

Chitosan-based Biogenic Nanoparticles for Wastewater Remediation: Synthesis, Characterization, and Applications - A Review[#]

Usman Lawal Usman^{1*}, S. Ellumalai², Anandh, S³ and Venkatesan Madha Suresh⁴

¹Department of Biology, Umaru Musa Yar'adua University Katsina, Nigeria

²Department of Biotechnology and The Registrar, University of Madras, Chennai India

³Centre for Environmental Sciences, University of Madras, Chennai India

⁴Department of Civil Engineering, SRM University, Chennai India

*Email: usman.usman@umyu.edu.ng

Received: 20.9.2023, Revised: 2.11.2023, 26.1.24, Accepted: 27.1.24

Abstract

Wastewater pollution has been a global concern, necessitating the development of efficient and sustainable treatment technologies. Biogenic nanoparticles have emerged as a potential solution owing to their low cost, eco-friendliness, and high remediation efficiency. Among these nanoparticles, chitosan-based nanoparticles have gained significant attention for the treatment of wastewater. Chitosan, a biodegradable polysaccharide derived from chitin, possesses unique physicochemical properties that make it a suitable candidate for nanoparticle synthesis. The present review focuses on the synthesis, characterization, and applications of chitosan-based biogenic nanoparticles for wastewater remediation. Various synthesis methods, such as chemical cross-linking, precipitation, biosynthesis, solvent evaporation, and emulsion techniques are discussed. The characterization of chitosan nanoparticles using techniques such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD), and, Fourier-transform infrared spectroscopy (FTIR) among others were also highlighted to provide insights into their structural and chemical properties. Additionally, the applications of chitosan-based nanoparticles in wastewater remediation, including the adsorption of heavy metals, organic dyes, and pharmaceuticals, are discussed. Finally, the review concludes with a discussion of the prospects and potential research directions in employing chitosan-based nanoparticles for wastewater remediation.

Keywords: Chitosan, Biogenic Synthesis, Nanoparticles, Wastewater, Remediation, Characterization

Paper presented during 3rd International Conference on Recent Trends in Analytical Chemistry (26-28 June 23) organized by Department of Analytical Chemistry, University of Madras, Chennai and ISAS Tamilnadu Chapter

Introduction

Wastewater pollution has emerged as a pressing global concern, necessitating the development of efficient and sustainable treatment technologies. Rapid industrialization, urbanization, and population growth in recent decades have contributed to the generation of large volumes of wastewater, which often contains a diverse range of contaminants¹. These contaminants can include heavy metals, organic dyes, pharmaceuticals, pathogens, and various other pollutants derived from industrial, domestic, and agricultural activities. The discharge of untreated or inadequately treated wastewater into the environment has severe implications for human health, ecosystem integrity, and overall environmental quality². Contaminants present in wastewater can enter water bodies, leading to the contamination of drinking water sources, rivers, lakes, and oceans. This not only poses a direct threat to aquatic organisms but also has the potential to enter the food chain, affecting human populations. Traditional wastewater treatment techniques, such as physical, chemical, and biological processes, have been widely employed. However, these methods often suffer from drawbacks like high cost, energy intensity, and the generation of large amounts of sludge or secondary pollutants³. Therefore, there is a rising need for alternate and sustainable approaches to address the challenges of wastewater pollution effectively. In recent years, biogenic nanoparticles have emerged as a promising solution for wastewater remediation owing to their low cost, eco-friendliness, and high remediation efficiency. These nanoparticles are derived from natural materials and possess unique properties that make them suitable candidates for pollutant removal. Chitosan-based nanoparticles, in particular, have garnered significant attention in the field of wastewater remediation⁴. Chitosan, a biodegradable polysaccharide derived from chitin, exhibits remarkable physicochemical properties, including high surface area, adsorption capacity, biocompatibility, and antimicrobial activity. These properties make chitosan an ideal material for the synthesis of nanoparticles with tailored characteristics and enhanced performance in wastewater treatment applications. Natural chitosan is known to be insoluble in water, organic solvents, and alkaline media, but soluble in organic acids at pH values below 6. Preparation of different water-soluble chitosan salts is possible by its neutralization with hydrochloric acid, acetic acid, lactic acid, or formic acid⁵. Its solubility in diluted aqueous solutions can be correlated with the conversion of glucosamine units into the soluble form of R-NH³⁺. Experimental data proved that water-insoluble chitosan shows antimicrobial activity in acidic medium, being appropriate for use as a preservative in acidic foods. Economic aspects and cost considerations associated with chitosan-based nanoparticles revolve around raw material costs, manufacturing and

processing expenses, customization for specific applications, regulatory compliance, market demand, and competitive pricing strategies. Balancing these factors is essential for determining the economic viability of chitosan-based nanoparticles in various industries.

This review aims to provide a comprehensive analysis of chitosan-based biogenic nanoparticles for wastewater remediation. We will delve into the synthesis methods used to fabricate these nanoparticles, explore the characterization techniques employed to evaluate their properties and examine their diverse applications in pollutant removal. By understanding the fabrication, characterization, and applications of chitosan-based biogenic nanoparticles, researchers can harness their potential to develop efficient and sustainable technologies for wastewater treatment. By bridging the gap between research and application, this review seeks to contribute to the advancement of wastewater remediation strategies and promote the synthesis of eco-friendly nanoparticles. Ultimately, the utilization of chitosan-based biogenic nanoparticles has the potential to significantly improve water quality, protect ecosystems, and safeguard public health in the face of the rising challenge of wastewater pollution.

Chitosan

Chitosan is a natural biopolymer that is made up of a linear polysaccharide that contains a primary amino group and it's considered a derivative of chitin. Chitin is considered one of the most ubiquitous natural biopolymers which form an integral part of the crustaceans. Similar to that cellulose chitin is a polysaccharide, strong, inelastic, and whitish with a replaced acetamide group at their carbon-2 position from the hydroxyl group⁶. The process of deacetylation of the chitin marks the production of the novel chitosan biopolymer.

Sources of the Biopolymer – Chitosan

The enormous chitin distribution is majorly found among crustacean shells such as lobsters, shrimps, and crabs. Similarly, they are also found in the exoskeleton structure of some marine species such as squid pens, jellyfish, and corals (Fig. 1). In addition, some microbes such as the yeast species and other fungi are known to possess chitin on their cell wall. The crustacean shells are made up of about 15 to 40% chitin, and 20 to 40% protein while calcium carbonate (CaCO_3) and magnesium carbonate (MgCO_3) compose of 20 to 50%, and other trace amounts of minerals, astaxanthin, and lipids⁷. Thus, chitosan derived from the shells of crustaceans has a high amount of molecular weight hence resulting in the removal of a large quantity of waste materials from the shells⁸. Crab shells are also a source of chitosan, they are

considered to have carotenoids which provide colouration for the shells⁹. The majority of the available chitosan (α -form) that is commercially synthesized is extracted from crab shells. Recently, chitosan of nanoparticle size has been extracted via encapsulation techniques from the shells of crabs¹⁰. The shells of shrimps are considered the second most common source of chitosan among crustaceans. Various shrimp species are well distributed across the entire world which increases their commercial production. A large number of shrimp heads and skin are generated as biological waste materials with less cost value. However, great value is obtained from this biowaste through the extraction of chitosan. The shells of lobster are known for their large and commercially scale chitosan synthesis. One of the most imperative merits of chitosan extraction from lobster shells is that the chitin steps during the extraction process can be removed¹¹. This helps to reduce the lengthy procedure thereby making the synthesis feasible and time efficient. The shells of the mussel are commonly found along the river banks which differs from that of crustaceans that are only found in the sea. The calcium carbonate content in the shells of the mussels is considered to be higher compared to that of other crustaceans like crabs and shrimps and is slightly less in the cuticles of the oyster¹². The chitin synthesis from the shells of the mussels loses much content moisture during the deacetylation stage of the chitosan extraction.

Significance of Chitosan Nanoparticles

The main constituents of the exoskeletons of terrestrial insects like honeybees and silkworms, as well as the cell walls of moulds, yeast, and ray fungus like *Streptomyces*, are chitin and chitosan. Crustacean shells include those of prawns, squid, crab, and lobster. Chitin can be fully or partially deacetylated to yield chitosan, a cationic biopolymer. Deacetylation of N-acetyl-D-glucosamine yields (1–4)-linked 2-amino-2-deoxy- β -D-glucopyranose, which forms a linear polysaccharide¹³. Because of their degree of deacetylation, polymerization, molecular mass, viscosity, and acid dissociation constant, or pka, chitosan refers to a collection of co-polymers rather than a single substance¹⁴. The chitosan obtained from microbial sources is seen to be promising since the underlying method may be adjusted to produce a clean, consistent product with the required unique properties¹⁵. It is a very adaptable biopolymer with a wide range of applications in the fields of sewage treatment, cosmetics, agriculture, medicine, and food industry¹⁶. Because of its distinct polymeric cationic character, capacity to enhance absorption, mucoadhesive properties, biocompatibility, and biodegradability, Chitosan nanoparticles are utilised extensively. Chitosan is an important biopolymer for the creation of nanoparticles because it is a modified

linear polysaccharide with variable amounts of free amino groups in its polymeric chain and cationic characteristic. This allows it to provide ionic cross-linking of multivalent anions¹⁷. In vivo, the positively charged Chitosan nanoparticles target particular sites with greater affinity for the negatively charged cellular membrane. When chitosan is applied to soil or foliage, it can cause the plant to produce phytoalexins, antifungal hydrolases, or lignin-like substances, which can induce structural barriers and help the plant fend off insect and disease attack¹⁸. Furthermore, the plant's biophysical characteristics are greatly improved by Chitosan nanoparticles. When nano-chitosan is applied, it accelerates photosynthesis, causes root nodulation, increases nutrient absorption, speeds up seed germination, and increases plant vigour¹⁸.

Furthermore, chitosan enhances the gradual release of the medication with improved solubility, it is frequently utilised in the administration of insecticides, fertilisers, and micronutrients¹⁴. Similarly, because it forms a complex by electrostatic contact and shields the nucleic acid from nuclease destruction, chitosan-mediated genetic transformation is also effective. In comparison to plants developed using conventional gene delivery techniques, chitosan-mediated results in plants that are stable transformed¹⁹.

Chitosan Extraction methods

The chitosan extraction from the organism's sources involves a unique successive technique. The general procedure comprised demineralization, deproteinization, decolourization, and deacetylation²⁰.

Demineralization

The process that involves the reduction of the minerals present in a substance is known as demineralization. This is commonly achieved via the treatment of the exoskeleton or shells of some organisms e.g., shrimps, lobster, fungi, etc. with a hydrochloric acid (HCl) solution. The acid concentration controls the demineralization process and it differs according to the content of minerals present in the different sources of materials used for the extraction process. Hydrochloric acid and lactic acids are the major acids used in the process of demineralization of crab and shrimp shells. However, the biological techniques used for the demineralization of the shells involve the use of Lactobacillales (lactic acid bacteria) for treating the shell extract²¹. In the demineralization process, chitosan with less viscosity is synthesized when using a high concentration of acid during the extraction. However, the synthesized chitosan's solubility is neither influenced by the acid type nor the concentration of the acid used during the demineralization process²².

Deproteinization

Deproteinization is the process of treating the shells with a strong solution of alkali for a longer time, which is followed by the dropping of the pH solution to the neutral range. This procedure can be carried out before or after demineralization. In this stage, the protein is removed from the shells alongside the ones that are bound with chitin due to the recurrent pH change. The repeated deproteinization procedure will result in a better chitin yield which will give rise to the chitosan from the animal shells. Similarly, following the deproteinization process, the amount of amino acid that is acidic will be greater compared to that of the alkaline amino acid²³.

Decolourization

The decolourization process is generally carried out among marine chitosan sources. This is conducted to remove the excess pigment found on the shells of marine crustaceans. The short period of the shells making contact with the weak acid solution results in the removal of the colour or bleaching of the shells²⁴. Thus, before the decolouration process, the extracted chitin usually has a slightly pink colour.

Deacetylation

The elimination of the acetyl group from the extracted chitin to give rise to chitosan is a term for the deacetylation process. This is considered the major step that involves the chitosan production process. The amount of deacetylation of the chitin determines the physical characteristics and chemical properties alongside the quality of the synthesized chitosan. The deacetylation techniques comprise long chitin treatment using a strong concentrated solution of alkaline. Continual deacetylation under heat helps in the synthesis of chitosan which has a higher rate of solubility and a degree of deacetylation (DDA). The deacetylated chitin that ascribes to the hydroxyl and amine groups tends to make the extracted chitosan more reactive compared to the chitin²⁵.

Types of Biopolymer Chitosan

Chitosan is majorly categorized into three forms according to their structural orientation, these are alpha (α), beta (β), and gamma (γ) chitosan. Most of the chitosan that is commercially produced are alpha (α) types which are extracted from the crustacean's shells. While the beta (β) type which is mostly reactive is extracted from the pens of squid. Generally, all three (α , β , and γ) forms of chitosan are soluble in acid. In addition, the alpha type of chitosan demonstrates a greater bactericidal effect as compared to the beta and gamma types²⁶. Table 1. depicts different properties among the various types of Chitosan.

Chitosan Properties and Chemical Structure

Chitosan is a natural polysaccharide substance composed of a glucosamine copolymer that is partly deacetylated polymer is derived via the process of deacetylation of chitin using an alkaline solution, for example, a biopolymer that is extracted from shell-fish. The chitosan structure is composed of a rigid material containing a unit of glucosamine substance and acetyl glucosamine polymer β : -1-4, 2-amino-2-deoxy-D-glucose with a structure of linear hydrophilic amino-polysaccharide groups as shown in Fig. 2. Natural chitosan tends to become insoluble in water or organic solvent, however, after protonating the free amino group and the pH range is decreased to below 5 scales, the chitosan now becomes soluble and possesses a high-density charge of positive polymers²⁷. Nevertheless, the protonated polymer having positive charges can be able to effectively flocculate and neutralize the negatively charged particles that are suspended in the form of colloidal materials. Thus, reducing the concentration of chlorides, turbidity, and chemical oxygen demand in the contaminated water²⁸.

Synthesis Methods for Chitosan-based Nanoparticles

Several specific techniques are employed for the fabrication of chitosan-based biogenic nanoparticles, each offering distinct advantages in terms of control over nanoparticle properties and scalability. Because they are more stable and have a higher adsorption capacity and better transport capability, chitosan nanoparticles enhance the benefits of chitosan²⁹. The past several decades has seen the use of a variety of techniques, including ionic gelation, microemulsions, emulsification, cross-linking, and polymerization, to generate chitosan nanoparticles. The chitosan particle size and molecular structure produced by this approach vary depending on some changes in the procedure. 300 nm to 500 nm is typically the range of findings from such procedures²⁹. The synthesis of nanoparticles via biogenesis is favoured over chemical or physical methods because it is a less time-consuming, safe, environmentally benign, economically and energetically feasible process that maximises the redox potential of metabolites produced by biological entities to transform macromolecules into nano form³⁰. Below are some of the commonly used techniques for nanoparticle fabrication:

i. *Chemical cross-linking method*: The chemical cross-linking method involves the cross-linking of chitosan molecules using cross-linking agents such as tripolyphosphate (TPP), sodium sulfate (Na_2SO_4), glutaraldehyde, citric acid, genipin, ethylenediamine (EDA), etc. Chitosan and the cross-linking agent are mixed, and the reaction leads to the formation of a three-dimensional network, resulting in nanoparticle formation. One commonly used cross-

linking agent for chitosan is tripolyphosphate (TPP). When chitosan and TPP are mixed, the interaction between chitosan amino groups and TPP phosphate ions forms strong ionic bonds, resulting in the formation of a three-dimensional network structure. This network traps the chitosan molecules and stabilizes them in a nanoparticle configuration. Sodium sulfate is another agent used similarly for cross-linking chitosan, leading to the creation of chitosan nanoparticles³¹. Glutaraldehyde is a cross-linking agent that forms covalent bonds with chitosan molecules, producing more stable and rigid nanoparticles. It can be utilized to tailor the mechanical properties and biodegradability of chitosan-based nanoparticles. Citric acid, genipin, and EDA are other cross-linking agents employed for chitosan modification, each offering unique advantages and characteristics for different applications. The size and properties of the chitosan nanoparticles can be adjusted by varying several factors, including the concentration of chitosan, the type and concentration of the cross-linking agent, and the reaction time. A higher concentration of chitosan typically results in larger nanoparticles, while increasing the cross-linking agent concentration or extending the reaction time can lead to smaller nanoparticles with enhanced stability³².

ii. *Biosynthesis using microorganisms*: Biosynthesis involves the use of microorganisms, like bacteria or fungi, as living factories for the production of chitosan-based nanoparticles. The redox processes that take place when a biological entity or microbe absorbs a metal ion and converts it to an element metal through cellular enzymatic activity form the fundamental basis for the biogenic creation of nanoparticles. Both external and intracellular synthesis fall under this category. The process of intracellular synthesis involves the transportation of metal ions into the cell, thereafter the reduction reaction takes place within the cell wall, cytoplasm, or periplasmic space. As a result, the resulting nanoparticles are formed inside the cell. But in the second case, the foreign object that has been trapped causes the reductase enzyme to catalyse the creation of nanoparticles on the cell surface³³. Extracellular synthesis of nanoparticles is often preferred over intracellular production due to the laborious procedure of harvesting nanoparticles from the cell matrix. Because of their capacity to produce nanoparticles on a vast scale, with relative control over their size and form (controlled by surrounding environmental conditions) and a simplified process of manufacturing, microorganisms are sometimes described as environmentally benign green nano-factories³⁰. Typically, a magnetic stirrer is used to constantly mix the plant component extract after the metal ions are injected during the phytofabrication of nanoparticles. When the resulting solution changes colour, the creation of nanoparticles may be visually verified³⁴. The metal ion is affected by antioxidants like polyphenols, flavonoids, and phytoalexins, which change

it into a harmless element metal in nanoform. Therefore, for the phytofabrication of nanoparticles, a plant with a larger percentage of naturally reducing elements is preferred. El-Naggar et al.¹³ combined an equivalent amount of *P. graveolens* phytoextract with chitosan solution, then incubated the combination in a rotating shaker at 50°C. After that, the turbid solution that remains after the redox reaction is created is centrifuged for 10 minutes at $10,000 \times g$. To get rid of the unreacted chitosan, it was further cleaned with an acetic acid solution. The Chitosan nanoparticles in the solution were then extracted by freezing and drying it. Similarly, fungal sources rich in chitosan were used to biogenically synthesise Chitosan nanoparticles by Boruah and Dutta¹⁸. The fungal biomass was subjected to a series of controlled alkali and acid treatments to isolate the chitosan. After first treating the fungal biomass with NaOH, an alkali-insoluble material (AIM) was produced. To extract the fungal chitosan, this AIM was next treated with an acid. Next, it was mixed with 1% TPP solution in a magnetic stirrer to create nano-chitosan.

iii. *Enzymatic synthesis*: Enzymatic synthesis utilizes enzymes, such as lysozyme or chitinase, to degrade chitosan into nanoparticles. Enzymes break down the chitosan polymer chains, resulting in the formation of nanoparticles. This method allows for precise control over the size and morphology of the nanoparticles by adjusting the enzyme concentration, reaction conditions, and chitosan properties. Enzymatic synthesis offers advantages such as mild reaction conditions, high specificity, and the potential for tailoring nanoparticle properties through enzyme engineering. These techniques provide researchers with a range of options for fabricating chitosan-based biogenic nanoparticles with specific properties and characteristics. However, it is important to note that the choice of technique depends on various factors, including the desired nanoparticle size, shape, surface properties, and specific application requirements.

iv. *Precipitation method*: The precipitation method involves the dissolution of chitosan in a suitable solvent, followed by the addition of a precipitating agent. Commonly used precipitating agents include sodium tripolyphosphate and calcium chloride. The addition of the precipitating agent causes chitosan to undergo phase separation and form nanoparticles. The size and morphology of the nanoparticles can be regulated by adjusting the concentration of chitosan, the precipitating agent, and the rate of mixing. This method offers simplicity, scalability, and the ability to produce chitosan-based nanoparticles with a narrow size distribution³⁵.

v. *Emulsion method*: The emulsion method utilizes the emulsification of chitosan in an organic solvent followed by the formation of nanoparticles through solvent evaporation.

Chitosan is dissolved in an organic solvent, and the resulting solution is emulsified in an aqueous phase using surfactants. The organic solvent is then evaporated, resulting in the formation of nanoparticles. The size and morphology of the nanoparticles can be controlled by adjusting the emulsion droplet size, surfactant concentration, and the rate of solvent evaporation. This method allows for the production of chitosan-based nanoparticles with controlled sizes and shapes.

vi. *Solvent evaporation method*: In the solvent evaporation method, chitosan is dissolved in a volatile organic solvent and then added dropwise to an aqueous solution under stirring. The organic solvent rapidly evaporates upon contact with the aqueous solution, leading to the precipitation of chitosan and the formation of nanoparticles. The size and morphology of the nanoparticles can be controlled by adjusting the chitosan concentration, solvent composition, and the rate of solvent addition. This method offers simplicity and scalability for the fabrication of chitosan-based nanoparticles. Gallic acid and chitosan were used in the combination of ultrasonication and chemical reduction methods by Guzman et al.³⁶ to synthesise colloidal AgNps. Upon characterization, they discovered that the resulting gallic acid-chitosan-modified silver nanoparticles (GC-AgNps) were spherical, monodispersed, and stable for four weeks without exhibiting any discernible changes in size. After 120 minutes of exposure, it was discovered that GC-AgNps had great efficacy against *Escherichia coli* even at 1 µg/mL³⁶.

In addition to the aforementioned techniques, other methods can be employed for chitosan-based nanoparticle fabrication. These include electrostatic assembly, template-assisted synthesis, spray drying, and supercritical fluid technology. Electrostatic assembly involves the layer-by-layer deposition of chitosan and oppositely charged polymers or nanoparticles, resulting in the formation of chitosan-based nanoparticles with controlled layer thickness and surface functionality. Template-assisted synthesis utilizes templates or molds to shape chitosan solutions into specific nanoparticle structures, offering precise control over size and shape. Spray drying involves atomizing chitosan solutions into fine droplets, which subsequently dry and form nanoparticles³⁷. Supercritical fluid technology employs supercritical fluids to dissolve chitosan and then rapidly depressurize it to induce nanoparticle formation. Moreover, various modifications and advancements can be incorporated into these techniques to enhance the fabrication process and nanoparticle properties. For instance, the introduction of microfluidic devices enables precise control over reactant flow rates, resulting in uniform nanoparticle size and shape. Ultrasonication can be utilized to aid in the dispersion and homogenization of chitosan solutions, promoting uniform nanoparticle formation.

Furthermore, the integration of green chemistry principles, such as using environmentally friendly solvents and reducing waste generation, contributes to the development of sustainable and eco-friendly nanoparticle fabrication methods. Table 2: depicts some chitosan nanoparticle synthesis techniques alongside their advantages and disadvantages.

Characterization Techniques for Chitosan-based Nanoparticles

Characterization techniques are essential tools for assessing the properties of chitosan-based nanoparticles and evaluating their suitability for various applications, including wastewater remediation. These techniques provide valuable insights into the structural, morphological, surface, and physicochemical properties of the nanoparticles, allowing researchers to understand their behavior, performance, and interactions with pollutants³⁸. Below are various characterization techniques commonly used to assess chitosan-based nanoparticles.

i. Spectroscopic Techniques: Spectroscopic techniques are widely employed to investigate the structural properties of chitosan-based nanoparticles. These techniques provide information about the chemical composition, functional groups, and molecular interactions within the nanoparticles. Some commonly used spectroscopic techniques include:

a) Fourier Transform Infrared Spectroscopy (FTIR): FTIR is a powerful technique for analyzing the functional groups and chemical bonds present in chitosan-based nanoparticles. It measures the absorption of infrared light by the nanoparticles, providing information about their molecular structure, degree of deacetylation, and any modifications or surface functionalization. FTIR provides valuable insights into the interactions between bonds and their respective functions. The fundamental idea behind the FTIR approach is that molecules, whether inorganic or organic, absorb electromagnetic radiation in the infrared range (4000–400 cm^{-1}). When a molecule absorbs infrared light, its dipole moment should alter, making it infrared active. An interference pattern is recorded when the data is measured in terms of wavenumbers, and this data is then transformed into a spectrum (transmittance/absorbance form)³¹. Fig. 3 provides a schematic illustration of the procedures involved in FT-IR analysis²⁰. The type of bonds, functions related to molecular structures, and interactions may all be determined with the aid of the spectrum, which is often represented by separate lines that can be broad or thin and correspond to a certain frequency³¹. Samples can be liquid, solid, or gaseous; however, KBr (100:1) is typically used to make solid or liquid samples, and a hydraulic press machine is used to create the pellet. First, at room temperature, a blank KBr background is recorded on the instrument, and then a KBr pellet containing the samples of

interest let's say chitosan nanomaterial/NPs in an absorbance/transmittance mode is recorded. It is important to note that the FTIR method is a very powerful analytical instrument that has been used to better understand the functions of NPs made of chitosan³⁹.

b) Ultraviolet-Visible Spectroscopy (UV-Vis): UV-Vis spectroscopy is used to determine the optical properties of chitosan-based nanoparticles. It measures the absorption or scattering of UV or visible light by the nanoparticles, providing information about their size, aggregation state, and optical behavior. UV-Vis spectroscopy can be used to assess the stability, dispersion, and surface plasmon resonance of the nanoparticles.

c) X-ray Diffraction (XRD): XRD Chitosan-based nanomaterials/NPs are not simple molecules, like any other nanoparticle system; instead, they are organised into three primary layers. First and foremost is the surface layer, which can be functionalized appropriately by coating with surfactants, encasing metal ions, etc. The third layer, which is effectively the core and is the central/innermost segment of chitosan-based NPs, is chemically separate from the second layer, also known as the middle layer or shell layer. The solid-state characteristics of crystallinity, composition, defect structure, grain size, etc. may all be efficiently used to shed light on the molecular dynamics of chitosan-based nanoparticles, or NPs. One effective method for examining the solid-state characteristics of chitosan-based nanomaterials/NPs is X-ray diffraction (XRD)^{40,41}.

Determining the crystallographic structure of nanoparticles is a crucial step in the field of material research. Max Von Laue made a significant finding in 1912 that the behaviour of a substance's two-dimensional diffraction gratings (also known as nanomaterials or NPs) in the presence of an X-ray wavelength is comparable to that of a crystal lattice's plane spacing. With the use of X-ray diffraction (XRD), a non-destructive analytical method, it is possible to identify the surface or phase of chitosan-based nanomaterials and get data on their cell sizes and atomic spacings. The incoming X-rays from the 1.5418 Å cathode ray "Cu X-ray tube" in XRD are filtered to create monochromatic radiation, which is then collimated to concentrate and utilised to irradiate the sample (a nanomaterial based on chitosan)^{42, 43}. The interaction between the incoming rays and the chitosan-based nanomaterial results in a diffracted ray and constructive interference after meeting Bragg's equation ($n\lambda = 2d \sin\theta$). According to Bragg's equation, the wavelength of an incoming ray (λ) may be correlated with the diffraction angle (θ) and lattice spacing (d) to determine the scattering angle and intensity that are diffracted from the sample⁴². The chitosan-based nanomaterial sample material, whether it is in the form of a powder or a film, has to be homogenised. It is possible to obtain a finite diffraction

direction for the lattice by varying the 2θ (2 theta) angles to all possible points. Every material has a distinct set of d-spacings, and the International Centre for Diffraction Data (ICDD) or, formerly, the Joint Committee on Powder Diffraction Standards JCPDS data, may provide the appropriate places and intensities as a reference pattern or database⁴². Therefore, it is simple to determine the particle size, content, crystalline nature, form of a unit cell, and other characteristics of chitosan-based nanomaterials/NPs by converting diffraction peaks to d-spacings⁴³. Notably, the size, shape, and nature of a unit cell of chitosan-based nanomaterials or NPs determine the direction of the diffraction angle, while the internal structural arrangements of the atoms determine the intensities of the diffraction patterns. The XRD spectra of chitosan powder were successfully reported by Usman et al.²⁰. The spectra showed features peaks corresponding to 2θ values of 10.9° and 19.8° , which correlate to the amorphous structure of chitosan (Fig. 4). The literature is widely aware that chitosan's internal lattice structure configurations are changed by structural alterations. Changes in the structural arrangements of chitosan are caused by several factors, including the degree of deacetylation, molecular weight, drying, precipitation, chemical processing, and dissolving process⁴⁴.

ii. Imaging Techniques: Imaging techniques allow for the visualization and characterization of the morphology, size, and shape of chitosan-based nanoparticles. These techniques provide valuable information about the physical characteristics and surface properties of the nanoparticles. Some commonly used imaging techniques include:

a) Transmission Electron Microscopy (TEM): Transmission electron microscopy, or "TEM," is a widely used technology for characterising chitosan-based nanomaterials because of its better spatial resolution, or equivalency with atomic dimensions. TEM offers chemical information and internal structural arrangements in an image format. A highly charged electron beam is sent through a thin foil in a TEM, changing its shape from elastic to inelastic. These altered electrons subsequently interact with the specimen, which contains a sample of chitosan nanomaterial. Particles that were transmitted or reflected were released as a result of the interactions between the inelastic/elastic electrons and the specimen. Following the detection of the energy difference, the camera produces better-resolution magnified pictures, which are subsequently visualised on a monitor. It is important to note that the ratio of the specimen, image plane, and objective lens-which has to be amplified by the lens- is taken into consideration⁴⁵. In comparison to SEM, TEM offers higher resolution (down to 0.2 nm) and higher-quality analytical measurements. This is because both methods show the

visualisation of nanomaterials, including how they appear, how their atoms are arranged, the degree of aggregation, and details about their size and dimensions. Because TEM uses energetic electrons to infer composition, morphology, imaging, and crystallographic analysis, it may yield comprehensive information. Consequently, TEM employed three primary methods, namely, diffraction pattern, imaging, and electron microscopy. For TEM analysis, sample preparation is rather straightforward: following sonication, a drop or two of a suspension of chitosan-based nanomaterials is added to a carbon-coated copper grid, which is then dried or, in certain cases, exposed to infrared radiation. Additionally, TEM differentiates between chitosan-based nanomaterials/NPs that are polycrystalline, amorphous, and monocrystalline⁴⁰. It is important to note that while there is a possibility of aggregation during the sample preparation procedure, the size distribution or size estimation of nanomaterials using TEM is precise rather than exact. Cryo-TEM is better than traditional TEM analysis because in the former case, a suspension of nanomaterial is solidified at cryogenic temperatures (-100 to -175 °C), and the visualisation is carried out when the specimen is frozen. By avoiding the issues of nanoparticle aggregation, heavy-metal contrasting agents, and solvation, the previously described technique produced a size estimation that was both exact and accurate⁴⁵.

The cryo-TEM is very helpful in determining if the self-assembly of chitosan-based nanomaterials is a natural occurrence or whether it only happens during sample preparation, in which case solvation/evaporation processes come into play⁴⁵. TEM analysis has been used for chitosan-based nanomaterials/NPs in some papers in the literature. By adopting a benzyl alcohol/water emulsion technique, Facchi et al.⁴⁶ effectively reported the synthesis of N-modified chitosan NPs for curcumin administration. TEM investigation further reveals that the average particle size of TMC/TPP NPs is around 99 nm, but it may reach up to 317 nm for DMC/TPP NPs⁴⁶. Using a reverse micellar system comprising surfactant, namely sodium bis (ethylhexyl) sulfosuccinate (AOT) and n-hexane, Banerjee et al.⁴⁷ reported the production of ultrafine chitosan nanoparticles in which the amine groups are cross-linked (10% or 100%)⁴⁷. According to the TEM study, the particles have a spherical shape and aggregate with dimensions of 30 nm when 10% amine functionalization (for chitosan NPs) is cross-linked, but the particle size increases to 110 nm when 100% cross-linking is applied⁴⁷. Several additional studies have reported on the spherical-type morphologies of chitosan-based nanoparticles⁴⁸ and their combination with chitosan to encapsulate metals like Cu⁴⁹.

b) Scanning Electron Microscopy (SEM): SEM is an effective technique for determining surface visualizations, studying functionalized/ agglomerated chitosan nanoparticles (NPs) in

detail, and estimating sample composition by energy-dispersive X-ray spectroscopy (EDX) is scanning electron microscopy (SEM). SEM achieves resolutions in the nanometer range by using an energised electron beam (usually 1–30 eV) to scan the sample's surface in a raster pattern and identify secondary emitted or backscattered electrons. Different types of radiated electrons are produced at or on the surface of the sample depending on the electronic interaction between the sample (chitosan nanoparticles) and the sample. The electrons of various energies that are detected are processed and shown as a pixel on the monitor, allowing the visualisation of three-dimensional pictures or the makeup of nanomaterials. Field emitter scanning electron microscopy (FE-SEM) is the term used when these electrons are beamed under a strong electric field⁵⁰. Traditionally, high vacuum conditions are employed to apply an ultrathin coating of noble metals, such as gold (Au), silver (Ag), and platinum (Pt), to increase the electrical conductivity and contrast of nanomaterials⁵¹. SEM analysis is widely applied in the literature for chitosan-based nanomaterials/NPs to provide a graphical visualisation⁵¹. Poly (lactic acid)/chitosan (PLA/CS) nanoparticles were synthesised by Dev et al. in 2010⁵² by the use of solvent evaporation and emulsion methods. The surface shape of ZnO-Chitosan NPs is depicted in Fig. 5, which displays SEM images of the particles²⁰. Using a two-step procedure of oil-in-water emulsion followed by ionic gelation, Hosseini et al.⁵³ produced the essential oil-encased chitosan NPs⁵³

iii. Surface Analysis Techniques: Surface analysis techniques are employed to evaluate the surface properties, charge, and interactions of chitosan-based nanoparticles. These techniques provide insights into the surface chemistry, charge distribution, and surface energy of the nanoparticles. Some commonly used surface analysis techniques include:

a) Brunauer-Emmett-Teller (BET) Analysis: BET analysis is a technique used to determine the specific surface area and porosity of chitosan-based nanoparticles. It measures the adsorption of gas molecules onto the nanoparticles, providing information about their surface area, pore size distribution, and surface roughness. BET analysis is valuable for assessing the adsorption capacity, surface reactivity, and surface accessibility of the nanoparticles.

b) Zeta Potential Analysis: Zeta potential analysis measures the electrical charge and surface potential of chitosan-based nanoparticles. It provides information about the stability, dispersibility, and potential for aggregation of the nanoparticles. Zeta potential analysis is valuable for understanding the electrostatic interactions between the nanoparticles and pollutants, as well as their colloidal stability in aqueous solutions. By measuring the zeta

potential, researchers can gain insights into the surface charge, surface functionality, and the potential for nanoparticle-pollutant interactions.

iv. Thermal Analysis Techniques: Thermal analysis techniques are used to investigate the thermal stability, decomposition behavior, and thermal properties of chitosan-based nanoparticles. These techniques provide information about the thermal transitions, degradation temperature, and thermal stability of the nanoparticles. Some commonly used thermal analysis techniques include:

a) Thermogravimetric Analysis (TGA): TGA measures the weight changes of chitosan-based nanoparticles as a function of temperature. It provides information about the thermal stability, decomposition kinetics, and thermal degradation behavior of the nanoparticles. TGA analysis can help assess the temperature range at which the nanoparticles maintain their structural integrity and stability.

b) Differential Scanning Calorimetry (DSC): DSC measures the heat flow into or out of chitosan-based nanoparticles as a function of temperature. It provides information about the phase transitions, melting points, glass transitions, and thermal behavior of the nanoparticles. DSC analysis can be used to determine the crystallinity, degree of cross-linking, and thermal stability of the nanoparticles.

v. Particle Size and Size Distribution Analysis: Characterizing the particle size and size distribution of chitosan-based nanoparticles is crucial for understanding their behavior, stability, and performance in wastewater remediation. Several techniques can be employed for particle size analysis, including:

a) Dynamic Light Scattering (DLS): A flexible and effective technique for analysing the diffusion behaviour of molecules in the solution phase is dynamic light scattering (DLS), also known as quasi-elastic light scattering (QELS) or photon correlation spectroscopy (PCS). The DLS or PCS approach determines the hydrodynamic radii or diffusion coefficient, which in turn affects the size distribution, particle size, surface charge, and morphologies of the particles (nanoparticles/macromolecules)⁵⁴. Since the sample to be analysed in DLS often needs to be in the solution phase, the size is determined by observing the Brownian motion of suspended particles with varying scattered angles, or " θ "⁵⁴. The constant bombardment of solvent molecules around suspended particles and molecules causes their random motion. Particles with dimensions less than one nanometer can be measured using the DLS method. The Stoke-Einstein equation was utilised in DLS to determine the NPs' size. Chitosan nanomaterials, or NPs, have hydrodynamic radii " d_h " that may be calculated from particle diffusion (D) using the formula $d_h = kT/3\pi\eta D$, where η represents the medium viscosity, T is

the absolute temperature, and k is the Boltzmann constant⁵⁴. Numerous scientific studies have previously been published in the literature, and they show that the DLS approach is useful in determining surface charge characteristics and size distribution⁵⁵. It is crucial to combine the DLS approach with more complex methods like microscopy to obtain trustworthy results. Reliable and reproducible data may be acquired for completely dispersed chitosan nanoparticles/nanomaterials, eliminating the idea of aggregation. Because chitosan is polymeric in nature and the DLS technique's fundamental theory is based on the Brownian motion of particles, the real size of the particle is always smaller than the average particle that is detected. Using the DLS approach, Li et al.⁵⁶ determined the average particle size distribution of ferrite-coated chitosan NPs, which range in size from 10 to 100 nm⁵⁶

b) Laser Diffraction: Laser diffraction is a technique that measures the scattering pattern of a laser beam passing through a dispersion of chitosan-based nanoparticles. It provides information about the particle size distribution and the volume-based particle size of the nanoparticles. Laser diffraction analysis is valuable for assessing the size range, polydispersity, and agglomeration state of the nanoparticles.

c) Atomic Force Microscopy (AFM): AFM is a high-resolution imaging technique that can be used to determine the size and topographical features of chitosan-based nanoparticles. It provides three-dimensional images and surface profiles of individual nanoparticles. AFM analysis allows for the direct measurement of particle size, height, and surface roughness.

vi. Rheological Analysis: Rheological analysis is used to assess the flow behavior, viscosity, and rheological properties of chitosan-based nanoparticle suspensions or gels. It provides information about the stability, dispersion, and potential for aggregation of the nanoparticles in complex fluids. Rheological measurements can be performed using techniques such as:

a) Rheometry: Rheometry involves the measurement of the flow and deformation of chitosan-based nanoparticle suspensions under controlled shear or stress conditions. It provides information about the viscosity, shear-thinning behavior, viscoelastic properties, and gelation characteristics of the nanoparticle systems. The rheological analysis is crucial for understanding the flow behavior and stability of nanoparticle suspensions in practical applications.

vii. Elemental Analysis: Elemental analysis is used to determine the elemental composition and elemental distribution within chitosan-based nanoparticles. It provides information about the presence of impurities, functional groups, and chemical elements incorporated into the nanoparticles. Some commonly used elemental analysis techniques include:

a) *Energy-dispersive X-ray spectroscopy (EDS)*: EDS is used in conjunction with scanning electron microscopy (SEM) to analyze the elemental composition of chitosan-based nanoparticles. It provides qualitative and quantitative information about the presence of different elements within the nanoparticles.

b) *Inductively Coupled Plasma Mass Spectrometry (ICP-MS)*: ICP-MS is a highly sensitive technique used to measure the elemental composition and trace element concentrations in chitosan-based nanoparticles. It provides quantitative information about the presence of various elements and can detect impurities or contaminants in the nanoparticles. An inductively coupled plasma is used to atomize the sample in inductively coupled plasma mass spectroscopy, or "ICP-MS." It atomizes to produce atomic and small-polyatomic ions, which were subsequently discovered. A sample solution is first introduced into the system, processed through a nebulizer, and then an inductively coupled plasma is created in the presence of an inert gas, such as argon. Following the atomization of the material by the highly ionizable plasma, polyatomic ions (fine aerosol form) were extracted to an interface area using ion optics. The polyatomic ions are focused into a quadrupole mass analyzer by the electrostatic lens of an ion optic. The detection and separation of ions occur in line with the mass by charge ratio, or "m/z ratio." In the case of AAS sample preparation, for example, the samples to be analysed are also similarly digested in an aqueous phase before being sent to ICP-MS. Deionized water is used as the diluent in sample preparation, which also involves the use of hydrochloric or nitric acid, or in certain situations, alkaline solutions⁵⁷. In 2017, Palomo-Siguero et al. synthesised Selenium NPs in the presence of stabilisers such as non-ionic surfactant (Triton X-100) or chitosan (polysaccharides) using a solution-phase approach⁵⁸.

c) *X-ray Photoelectron Spectroscopy (XPS)*

XPS techniques is one of the popular surface science methods for determining or analysing the chemical state, electrical states, and elemental composition/empirical formula of nanomaterials is called "XPS." The photoelectric effect was discovered in 1887 by Hz, and this discovery is the foundation of the XPS operating concept. The study was expanded to surface analysis later in the 1960s by Siegbahn and his research group at Uppsala University in Sweden. They also invented the acronym XPS, or electron spectroscopy for chemical analysis, for which Siegbahn was awarded a Nobel prize in physics in 1981. In qualitative analysis, the sample is bombarded with single energy, monochromatic, or monoenergetic X-ray photons (Mg K α 1253.6 eV or Al K α 1486.6 eV, line width \approx 0.7–0.85 eV), and the kinetic energy of the electrons that are emitted from the sample's topmost layer (1–10 nm) is

then calculated⁵⁹. The XPS spectrum shows distinctive peaks for the electrons released from outermost/surface atoms, making it possible to identify and measure the surface elements (apart from hydrogen and helium) that are present. By measuring or examining the minute differences that occur in the binding energies of emitted photoelectrons, auger electrons, multiple splitting, satellite peaks, etc., one can easily identify and quantify the chemical states of the elements contained inside. With the use of XPS, an extremely sensitive and high vacuum method, it is possible to identify certain elements or species by simply detecting changes in the electrical configuration of atoms or chemical bonds. Elements like C, N, O, and P are easily distinguished in the case of chitosan-based nanomaterials as they make up the majority and have distinctive peaks that match their binding energies⁶⁰. The XPS for the mildly wet synthesised gold/silver NPs in an aqueous chitosan solution was reported by Boufi et al. in 2013⁵⁹.

d) Atomic Absorption Spectroscopy (AAS)

Atomic absorption spectroscopy, or "AAS," is a very sensitive elemental analytical method that is used to ascertain the metal content at the pictogram level in a range of substances. The AAS measures the decrease in the optical radiation intensity of a cell that contains gaseous atoms from the samples. An analyte in AAS typically absorbs particular wavelengths generated by a hollow cathode lamp, or "HCL," which is utilised as a light source⁶¹. The two most often utilised atom-cells in AAS are either flames or graphitic furnaces/electrothermal atomizers, or "ETAs." In comparison with ETAs, which have a temperature that can be monitored by a power source, flames have a highly regulated combustion environment, so their sensitivity is comparatively lower. Use of inert gas, such as argon, is used to stop the combustion process at high temperatures. The sample/analyte in a "atom-cell" absorbs the particle wavelength and becomes gaseous. It then moves to a detector, which measures and separates the wavelengths of interest before processing the data in a computer/control instrumentation operation. The analyte or samples are produced by digestive processes and are often of a certain concentration in an aqueous phase in order to give maximum accuracy, precision, and fewest interferences⁶¹. It is noteworthy to highlight that each element has a unique absorption of electromagnetic radiation wavelengths for the HCL source. Put another way, the standards are used to quantify how specific and unique an element's absorbance is for the absorbing wavelength of interest. The unknown sample may be processed and the concentration can be retrieved from the digital output display unit when the standards are measured, indicating that the instrument is calibrated for specific components of interest. The underlying concept behind AAS is ionisation energy, which is essentially the energy needed

to excite an electron and varies depending on the element. Every atom in chitosan-based nanomaterials/NPs has a unique fingerprint area, which makes qualitative AAS analysis of the material possible⁶². AAS analysis may be performed against several reaction parameters, including agitation duration, reaction rate, pH, and so on because chitosan-based nanomaterials have been widely employed to encapsulate the metal ions⁶³. Generally speaking, one may compute the Concentration of encapsulated metals (%) by multiplying the ratio of released metal ions by the total amount of metals in a nanomaterial, and then storing the result. AAS was subsequently used to determine the number of metal ions after Liu et al.⁶⁴ proved the utilisation of chitosan-based nanomaterials for the metal encapsulation⁶⁴.

Applications of Chitosan-based Biogenic Nanoparticles in Wastewater Remediation

Chitosan-based biogenic nanoparticles have gained significant attention for their potential applications in wastewater remediation due to their unique properties, such as large surface area, high adsorption capacity, and versatile surface chemistry. These nanoparticles offer promising solutions for the removal of numerous contaminants from wastewater which are sourced from various routes such as natural incidence comprising of geological parental materials or outcropping of rocks, sources from agricultural activities such as application of organic and inorganic fertilizers, depositions from atmospheric emissions such as exhaust of vehicles, wear of asphalt, tires, leakage of oil or gasoline, etc. (Fig. 6) and sources from industrial activities such as mining, metals smelting, finishing, recycling, etc.⁶⁵.

Below are some of the applications of chitosan-based biogenic nanoparticles in wastewater remediation.

i. Heavy Metal Removal: Chitosan-based nanoparticles have demonstrated excellent efficiency in the removal of heavy metal ions from wastewater. The amino and hydroxyl functional groups on the chitosan surface provide active sites for chelation and complexation interactions with heavy metal ions. The large surface area and high adsorption capacity of the nanoparticles allow for the effective removal of heavy metals, comprising cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As)⁶⁶. Chitosan-based nanoparticles offer a cost-effective and environmentally friendly solution for heavy metal remediation in wastewater.

ii. Dye Degradation: Chitosan-based nanoparticles have shown remarkable potential in the degradation and removal of dyes from wastewater. The nanoparticles can adsorb and catalytically degrade a wide range of dyes, including synthetic dyes used in textile, printing, and dyeing industries. The adsorption capacity of chitosan-based nanoparticles enables the

removal of dyes from wastewater, while their catalytic properties promote the degradation of dyes through chemical reactions or photocatalysis⁶⁷. The use of chitosan-based nanoparticles for dye degradation contributes to the reduction of color and toxicity in wastewater.

iii. Organic Compound Adsorption: Chitosan-based nanoparticles have been widely investigated for the adsorption of various organic compounds present in wastewater. These nanoparticles can effectively adsorb organic pollutants such as pharmaceuticals and pesticides. The functional groups on the chitosan surface facilitate interactions, including hydrophobic interactions, hydrogen bonding, and electrostatic interactions, enabling the efficient removal of organic compounds. Chitosan-based nanoparticles offer a sustainable and efficient approach to the removal of organic pollutants from wastewater⁶, antibiotics and pharmaceutical compounds from wastewater. These nanoparticles can effectively adsorb and bind to antibiotics and pharmaceuticals, reducing their concentration in wastewater. The surface properties of chitosan, such as its positive charge, enable electrostatic interactions with negatively charged pharmaceutical compounds. By removing antibiotics and pharmaceuticals, chitosan-based nanoparticles contribute to the reduction of potential ecological risks and the prevention of antibiotic resistance in aquatic environments⁶⁸.

iv. Nutrient Removal: Chitosan-based nanoparticles have shown potential for the removal of nutrients, particularly nitrogen, and phosphorus, from wastewater. These nutrients contribute to water eutrophication and can have detrimental effects on aquatic ecosystems. Chitosan-based nanoparticles can adsorb and immobilize nutrients, preventing their release into receiving water bodies. The large surface area and high adsorption capacity of the nanoparticles make them suitable for nutrient removal, contributing to the preservation of water quality and the prevention of ecological imbalances⁶⁹.

v. Antibacterial Applications: Chitosan-based nanoparticles possess inherent antibacterial properties, making them effective in controlling microbial contamination in wastewater. The cationic nature of chitosan enables interactions with bacterial cell membranes, leading to cell disruption and inhibition of bacterial growth. Incorporating chitosan-based nanoparticles into wastewater treatment systems can help reduce bacterial contamination, prevent the spread of waterborne diseases, and enhance the overall microbiological safety of treated wastewater⁷⁰.

The efficiency of chitosan-based nanoparticles for pollutant removal

Chitosan-based nanoparticles have emerged as promising materials for pollutant removal in wastewater treatment due to their unique properties, including high adsorption capacity, versatile surface chemistry, and biodegradability. The efficiency of chitosan-based

nanoparticles in removing various pollutants depends on several factors, including the type of pollutant, nanoparticle characteristics, operating conditions, and the specific wastewater treatment process⁷¹. Below is the efficiency of chitosan-based nanoparticles for pollutant removal.

a. Adsorption Capacity: Chitosan-based nanoparticles exhibit a high adsorption capacity for a wide range of pollutants. The amino and hydroxyl groups on the chitosan surface provide active sites for adsorption through electrostatic interactions, van der Waals forces, and hydrogen bonding. The large surface area of nanoparticles allows for increased contact with the pollutants, enhancing the adsorption efficiency. The adsorption capacity of chitosan-based nanoparticles can be further improved by modifying their surface properties through functionalization or incorporation of other materials⁶.

b. Selectivity: The selectivity of chitosan-based nanoparticles refers to their ability to preferentially adsorb specific pollutants while ignoring or minimizing the adsorption of other constituents in wastewater. The surface chemistry of chitosan nanoparticles can be modified to enhance selectivity towards target pollutants. Functional groups or coatings can be introduced to promote specific interactions with the desired pollutants, allowing for more efficient removal while minimizing interference from other substances. The selectivity of chitosan-based nanoparticles contributes to their effectiveness in treating complex wastewater matrices⁷².

c. pH and Ionic Strength: The pH and ionic strength of wastewater play a crucial role in the efficiency of chitosan-based nanoparticles for pollutant removal. The surface charge of the nanoparticles and the charge distribution of the pollutants can be influenced by pH, affecting the electrostatic interactions and adsorption capacity. Adjusting the pH within the optimal range can enhance the adsorption efficiency. Additionally, the ionic strength of the wastewater affects the competition between ions and pollutants for adsorption sites. Proper consideration of pH and ionic strength is essential for optimizing the efficiency of chitosan-based nanoparticles in pollutant removal⁷².

d. Regeneration and Reusability: One of the advantages of chitosan-based nanoparticles is their potential for regeneration and reusability. After adsorbing pollutants, the nanoparticles can be regenerated through desorption or other methods, allowing for repeated use in wastewater treatment. Regeneration techniques such as pH adjustment, solvent extraction, or thermal treatment can be employed based on the nature of the pollutant and nanoparticle characteristics. The ability to regenerate and reuse chitosan-based nanoparticles enhances their cost-effectiveness and sustainability as compared to single-use adsorbents²⁰.

Chitosan-based incorporated Nanoparticles

In recent years, various studies have explored the synthesis of chitosan-based biogenic nanoparticles by incorporating different types of nanoparticles. This approach aims to enhance the properties and functionality of chitosan nanoparticles for efficient wastewater remediation. Here, we discuss the synthesis and adsorption potential of chitosan-based biogenic nanoparticles incorporated with other nanomaterials reported in the literature as shown in Table 3.

i. Metal Nanoparticles:

a. Silver Nanoparticles (AgNPs): The synthesis of chitosan-based biogenic nanoparticles incorporating silver nanoparticles has been widely investigated. One common method involves the reduction of silver ions using chitosan as a reducing and stabilizing agent. The resulting chitosan-silver nanoparticles exhibit antimicrobial properties, making them effective for the removal of bacteria and pathogens from wastewater⁷³.

b. Iron Oxide Nanoparticles (Fe₃O₄ NPs): Chitosan-based biogenic nanoparticles combined with iron oxide nanoparticles have been developed for the removal of heavy metals from wastewater. The synthesis typically involves the co-precipitation of chitosan and iron salts, followed by the reduction of the iron ions. The resulting chitosan-iron oxide nanoparticles exhibit excellent adsorption capacity for heavy metal ions, such as lead and cadmium⁷⁴.

c. Cobalt Ferrite Nanoparticles (CoFe₂O₄ NPs): Chitosan-based biogenic nanoparticles combined with cobalt ferrite nanoparticles have shown promising potential for wastewater treatment. The synthesis typically involves the co-precipitation of chitosan and metal salts, followed by the addition of ferrite precursors and subsequent annealing. The resulting chitosan-cobalt ferrite nanoparticles exhibit high adsorption capacity and magnetic responsiveness, enabling the effective removal of pollutants, particularly heavy metal ions⁷⁵.

ii. Carbon-based Nanoparticles:

a. Graphene Oxide Nanoparticles (GO NPs): Chitosan-based biogenic nanoparticles incorporating graphene oxide have gained attention for their excellent adsorption properties. The synthesis involves the dispersion of graphene oxide in a chitosan solution, followed by the crosslinking of chitosan chains to form nanoparticles. The resulting chitosan-graphene oxide nanoparticles possess a large surface area and high adsorption capacity for organic pollutants, including dyes and pharmaceutical compounds⁷⁶.

b. Carbon Nanotubes (CNTs): Chitosan-based biogenic nanoparticles combined with carbon nanotubes have been investigated for wastewater treatment applications. The synthesis

typically involves the dispersion of carbon nanotubes in a chitosan solution, followed by the formation of nanoparticles through crosslinking or precipitation methods. The resulting chitosan-carbon nanotube nanoparticles exhibit enhanced adsorption capacity for various pollutants, like heavy metals and organic compounds⁷⁷.

iii. Semiconductor Nanoparticles:

a. Titanium Dioxide Nanoparticles (TiO₂ NPs): Chitosan-based biogenic nanoparticles incorporating titanium dioxide nanoparticles have been widely studied for photocatalytic wastewater treatment. The synthesis typically involves the dispersion of TiO₂ nanoparticles in a chitosan solution, followed by the formation of nanoparticles through gelation or precipitation methods. The resulting chitosan-TiO₂ nanoparticles exhibit photocatalytic activity and can efficiently degrade organic pollutants under light irradiation⁷⁸.

b. Zinc Oxide Nanoparticles (ZnO NPs): Chitosan-based biogenic nanoparticles incorporating zinc oxide nanoparticles have also been explored for wastewater remediation. The synthesis involves the dispersion of ZnO nanoparticles in a chitosan solution, followed by the formation of nanoparticles through chemical or physical methods. The resulting chitosan-ZnO nanoparticles exhibit adsorption and photocatalytic properties, making them effective for the removal of pollutants in wastewater²⁰.

iv. Composite Nanoparticles:

a. Hybrid Nanoparticles: Chitosan-based biogenic nanoparticles can be synthesized by combining chitosan with multiple types of nanoparticles, creating hybrid systems with enhanced functionalities. For example, a chitosan-based hybrid nanoparticle could incorporate both metal nanoparticles and carbon-based nanoparticles, combining the adsorption and catalytic properties of different materials. This approach allows for synergistic effects, improving pollutant removal efficiency in wastewater treatment⁷⁹.

b. Core-Shell Nanoparticles: Chitosan-based biogenic nanoparticles can also be designed as core-shell structures, where a chitosan core is coated with a layer of another nanoparticle material. This design provides additional advantages such as controlled release of active components and protection of the core material from degradation. For example, a chitosan core coated with silver nanoparticles can provide both antimicrobial properties and adsorption capabilities for wastewater treatment⁸⁰.

v. Quantum Dots: Quantum dots (QDs) are semiconductor nanoparticles with unique optical and electronic properties. Incorporating QDs into chitosan-based biogenic nanoparticles offers exciting possibilities for pollutant removal and detection in wastewater. One approach involves the synthesis of chitosan-QD hybrid nanoparticles through electrostatic interactions

or covalent bonding. The resulting chitosan-QD nanoparticles can effectively adsorb heavy metals and organic pollutants, while the QDs provide a fluorescence-based detection platform for monitoring pollutant concentrations in real-time⁸¹.

vi. Clay Nanoparticles: Clay nanoparticles, such as montmorillonite and halloysite, possess a high surface area and a layered structure, making them suitable for pollutant adsorption. Chitosan-based biogenic nanoparticles incorporating clay nanoparticles have been developed for wastewater treatment. The synthesis typically involves the dispersion of clay nanoparticles in a chitosan solution, followed by crosslinking or gelation. The resulting chitosan-clay nanoparticles exhibit enhanced adsorption capacity for a wide range of pollutants, including heavy metals, dyes, and organic compounds⁸².

vii. Metal-Organic Frameworks (MOFs): Metal-organic frameworks are porous materials made up of metal ions or clusters coordinated with organic ligands. Combining MOFs with chitosan-based biogenic nanoparticles offers a synergistic effect for pollutant removal and degradation. The synthesis typically involves the encapsulation of MOFs within the chitosan matrix or the functionalization of chitosan with MOF particles. The resulting chitosan-MOF nanoparticles exhibit high surface area, porosity, and selective adsorption properties, making them efficient for removing various pollutants, such as volatile organic compounds, dyes, and heavy metals⁸³.

Drawbacks and Challenges of chitosan-based nanoparticles

Chitosan-based nanoparticles have gained attention in recent years as a promising solution for wastewater treatment due to their unique properties, including biocompatibility, biodegradability, and excellent adsorption capabilities. However, like any technology, they also come with their share of drawbacks and challenges. Below are some of the key limitations and obstacles associated with chitosan-based nanoparticles in wastewater treatment:

- i. Limited stability and agglomeration: Chitosan-based nanoparticles can exhibit poor stability in aqueous environments, leading to aggregation or precipitation. This can reduce their effectiveness in adsorbing contaminants and may require the addition of stabilizing agents.
- ii. Cost and production: The production of chitosan-based nanoparticles can be expensive, especially when obtaining high-quality chitosan from natural sources like crustacean shells. Additionally, the synthesis of nanoparticles often involves energy-intensive processes, increasing operational costs.

iii. Difficult recovery and regeneration: Once chitosan-based nanoparticles adsorb contaminants in wastewater, their recovery and regeneration for reuse can be challenging. Regenerating the nanoparticles typically requires harsh chemical treatments, which may decrease their efficiency over time and generate additional waste.

iv. Limited selectivity: Chitosan-based nanoparticles are generally effective at adsorbing a wide range of contaminants, but they may not exhibit high selectivity for specific pollutants. This lack of selectivity can lead to the removal of both harmful and beneficial substances from wastewater.

v. pH and ionic strength sensitivity: The adsorption capacity and stability of chitosan-based nanoparticles can be influenced by the pH and ionic strength of the wastewater. These sensitivity issues may limit their applicability in treating wastewater with varying chemical compositions.

vi. Competitive adsorption: In complex wastewater matrices, chitosan-based nanoparticles may compete with other ions and molecules for adsorption sites. This competition can reduce their efficiency in removing target pollutants.

vii. Limited scalability: While chitosan-based nanoparticles have shown promise in laboratory studies, scaling up their production and application for industrial wastewater treatment can be challenging. Ensuring consistent performance and cost-effectiveness at larger scales is a significant hurdle.

Conclusion and Future Research Directives

Chitosan-based nanoparticles offer versatile functions for wastewater remediation owing to their unique properties, including biodegradability, biocompatibility, and high surface area. Various synthesis methods have been explored to fabricate chitosan-based nanoparticles, incorporating different types of nanoparticles, such as metal nanoparticles, metal oxides, or carbon-based nanoparticles. Characterization techniques have been employed to assess the physicochemical properties, including size, shape, surface area, and porosity, of chitosan-based nanoparticles. The efficiency of chitosan-based nanoparticles in pollutant removal has been demonstrated through their applications in heavy metal removal, dye degradation, and organic compound adsorption. The mechanisms involved in pollutant adsorption and degradation by chitosan-based nanoparticles have been elucidated, including chemical interactions, electrostatic attraction, and catalytic processes. The significance of chitosan-based biogenic nanoparticles in wastewater remediation cannot be overstated. They offer a sustainable and cost-effective solution for the removal of various pollutants from wastewater,

contributing to the protection of our environment and human health. The unique properties of chitosan-based nanoparticles, coupled with their ability to be synthesized with different nanoparticles, enable tailored approaches for specific pollutant removal. In light of the findings presented in this review, several suggestions for further research can be proposed. Firstly, exploring the potential of chitosan-based nanoparticles in the removal of emerging contaminants and microplastics is an important area of investigation. These pollutants pose significant challenges to conventional wastewater treatment methods and require innovative approaches for effective removal. Additionally, the development of scalable synthesis techniques, optimization of process parameters, and integration of chitosan-based nanoparticles with other treatment technologies should be further explored to enhance their applicability in real-world wastewater treatment systems. Furthermore, comprehensive studies on the long-term environmental impacts and the fate of chitosan-based nanoparticles in wastewater treatment are essential. Understanding their potential accumulation, biodegradability, and ecotoxicity is crucial to ensure their safe and sustainable use.

Figures:



Fig. 1: Various shells and cell walls sources of biopolymer chitosan

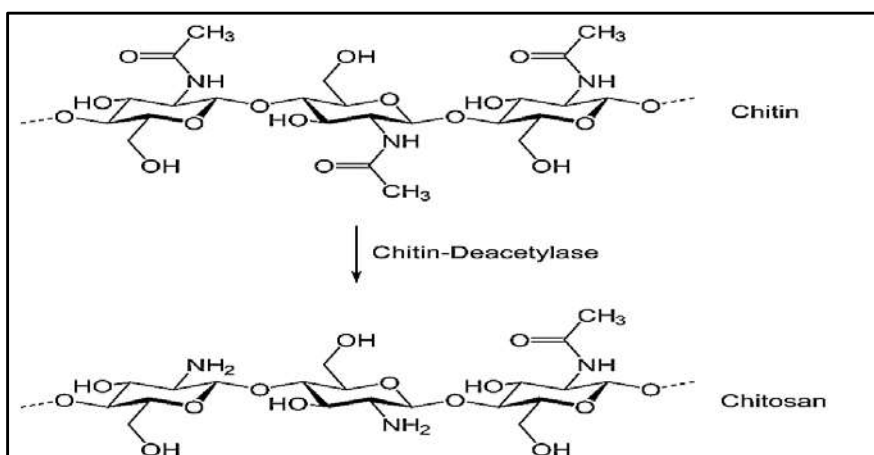


Fig. 2: The structural configuration of Chitin and Chitosan biopolymer

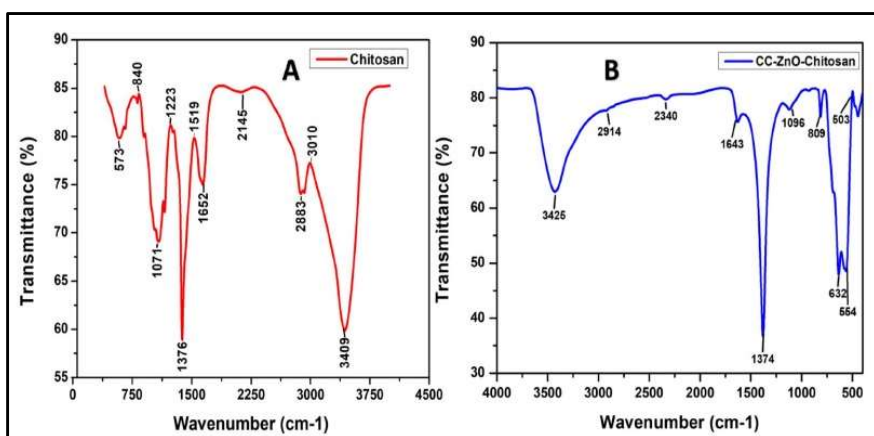


Fig. 3: FTIR spectra of synthesized nanomaterials. A) Chitosan, (B) CC-ZnO-Chitosan²⁰.

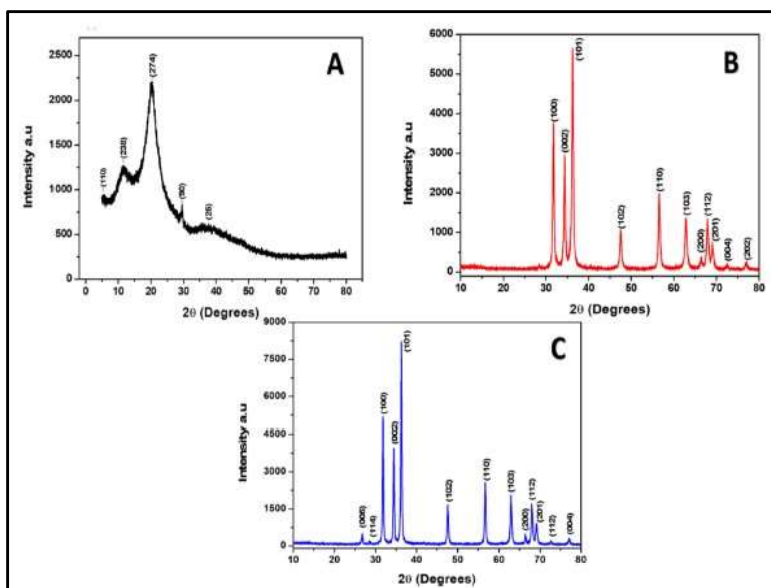


Fig. 4: XRD spectra of a synthesized nanomaterial. A) Chitosan, (B) ZnO, (C) ZnO-Chitosan²⁰

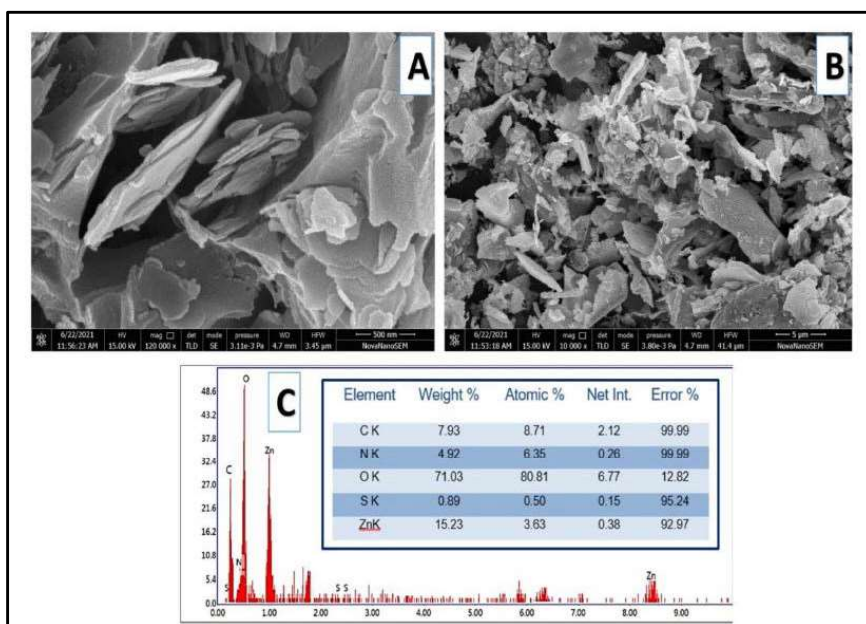


Fig. 5: A) FE-SEM of synthesized CC-ZnO-Chitosan. B) EDX of Synthesized ZnO-Chitosan²⁰

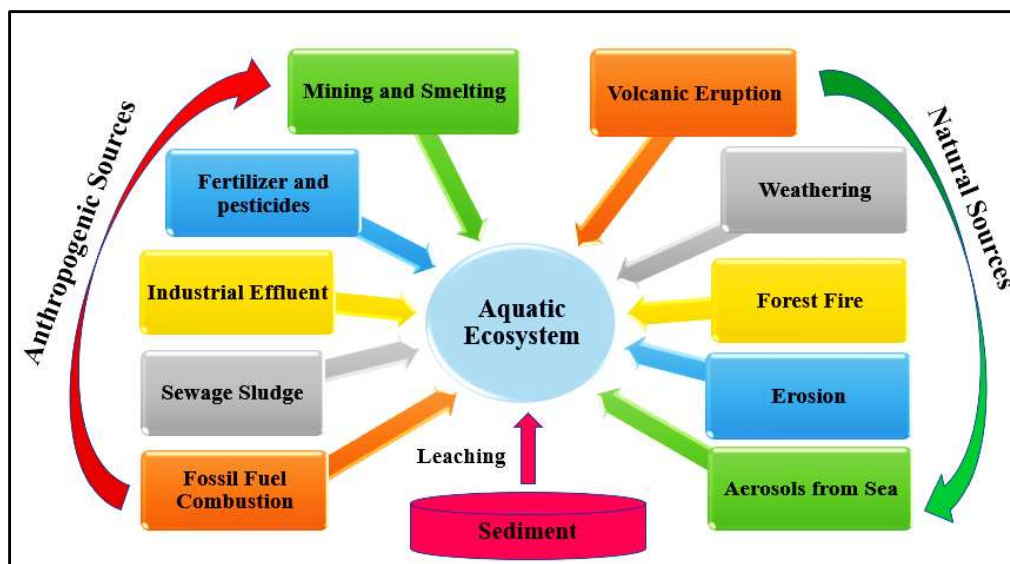


Fig. 6: Sources of heavy metals pollution in the aquatic ecosystem

Tables:

Table 1: Different types of chitosan and their characteristics

Chitosan Properties	α -Type Chitosan	β -Type Chitosan	γ -Type Chitosan	References
Chitosan source	Shrimps and Crabs	Squid's pens	Fungi cell wall	84
Degree of crystallinity	Moderately Lower crystallinity	Higher crystallinity	Moderately lower	85
Rate of stability	Higher stability rate	Least stability rate	Lower stability rate	86
Medium of solubility	Soluble in acid medium	Soluble in acid medium	Soluble in acid medium	87
Molecular weight volume	Highest volume	Higher volume	Lower volume	7
Structural shape	Strands are antiparallel	Strands are Parallel	Contain both parallel antiparallel strands	84
Rate of reactivity	More reactive	Most reactive	More reactive	88

Table 2: Preparation techniques for synthesizing chitosan nanoparticles

Technique	Principle	Merit (s)	Demerit (s)	References
Spray drying	Atomization	simple and speedy process, other drying or separation steps not required	particle size is large, incompatible with sensitive temperatures, substances	⁸⁹
Emulsification and crosslinking	Covalent crosslinking	Simple processing	harmful chemicals involved	90
Phase inversion precipitation	Precipitation	High encapsulation of some compounds	shear force is required, harmful chemicals involved	91
Reversed micelles	Covalent crosslinking	NPs Ultrafine less than 100 nm	Complex application, harmful chemicals involved, Time-consuming process	92
Ionic gelation with radical polymerization	Polymerization and crosslinking	-	Complex application, Time-consuming process	93
Supercritical-CO ₂ -assisted solubilization and atomization (SCASA)	Atomization	Non-harmful solvent, other drying or separation steps not required	Complex application, Time-consuming, harmful chemicals involved, specially designed system required, large particle size involved	94
Emulsion-droplet coalescence	Precipitation	-	high shear force required, harmful chemicals involved,	95
Self-assembly	Hydrophobic and/or electrostatic interaction	NPs Highly stable, with mild chemicals involved, and Modifiable procedure	Difficulty in controlling at large scale	96

Ionic gelation	Ionic crosslinking	Simple process, mild Chemicals involved, NPs adjusting	-	97
Top-down	Acid hydrolysis and deacetylation	-	Complex application, Time-consuming, extra step for drug loading is required	98

Table 3: Chitosan-based incorporated nanoparticles and removal efficiency from literature

Adsorbent	Adsorbate	Adsorption Capacity (mg/g)	Reference
ZnO/CHS	Methylene Blue	99%	20
CdO/NiO	Rhodamine B	90%	67
CSGO aerogels	Methyl orange	686.89	99
	Amido black 10B	573.47	99
ZnO-Chitosan	Malachite green	54%	100
Ce-ZnO-Chitosan	Malachite green	87%	100
Fe ₃ O ₄ /GO/CHS	Reactive Blue 19	102.06	101
Fe ₃ O ₄ -CHS	Pb(II)	78.58 -487.5	71
CHS/Fe ₃ O ₄	Cr(VI)	162	102
Fe ₃ O ₄ /GO/CHS	Ni(II)	80.48	101
Fe ₃ O ₄ /CHS	Cd(II), Pb(II)	36.42, 79.24	103
SiO ₂ @CHS	As(V), Hg(II)	198.6, 204.1	104
CHS-EDTA	Pb(II), Cd(II), Cu(II)	370.37, 243.90, 227.27	105
TiO ₂ /CHS	Cu(II), Pb(II)	526.5-715.7, 475.5-579.1	106
Fe ₃ O ₄ @CHS@DMSA	Cd(II)	314.12	81

Magnetic xanthate CHS /polyacrylic acid	Cu(II), Cd(II), Pb(II), Co(II)	178, 168,140	107
3,3-diphenylpropylimine methyl benzaldehyde / CHS	Pb(II)	230.48	108
Glucan/CHS	Pb(II), Cu(II), Co(II), Cd(II), Ni(II)	395, 342, 232, 269, 184	109
NOCC-MNPs/CHS-citrate gel beads (CCGBs)	Cu(II)	294.11	110
CHS grafted with methyl methacrylate (M-CS)	Cu(II)	192.31	111
CHS/calcium alginate /bentonite	Pb(II), Cu(II), Cd(II)	434.89, 115.30, 102.38	112
CHS/g-acrylamide	Cr(VI), Cu(II)	178.34, 181.88	113
CHS grafted UiO-66-NH ₂	Pb(II), Cu(II)	555.56, 364.96	114
CHS microspheres	Cu(II)	75.52	115
AgNPs/GO/CHS	Mn(II)	1605	116
CHS -carboxyl groups	Cu(II), Zn(II)	220.5, 124.3	117
CHS microspheres/sodium alginate hybrid beads	Pb(II), Cr(VI)	180, 16	118
Magnetic thiolated /quaternized-CHS	Pb(II), As(V), Hg(II), As(III), Cu(II)	235.63, 66.27, 28.00, 67.69, 33.99	119

References

1. M. A. Onu, O. O. Ayeleru, B. Oboirien, and P. A. Olubambi. *Envi. Chall.* 10(6),86, 2023.
2. P. Rajasulochana, and V. Preethy. *Res. Tech.* 9(4), 69, 2016.
3. D. Dutta, S. Arya and S. Kumar. *Chemos.* 13(1), 245, 2021.
4. M. C. Goci, T. Leudjo, A. L. Martin and M. J. Klink. *Polym.* 15(3), 482, 2023.
5. E. I. Rabea, M. E. T. Badawy, C. V. Stevens, G. Smagghe and W. Steurbaut, 34(1), 30, 200.
6. D. C. Alves, B. Healy, L. A. Pinto, T. R. Cadaval and C. B. Breslin. *Mole.* 26(3), 594, 2021.
7. S. Kou, L. M. Peters and M. R. Mucalo. *Int. Jou. Biol Macromol.* 12(5), 784 2020.
8. L. Bastiaens, L. Soetemans, E. D'Hondt and K. Elst, Sources of chitin and chitosan and their isolation. Wiley Eastern Limited, edn. 4 (2019).
9. S. Kumari, P. Rath, S. H. Kumar and T. N. Tiwari. *Environ. Tech. Innov.* 6(25), 476, 2015.
10. D. Sakthivel, N. Vijayakumar and A. V. Hum. *Journals Res. Artic.* 13(5),2015.
11. M. A. García, P. Nilia, C. Castro, L. J. Rodríguez, M. R. Robin, Z. P. Gañán, C. J. Alicia *Radiat. Res. Appl. Sci.* 13(4), 2015.
12. L. Handayani, F. Syahputra, and A. Y. J. Kim. *Sains. dan Apl.* 21(4), 224-231 2018.
13. N. E. A. El-Naggar, W. E. A. Saber, A. M. Zweil and S. I. Bashir. *Sci. Rep.* 4 (15), 98, 2022.
14. M. Malerba and R. Cerana. *Int. Jou. of Mol. Sci.* 17(7), 996, 2016.
15. Z. K. Jugovic, Z. Petronijevic and A. Smelcerovic, *Bio. Act. and App.* 14(3), 981, 2010.
16. M. Yanat, and K. Schroën, *React. Funct. Polym.* 10(4), 849, 2021.
17. U. M. S. P. Perera, and N. Rajapakse 46(14), 9590, 2014.
18. S. Boruah and P. Dutta, *Indian Phytopathol.* 42(3), 60, 2021.
19. M. Iriti, and E. M. Varoni, *Environ. Sci. Pollut. Res.* 11(3), 56, 2015.
20. U. L. Usman, N. B. Singh, B. K. Allam and S. Banerjee, *Macrom. Sym.* 20(21), 357, 2023.
21. G. Ilangumaran, G. Stratton, S. Ravichandran, P. S. Shukla, P. Potin, S. Asiedu, and B. Prithiviraj. *Front. Microbiol.* 7(81), 593, 2017.

22. M. Abhinaya, R. Parthiban, P. S. Kumar and D. V. N. Vo, *Environ. Res.* 11(9), 96, 2021.
23. X. Jingwen, X. Wancui, J. Yu, R. Xin, Z. Shi, L. Song and X. Yang *Front. Microbiol.* 67(7), 126, 2021.
24. I. Younes and M. Rinaudo. *Marine Drugs* 13(3), 133, 2015.
25. S. C. M. Queiroz, A. Rayane, B. R. L. F. Paschoal, Lima, V. A. O. Í. de Farias R. Raid, E. P. N. Lima, R. J. L. da Silva, C. P. C. Andrés, and M. V. Lia Fook. *Mar. Drugs* 15(5), 141, 2017.
26. G. A. Martau, M. Mihai and D. C. Vodnar. *Polymers.* 11(18), 37, 2019.
27. N. A. Negm, H. H. H. Hefni, A. A. A. Abd-Elaal, E. A. Badr & A. Kana, M. T. H. *Int. Jou. of Bio. Macromo.* 2(1), 96, 2020.
28. S. Islam, M. A. R. Bhuiyan and M. N. Islam. *Jou. of Poly. and the Env.* 10(9), 24, 2017.
29. S., Syamdidi, H. Sidauruk, A. Munandar, and S. Haryati *Web of Conferences.* 14(7), 3025, 2020.
30. X. Li, H. Xu, Z. S. Chen and G. Chen, *Jou. of Nanoma.* 27(9), 74, 2011.
31. B. Andujar, Dr. thesis, UCL (University Coll. London). 2014.
32. S. Ying, Z. Guan, P. C. Ofoegbu, P. Clubb, C. Rico, F. He, J. Hong. *Env. Tech. and Inn.* 10(2), 336, 2022.
33. P. Mohanpuria, N. K. Rana and S. K. Yadav. *Jou. of Nanop. Res.* 11(5), 79275, 2008.
34. P. Dutta, and P. K. Kaman. *Int. J. Curr. Microbiol. Appl. Sci.* 60(6), 336, 2017.
35. D. Sharma, S. Kanchi and K. Bisetty. *Arab. Jou. of Chem.* 20(15), 11002, 2019.
36. K. Guzmán, B. Kumar, M. J. Vallejo, M. Grijalva, A. Debut, L. Cumbal. *Prog. Org. Coatings* 10(10), 1568, 2019.
37. C. Thambiliyagodage, M. Jayanetti, A. Mendis, G. Ekanayake, H. Liyanaarachchi, S. Vigneswaran. *Materials* 60(5), 2073, 2023.
38. L. Pellenz, C. R. S. de Oliveira, A. H.S. Júnior, L. J. S. Silva, L. Silva, A. A. U. Souza, S. M. Arruda, G. U. de Souza, F. H. Borba, A. Silva. *Sep. and Pur. Tech.* 12(2) 435, 2023.
39. M. M. López, A. R. Moraga, A. J. L. Jimenez, J. C G. Martínez, O. Ahrazem, L. G. Gómez, E. Niza *Carbohydr. Polym.* 11(8), 815, 2022.
40. S. Mourdikoudis, R. M. Pallares and N. T. K. Thanh, *Nanoscale* 21(2), 785, 2018.
41. F. Brunel, N. E. El Gueddari and B. M. Moerschbacher *Carb. Polym.* 20(12), 1025, 2013

42. W. Wong-Ng, H. F. McMurdie, C. R. Hubbard and A. D. J. Mighell Res. Natl. Inst. Stand. Technol. 10(6), 152, 2001.
43. M. Gonon, Encyclopedia of Materials: Techn. Ceram. and Glass. 81(8), 542, 2021.
44. J. W. Rhim, Hong, S. I., Park, H. M. and Ng, P. K. W. J. Agric. Food Chem. 6(6), 58, 2006.
45. A. Parupudi, S. H. R. Mulagapati and J. A. Nanoparticle Therapeutics 82(7), 574, 2022.
46. S. P. Facchi, D. B. Scariot, P. V. A. Bueno, P. R. Souza, L. C. Figueiredo, H. D. M.Follmann, C. S. Nunes, J. P. Monteiro, E. G. Bonafé, C. V. Nakamura, E. C. Muniz, A. F. Martins Int. J. Biol. Macromol. 10(16), 363, 2016.
47. T. Banerjee, Mitra, S., K. Singh, A., K. Sharma, R. & Maitra, A. Int. J. Pharm. 267(3), 378- 5173 2002.
48. V. Saharan, A. Mehrotra, R. Khatik, P. Rawal, S.S. Sharma, A. Pal Int. J. Biol. Macromol. 31(12), 236, 2013.
49. R. C. Choudhary, R. V. Kumaraswamy, S. Kumari, S. S. Sharma, A. Pal, R. Raliya, P. Biswas and V. Saharan Sci. Rep. 41(5), 98, 2017.
50. T. Jain, Srivastava, K., S. Kumar and P. K. Dutta, Plant Sciences 10(16), 9739, 2022.
51. R. C. Choudhary, R. V. Kumaraswamy, S. Kumari, A. Pal, R. Raliya, P. Biswas & V. Saharan. Nanotechnology 97(8), 981, 2017.
52. A. Dev, N. S. Binulal, A. Anitha, S.V. Nair, T. Furuike, H. Tamura, R. Jayakumar Carbohydr. Polym. 20(9), 12040, 2010.
53. S. F. Hosseini, Zandi, M., Rezaei, M. and Farahmandghavi, F. Carbohydr. Polym. 10(16), 684, 2013.
54. S. E. Harding, Methods Mol. Biol. 8(9), 603, 1994.
55. J. Stetefeld, S. A. McKenna, and T. R. Patel, Biophysical Reviews 25(5), 60218, 2016.
56. G. L. yin, Jiang, Y. ren, Huang, K. long, Ding, P. and Chen, J. J. Alloys Compd. 6(13), 270, 2008.
57. S. C. Wilschefski, and M. R. Baxter, Clin. Biochem. 3(3), 176, 2019.
58. M. Palomo-Siguero, P. Vera, Y. Echegoyen, C. Nerin, C. Cámara, Y. Madri. Spectrochim. Acta - Part B At. Spectrosc. 6(7), 239, 2017.
59. S. Boufi, Vilar, M. R., Ferraria, A. M. and Botelho do Rego, A. M. Colloids Surfaces Physicochem. Eng. Asp. 16(2), 836.

60. A. Trapani, E. De Giglio, D. Cafagna, N. Denora, G. Agrimi, T. Cassano, S. Gaetani, V. Cuomo, G. Trapani *Int. J. Pharm.* 10(16), 7036, 2011.
61. M.J Cal-Prieto, M Felipe-Sotelo, A Carlosena, J. M. Andrade, P López-Mahía, S Muniategui, D. P. Talanta. 39(9), 140434, 2002.
62. D. J. Butcher, *Encyclopedia of Analytical Science: Second Edition* 36(9), 397, 2004.
63. Y. A. Azarova, Pestov, A. V., Ustinov, A. Y. and Bratskaya, S. Y. *Carbohydr. Polym.* 6(15), 7086, 2015.
64. X. D. Liu, Tokura, S., Haruki, M., Nishi, N. and Sakairi, N. *Carbohydr. Polym.* 10(6), 8617, 2002.
65. M. S. Islam, Hossain, M. B., Matin, A. and I. Sarker, M. S. *Chemosphere* 8(3), 771, 2018.
66. L. Zhang, Zeng, Y. and Z. Cheng. *Journal of Molecular Liquids* 10(16), 654, 2015.
67. S. A. Qamar, Ashiq, M., Jahangeer, M., Riasat, A. and Bilal, M. *Chem. Environ. Eng.* 10(21), 3682, 2020.
68. A. M. Eltaweil, H. G. Omer, N. M. G. El-Aqapa, F. A. Nour, M. G. El-Subruiti, M. S. Mohy-Eldin, E. M. Abd El-Monaem. *Carbohydrate Polymers* 16(1), 8671, 2021.
69. M. Másson *Polymer Science* 10(11), 712, 2021.
70. E. M. Abd El-Monaem, A. S. Eltaweil, H. M. Elshishini, M. Hosny, M. M. Abou Alsoaud, Nour F. Attia, G. M. El-Subruiti, A. M. Omer. *Arab. Jou. of Chem.* 16(2), 103743, 2022.
71. U. L. Usman, Singh, N. B., Allam, B. K. and Banerjee, S. *Mater. Today Proc.* 2(2), 2311, 2022.
72. A. Benettayeb, F. Seihoub, P. Pal, S. Ghosh, M. Usman, C. Hua Chia, M. Usman and M. Sillanpää *Nanomaterials* 30(3), 447, 2023.
73. Y. Yu, Z. Zhou, G. Huang, H. Cheng, L. Han, S. Zhao, Y. Chen, F. Meng. *Water Research* 11(8), 901, 2022.
74. T. A. Aragaw, Bogale, F. M. and Aragaw, B. A. *Journal of Saudi Chemical Society* 10(1), 280, 2021.
75. V. Srivastava, Kohout, T. and Sillanpää, M. J. *Environ. Chem. Eng.* 16(6), 1342, 2016.
76. A. K. Sarkar, J. K. Bediako, J. W. Choi and Yun, Y. S. *NPG Asia Mater.* 41(4), 27018, 2019.
77. B. Arora and P. Attri, *Journal of Composites Science* 4(3), 135, 2020.

78. V. L. Punzi, V. Z. Kungne and Skaf, D. W. *Environ. Prog. Sustain. Energy* 13(4), 314, 2020.
79. M. Martínez-Cabanas, L. M., J. L. Barriada, R. Herrero and Sastre de Vicente, M. E. *Chem. Eng. J.* 16(4), 149, 2016.
80. K. S. Prasad, Gandhi, P. and K. Selvaraj, *Appl. Surf. Sci.* 140(9), 2442, 2014.
81. J. S. Algethami, A. A. Alqadami, S. Melhi, M. A. M. Alhamami, A. M. Fallatah, and M. A. Rizk. *Adsorp. Sci. & Techn.* 22(4), 8249, 2022.
82. S. Biswas, J. Fatema, T. Debnath and T. U. Rashid, *ACS ES and T Water* 2(11), 2075, 2021.
83. K. Tan, N.R Nijem, Y. Gao, S. Zuluaga, J. Li, T. Thonhauser and Y. J Chabal. *Cryst Eng Comm* 10(3), 940140, 2015.
84. L. D. Fernando, M. C. D. Widanage, J. P., Andrew S L., N. Washton, J. P. Latgé, P. Wang, L. Zhang, T. Wang *Front. Mol. Biosci.* 7(2), 7053, 2021.
85. M. Ioelovich, *Research and Reviews: Journ. of Chem.* 3(3), 938, 2014.
86. H. K. No, Kim, S. H., Lee, S. H., N. Y. Park, and W. Prinyawiwatkul, *Carbohydr. Polym.* 1(2), 1036, 2006.
87. D. P. Chattopadhyay and M. S. *Int. J. Polym. Sci.* 9(3), 9536, 2010.
88. M. B., Kaczmarek, S. K. Swita, Li, X., S. M. Antczak and M. Daroch, *Front. in Bioeng. and Biotech.* 2(4) 336, 2019.
89. E. Başaran, E. Yenilmez, M. S. Berkman, G. Büyükköroğlu, and Y. A. J. Yazan, *Micro.encapsul.* 8(5), 839, 2014.
90. Q. Wang, T., Cochrane, C. and McCarron, P. *Coll. Surf. B Bioint.* 10(1) 62024, 2005.
91. A. Grenha, *Journ. of Drug. Targ.* 6(5), 4121, 2012.
92. M. H. Kafshgari, M. Khorram, M. Mansouri, A. Samimi, and S. Osfour. *Iran. Polym. J. English Ed.* 13(7), 265, 2012.
93. S. Sajeesh, and C. P. J. Sharma, *Biomed. Mater. Res.* 3(3), 726, 2006.
94. N. Hijazi, N. Le Moigne, E. Rodier, M. Sauceau, T. Vincent, J. Benezet, J. Fages *Polym. Eng. Sci.* 2(4), 983, 2019.
95. H. Ichikawa, H. Tokumitsu, M. Miyamoto, and Y. Fukumori *Nanot. for the Lif. Sci.* 9(7), 835, 2003.
96. J. P. Quiñones, H. Peniche, and C. Peniche, *Polymers* 3(3), 30235, 2018.
97. W. Fan, Yan, W., Xu, Z. and Ni, H. *Coll. Surf. B Biointerfaces* 10(1), 62011, 2012.
98. R. N. Wijesena, N. Tissera, Y. Y. Kannangara, Y. Lin, G. A. J. Amaratunga, K. M. Nalin de Silva *Carbohydr. Polym.* 10(1), 62014, 2015.

99. Y. Wang, G. Xia, C. Wu, J. Sun, R. Song, W. Huang *Carbohydr. Polym.* 10(1), 1409, 2015.
100. A. M. Saad, M. R. Abukhadra, S. Abdel-Kader Ahmed, A. M. Elzanaty, A. H. Mady, M. A. Betiha, J. Shim, A. M. Rabie. *J. Environ. Manage.* 1(10), 3043, 2020.
101. T. T. Nhi Le, V. Thuan Le, M. U. Dao, Q. V. Nguyen, T. Thu Vu, M. H. Nguyen, D. L. Tran & H. Sinh Le *Chem. Eng. Commun.* 20(6), 1337–1352, 2019.
102. S. M. Pourmortazavi, H. Sahebi, H. Zandavar, and S. Mirsadeghi, *Compos. Part B Eng.* 10(7), 130, 2019.
103. H. L. Fan, S. F. Zhou, W. Z. Jiao, G. S. Qi and Y. Z. Liu. *Carbo. Polym.* 1(7), 7050, 2017.
104. J. Liu, Y. Chen, T. Han, M. Cheng, W. Zhang, J. Long and X. Fu. *Chemos.* 8(9), 172, 2019.
105. M. Verma, W. Ahmad, J. Park, V. Kumar, S. Mikhail, D. Vlaskin, G. Vaya and H. Kim *J. Water Process Eng.* 10(2), 989, 2022.
106. A. Razzaz, S. Ghorban, L. Hosayni, M. Irani, and M. J. Aliabadi. *Taiwan Inst. Chem. Eng.* 5(6), 5613, 2016.
107. L. Dong, C. Shan, Y. Liu, H. Sun, B. Yao, G. Gong, X. Jin, and S. Wang. *Int. J. Environ. Res. Public Health* 19(17), 11123, 2022.
108. S. Shahraki, Delarami, H. S., Khosravi, F. and Nejat, R. *J. Colloid Interface Sci.* 20(10), 5006, 2020.
109. C. Jiang, X. Wang, G. Wang, C. Hao, X. Li and T. Li. *Compos. Part B Eng.* 1(9), 3082, 2019.
110. Mi, F. L., Wu, S. J. and Chen, Y. C. *Carbohydr. Polym.* 1(5), 6031, 2015.
111. Z. A. Sutirman, E. A. Rahim, M. M. Sanagi, K. J. Abd Karim. and W. A. Wan Ibrahim. *Int. J. Biol. Macromol.* 20(3), 4115, 2020.
112. Z. Lin, Yang, Y., Liang, Z., Zeng, L. and Zhang, A. *Polymers (Basel).* 13(11), 1891, 2021.
113. S. Pavithra, G. Thandapani, S Sugashini, P.N. Sudha, H. Hussein, A. F. Alkhamis, M. H. Almutairi. *Chemos.* 12(9), 415, 2021.
114. S. Hu, Z. Huang, T. Zhang, N. Lei, Y. Zhou and Y. Wang. *Carbohydr. Polym.* 11(8), 809, 2022.
115. B. Wang, Z. Bai, H. Jiang, P. Prinsen, R. Luque, S. Zhao and J. Xuan *J. Hazard. Mater.* 18(10), 3024, 2019.

116. A. El Shahawy, M. F. Mubarak, M. El Shafie and H. M. Abdulla, RSC Adv. 20(4), 693, 2022.
117. A. M. Abu El-Soad, G. Lazzara, M. O. Abd El-Magied, G. Cavallaro, J. S. Al-Otaibi, M. I. Sayyed, and E. G. Kovaleva Int. J. Mol. Sci. 2(3), 42396, 2022.
118. E. H. Ablouh, Z. Hanani, N. Eladlani, M. Rhazi. and M. Taourirte. Sustain. Environ. Res. 42(8), 34019, 2019.
119. X. Song, Li, L., Zhou, L. and Chen, P. Chem. Eng. Res. Des. 8(6), 1425, 2018.