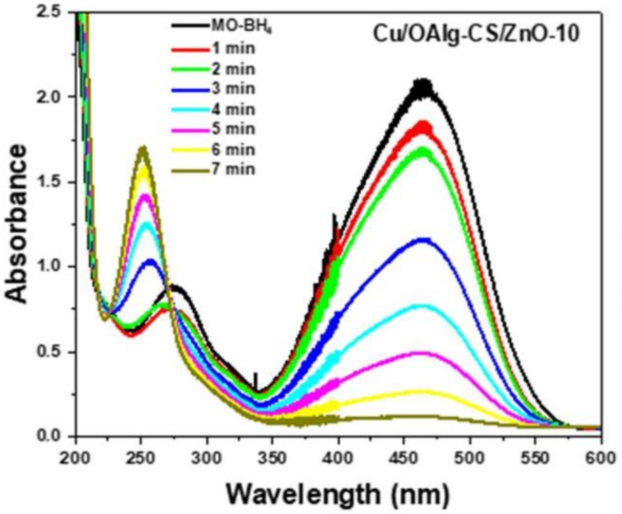
Figure F shows that reduction of 4-NP follows pseudo-first order.

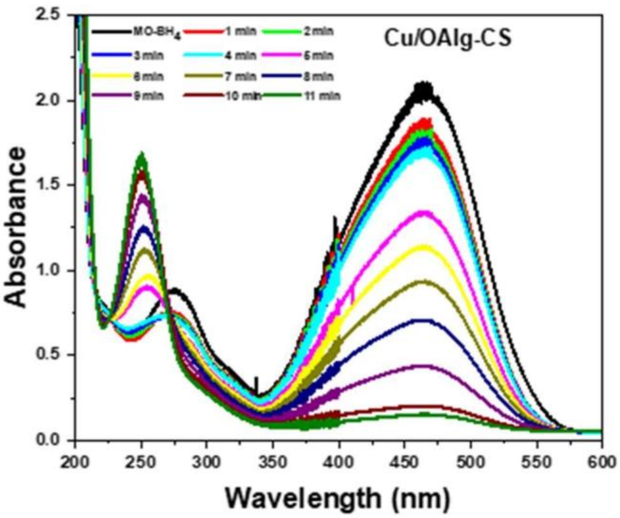
4.8. Degradation Analysis of Methyl Orange

Degradation studies were also carried out on methyl orange with all the prepared films under similar conditions. The maximum and characteristic absorbance intensity is at 450nm.


Cu/OAlg-CS/ZnO-20
MO-BH$_4$
1 min
2 min
3 min
4 min
5 min
Absorbance
Wavelength (nm)
0.0
0.5
1.0
1.5
2.0
2.5
200
250
300
350
400
450
500
550
600


Cu/OAlg-CS/ZnO-15
MO-BH$_4$
1 min
2 min
3 min
4 min
5 min
6 min
Absorbance
Wavelength (nm)
0.0
0.5
1.0
1.5
2.0
2.5
200
250
300
350
400
450
500
550
600


Cu/OAlg-CS/ZnO-10
MO-BH$_4$
1 min
2 min
3 min
4 min
5 min
6 min
7 min
Absorbance
Wavelength (nm)
0.0
0.5
1.0
1.5
2.0
2.5
200
250
300
350
400
450
500
550
600


Cu/OAlg-CS
MO-BH$_4$
1 min
2 min
3 min
4 min
5 min
6 min
7 min
8 min
9 min
10 min
11 min
Absorbance
Wavelength (nm)
0.0
0.5
1.0
1.5
2.0
2.5
200
250
300
350
400
450
500
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600

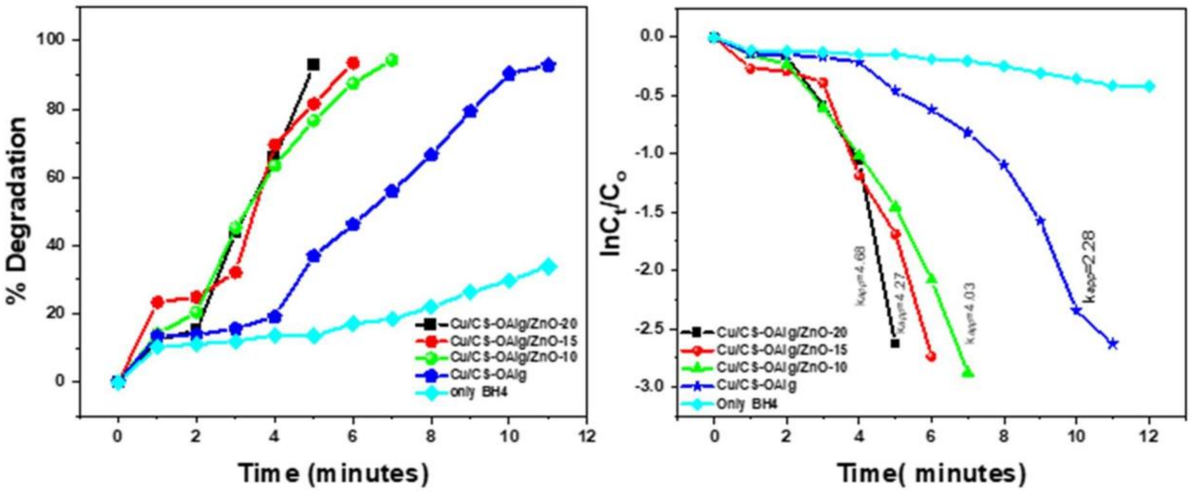
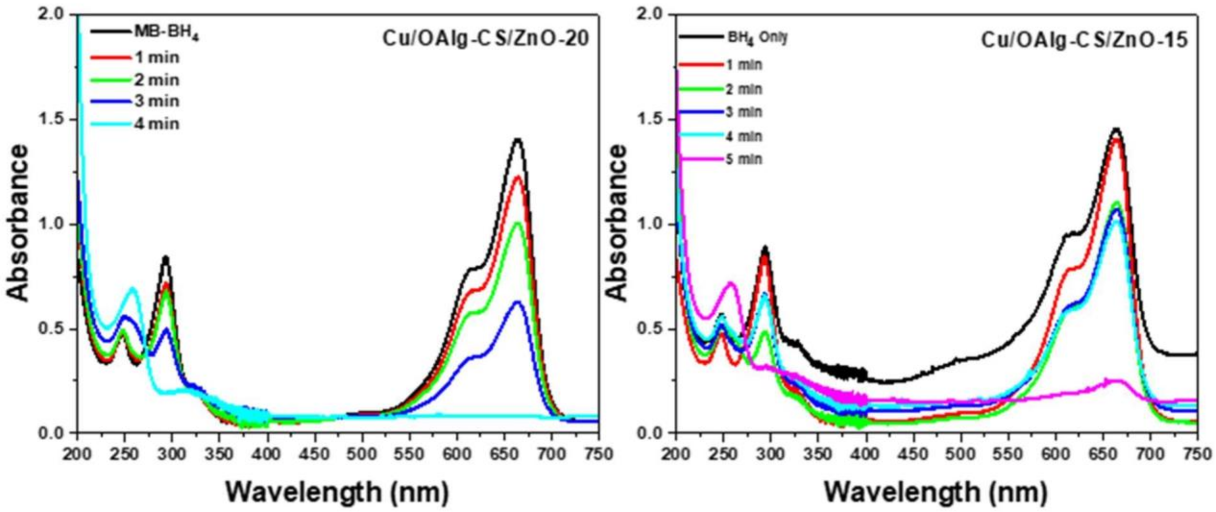


Figure 4. 18 Degradation Analysis of Methyl Orange

4.9. Degradation of Methylene Blue

Degradation studies were also carried out on methylene blue with all the prepared films under similar conditions. The maximum and characteristic absorbance intensity is at 664nm. Degradation of MB with bare and Cu loaded film is shown in figure. Following graphs show degradation of methylene blue in the presence of different polymer nanocopomosite.

All the polymer nanocomposite showed exceptional results but the onw with 20% of ZnO nanoparticles showed results in the less amount of time.



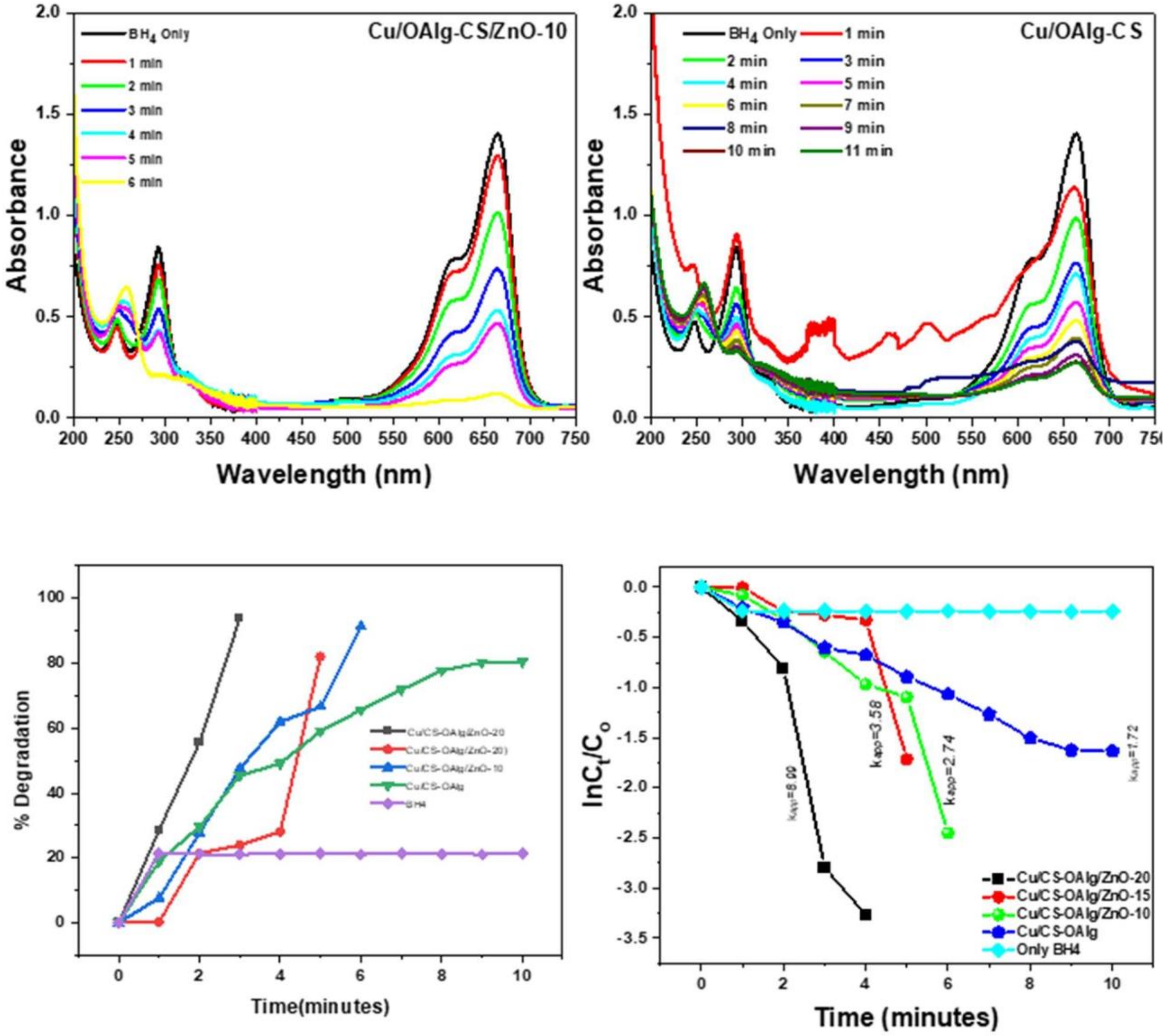


Figure 4. 19 Degradation Analysis of Methylene Blue

4.10. Antibacterial Activity

Recently synthesized polymers were found to have additional anti-bacterial capabilities. Zones of inhibition for two bacterial strains were found. The bacteria employed were both gram-positive (S. aureus) and gram-negative (E. coli). The experiment started with the preparation of nutrient agar plates in Petri dishes using sterilized agar media. A suitable growing medium for bacteria was made by letting the agar solidify. A sterile inoculating loop or brush was then used to apply the bacterial culture to the agar surface. The culture was evenly distributed throughout the agar surface with care. Little pressure was used to make contact with the agar. Then, for a further 24 hours, the agar plates were maintained at a proper temperature, typically 37°C (98.6°F). This allowed the antibiotics to function and the bacteria to multiply.

In this study, two different antibiotics—gentamycin for E. coli and erythromycin for S. aureus—were used. The antibiotic zone's surrounding bacterial inhibition zones were contrasted with the control. The films were tried on two different strain of two bacteria one was less resistant and the other one was more resistant. The zones of inhibition against less resistant were so good that they merged into one another. The anti-bacterial activity againt more resistant strain was also not bad. The graph given below shows zone of inhibition against more resistant strain. The zone of inhibition against less resistant strain cannot be measured because zones were so big and it gets merged.

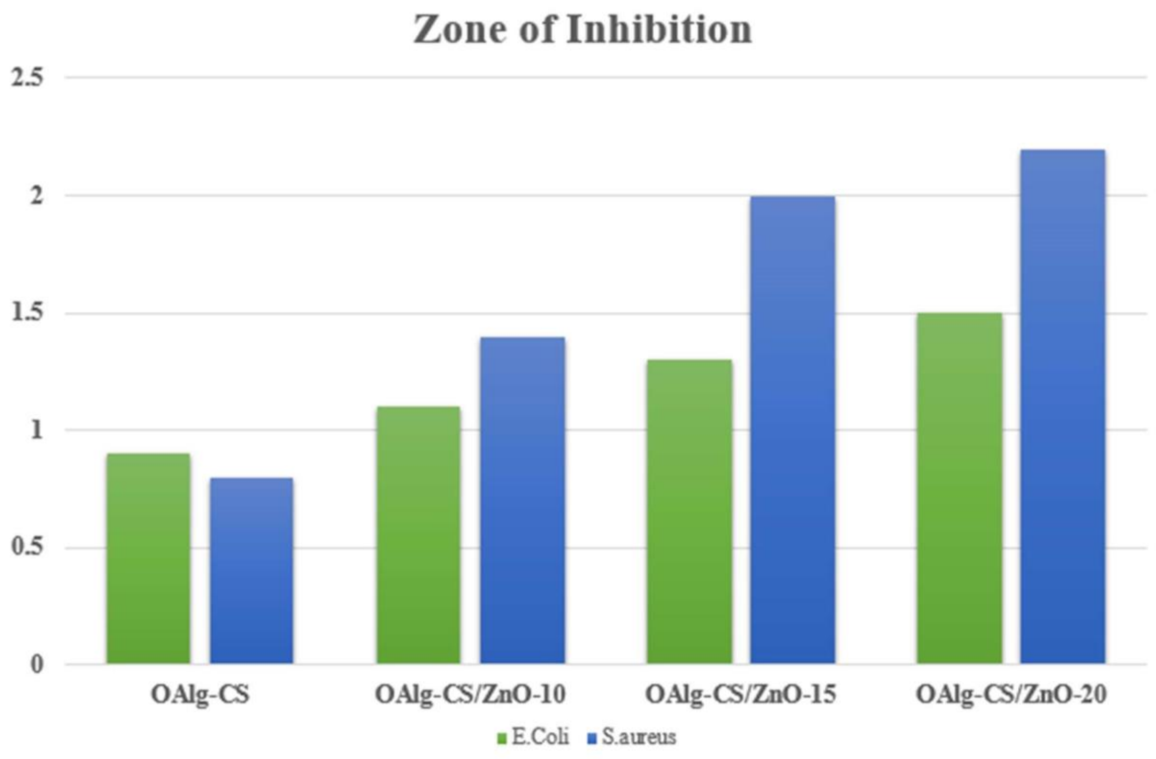


Figure 4. 20 Antibacterial activity of all synthesized films

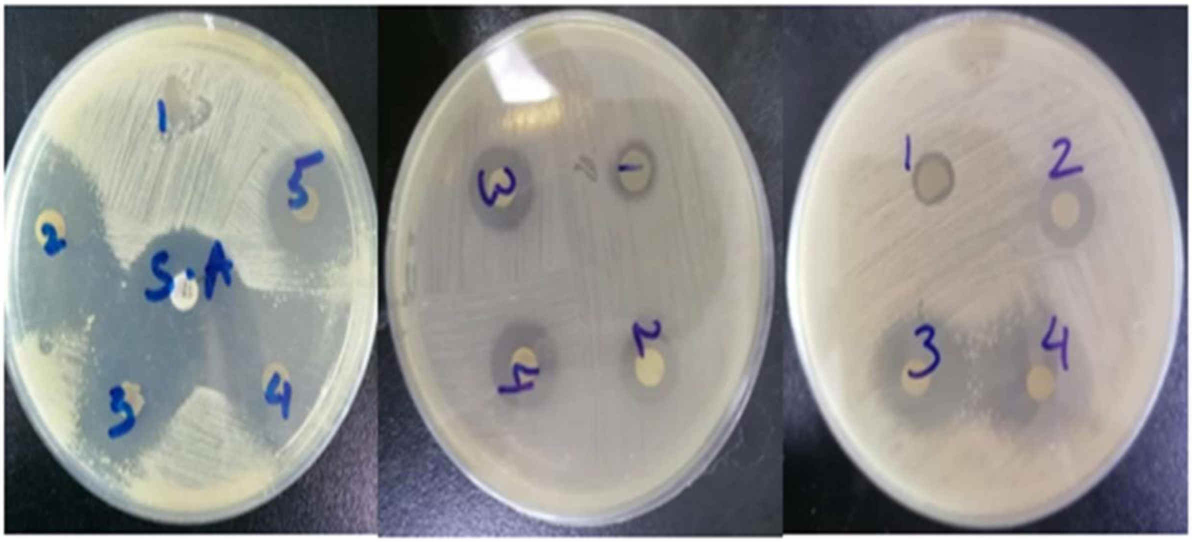


Figure 4. 21Antibacterial Activity

Conclusion

- Here oxidized alginate and chitosan modified ZnO polymer nanocomposite were successfully synthesized. OAlg-CS, OAlg-CS/ZnO-10, OAlg-CS/ZnO-20.
- OAlg-CS and polymer nanocomposites were employed for uptake of copper ions from aqueous solution.
- Films of OAlg-CS and its polymer nanocomposite was successfully prepared and used as supporting site for the production of zero valent copper nanoparticles.
- Chemical reduction method was used for the synthesis of Zero valent copper nanoparticles.
- OAlg-CS, OAlg-CS/ZnO-10, OAlg-CS/ZnO-20. showed excellent degradation efficiency in the presence of Cu^0 nanoparticles

REFERENCES

1. Younger, M., et al., The built environment, climate change, and health: opportunities for co-benefits. 2008. 35(5): p. 517-526.

2. Batchamen Mougnol, J.B., et al., Photodegradation of Polycyclic Aromatic Hydrocarbons from Coal Tar into Mine Wastewaters and Sewage Wastewater on a Flat-Bed Photoreactor. 2022. 2(3): p. 333-346.

3. Jeyaratnam, J.J.B.j.o.i.m., Health problems of pesticide usage in the Third World. 1985.
42(8): p. 505.

4. Varavipour, M., T. Asadi, and M.J.R.O.C. Mashal, Application of two layers of soil with different textures for decreasing pollution from landfills to the subterranean water table. 2011.
12(3): p. 833-838.

5. Anju, A., et al., Water pollution with special reference to pesticide contamination in India. 2010. 2010.

6. He, G., et al., The effect of air pollution on mortality in China: Evidence from the 2008 Beijing Olympic Games. 2016. 79: p. 18-39.

7. Vunain, E., A. Mishra, and B.J.I.j.o.b.m. Mamba, Dendrimers, mesoporous silicas and chitosan-based nanosorbents for the removal of heavy-metal ions: A review. 2016. 86: p. 570586.

8. Babel, S. and T.A.J.J.o.h.m. Kurniawan, Low-cost adsorbents for heavy metals uptake from contaminated water: a review. 2003. 97(1-3): p. 219-243.

9. Razali, M., et al., Sustainable wastewater treatment and recycling in membrane manufacturing. 2015. 17(12): p. 5196-5205.

10. Teh, C.Y., et al., Recent advancement of coagulation–flocculation and its application in wastewater treatment. 2016. 55(16): p. 4363-4389.

11. Pal, S., et al., Modified guar gum/SiO 2: development and application of a novel hybrid nanocomposite as a flocculant for the treatment of wastewater. 2015. 1(1): p. 84-95.

12. Chen, Q., et al., Precipitation of heavy metals from wastewater using simulated flue gas: sequent additions of fly ash, lime and carbon dioxide. 2009. 43(10): p. 2605-2614.

13. Labana, S., et al., Pot and field studies on bioremediation of p-nitrophenol contaminated soil using Arthrobacter protophormiae RKJ100. 2005. 39(9): p. 3330-3337.

14. Chang, Y.-C. and D.-H.J.J.o.h.m. Chen, Catalytic reduction of 4-nitrophenol by magnetically recoverable Au nanocatalyst. 2009. 165(1-3): p. 664-669.

15. Wang, J. and H.J.M. Gu, Novel metal nanomaterials and their catalytic applications. 2015. 20(9): p. 17070-17092.

16. Yang, J., et al., Nickel nanoparticle–chitosan-reduced graphene oxide-modified screenprinted electrodes for enzyme-free glucose sensing in portable microfluidic devices. 2013. 47: p.
530-538.

17. Gao, C., et al., Preparation and controlled degradation of oxidized sodium alginate hydrogel. 2009. 94(9): p. 1405-1410.

18. Kong, X., et al., Applications of oxidized alginate in regenerative medicine. 2021. 9(12): p. 2785-2801.

19. Sánchez-Morán, H., et al., Oxime cross-linked alginate hydrogels with tunable stress relaxation. 2019. 20(12): p. 4419-4429.

20. Yang, C., et al., Modification of collagen-chitosan membrane by oxidation sodium alginate and in vivo/in vitro evaluation for wound dressing application. 2019. 24(7): p. 619-629.

21. Yu, G., et al., Preparation and Properties of Self-Cross-Linking Hydrogels Based on Chitosan Derivatives and Oxidized Sodium Alginate. 2023.

22. Gao, D., et al., The modification of collagen with biosustainable POSS graft oxidized sodium alginate composite. 2022. 200: p. 557-565.

23. Ponzio, F., et al., Robust alginate-catechol@ polydopamine free-standing membranes obtained from the water/air interface. 2017. 33(9): p. 2420-2426.

24. Lu, L., et al., Study on partially oxidized sodium alginate with potassium permanganate as the oxidant. 2009. 113(6): p. 3585-3589.

25. Li, X., et al., Preparation of low molecular weight alginate by hydrogen peroxide depolymerization for tissue engineering. 2010. 79(3): p. 660-664.

26. Wang, W., et al., Immobilization of sodium alginate sulfates on polysulfone ultrafiltration membranes for selective adsorption of low-density lipoprotein. 2014. 10(1): p. 234-243.

27. Uysal, E., et al., Nd Recovery from Wastewater with Magnetic Calcium Alginate ((1, 4)β-d-Mannuronic Acid and α-L-Guluronic Acid) Hydrogels. 2023.

28. Zhao, J., et al., Low-viscosity sodium alginate combined with TiO2 nanoparticles for improving neuroblastoma treatment. 2021. 167: p. 921-933.

29. Xiang, S., et al., Green synthesis of an alginate-coated silver nanoparticle shows high antifungal activity by enhancing its cell membrane penetrating ability. 2019. 2(9): p. 4087-4096.

30. Dodero, A., et al., Alginate-based electrospun membranes containing ZnO nanoparticles as potential wound healing patches: biological, mechanical, and physicochemical characterization. 2019. 12(3): p. 3371-3381.

31. Li, P., J. Mei, and J.J.L. Xie, Chitosan-sodium alginate bioactive coatings containing εpolylysine combined with high CO2 modified atmosphere packaging inhibit myofibril oxidation and degradation of farmed pufferfish (Takifugu obscurus) during cold storage. 2021. 140: p. 110652.

32. Wu, Y., et al., Preparation and adsorption behaviors of sodium alginate-based adsorbent-immobilized β-cyclodextrin and graphene oxide. 2017. 7(50): p. 31549-31557.

33. Thakur, S., S. Pandey, and O.A.J.C.p. Arotiba, Development of a sodium alginate-based organic/inorganic superabsorbent composite hydrogel for adsorption of methylene blue. 2016.

153: p. 34-46.

34. Bendtsen, S.T. and M.J.J.o.M.C.B. Wei, Synthesis and characterization of a novel injectable alginate–collagen–hydroxyapatite hydrogel for bone tissue regeneration. 2015. 3(15): p. 3081-3090.

35. Hua, S. and A.J.C.P. Wang, Synthesis, characterization and swelling behaviors of sodium alginate-g-poly (acrylic acid)/sodium humate superabsorbent. 2009. 75(1): p. 79-84.

36. Al-Ghamdi, Y.O. and S.A.J.C. Khan, Stabilization of zero-valent Au nanoparticles on carboxymethyl cellulose layer coated on chitosan-CBV 780 zeolite Y sheets: Assessment in the reduction of 4-nitrophenol and dyes. 2020. 27(15): p. 8827-8841.

37. Lin, N., et al., TEMPO-oxidized nanocellulose participating as crosslinking aid for alginate-based sponges. 2012. 4(9): p. 4948-4959.

38. Fifield, F.W., Principles and practice of analytical chemistry. 2000.