Chitosan Hydrogel for Removing of Heavy Metal Ions from Water: A Review

Maysaa A. Ali a, Shemaa A. Soud b*, Anaheed H. Hameed c

a University of Technology, Department of Applied Science, Division of Biotechnology /Baghdad-Iraq
Maysaa.A.Ali@uotechnology.edu.iq
b University of Technology, Department of Applied Science, Division of Biotechnology /Baghdad-Iraq
Shemaa.A.Soud@uotechnology.edu.iq
c University of Technology, Department of Applied Science, Division of Biotechnology /Baghdad-Iraq
Anaheed.H.Hameed@uotechnology.edu.iq

*Corresponding author.

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A B S T R A C T

Industrial and agricultural discharges have been shown as important sources for a wide range of contaminants like heavy metals. This study focused on the assessment of the toxic effect of heavy metals and illustrates some methods of eliminating or avoiding these contaminants. In certain methods, heavy metals (Cu, Zn, Cr, Ni, Pb, Hg, and Cd) are recovered, and range of absorbents are tested. A number of studies have reported the complex interaction of polymers with metal ions with the polymers for eliminating heavy metals (e.g. cellulose, chitosan, and chitin). Metal ions are absorbed by these polymers by the amine and hydroxyl groups. They have unique features that incorporate polyxysalt arrangement, capacity to frame films, optical underlying qualities, and chelates metal particles. Chemical bonds, pH, crystallinity, and deacetylation are controlling the mechanism and performance of the absorption. Some methods increase the efficiency, selectivity, and quality of chitosan: the first is through chemical modification employing grafting and crosslinking, and the second by physical modification, such as manufacturing gel granules and reducing crystallization. This study aims to investigate the removal of heavy metals from water by the sorption method and to study and analyze literature dealing with this topic using chitosan hydrogel.

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1. INTRODUCTION

The increased exploitation of heavy metals, colloids, and smaller organic substances, for more than 20 toxic metals, has caused an increase in the level of harmful minerals flowing into the water [1]. The major risk lies in industrial wastewater resulting from some industries, such as fertilizers, batteries, thermoplastics, dyes, etc., because it is often filled with heavy metals (zinc, nickel, copper, mercury, lead, cadmium, and chromium) and is very harmful if it flows into the water or is...
discharged into nature induced by humans even at low concentrations, such contaminants have five times the gravity of water [2].

Such toxic metals are hard to degrade biologically, so they accumulate in nature. To complete this process requires an expensive treatment for gaining pure useful water for local usage [3]. There are classic techniques used to remove heavy metals from solutions and reduce environmental pollution. They are as follows: Solvent extraction, chemical and biological absorption [4], co-precipitation and precipitation [5], ion exchange [6], and electrochemical reduction [7].

Among these techniques, the adsorption method is used by using adsorbents to absorb organic materials and remove their ions because of the possibility of applying them on a large scale at reduced costs and replacing carbon with them because its cost is high compared to other types of adsorbents [8]. Some of these materials are chitin, rice husk ash, modified flax husks, chitosan, waste biomass, and others, in addition to materials that are still under development into new adsorbents [9].

Several basic parameters need to be taken into consideration in wastewater that must be measured by the municipality and they are chemical oxygen demand, alkalinity, pH, phosphorous and its compounds, conductivity, solids and suspended matter, organic carbon [10]. In recent, chitosan has been utilized as a crude material to synthesize the hydrogels in widely potential and reasonable applications like wastewater treatment [11].

Chitosan-based hydrogels are adjusted in chemical and physical methods through crosslinking [3], joining, impregnating, consolidating of hard fillers, blends, interpenetration, and ion-imprint techniques to be mechanically improved and of enhanced absorption [12]. Comprehension of such techniques gives helpful data in the plan of proficient chitosan-based hydrogels and the selection of suitable pollutants to remove.

This study aims to investigate the removal of heavy metals from wastewater by adsorption method and to study and analyze all kinds of literature dealing with this topic using chitosan hydrogel.

2. CHITOSAN

The use of ion exchange, chemical, or oxidation, or chemical precipitation to remove heavy metals is not feasible because such methods cause environmental pollution due to the synthetic chemical product used in these methods, such as sulfates and polymers [13, 14]. Therefore, "greener" alternatives such as chitin and chitosan are better than physical and chemical methods due to plenty of reactive functional groups (-NH2 and - OH) their biodegradability, toxicity, and flocculation potential [15]. Saifuddin and Kumaran (2005), concluded that the chitosan coating on acid-treated palm-peel charcoal acts as an inexpensive and environmentally safe bio-absorbent with high efficiency [16].

Sokker et al. (2011) reported the synthesis of the chitosan-polyacylamide synthesis through gamma radiation to adsorb raw petroleum from an aquatic solution. The outcomes showed an eliminating efficiency of the advanced hydrogel (2.3 g/g) of raw petroleum at pH-3 [17]. Zhuang and co-workers (2018) prepared the chitosan-g-maleic acid by gamma radiation for the adsorption of Co ions from solutions and the adsorption limit of chitosan was 2.78 mg/g [18].

Hasan et al. (2018) synthesized chitosan graft copolymer with poly[2-(acryloyloxy) ethyl trimethylammonium chloride] within the existence of potassium persulphate initiator using redox polymerization for antimicrobial application. It was discovered that the antimicrobial action and water dissolvability were upgraded after grafting [19].

Wang and colleagues (2019) successfully removed toxic metal ions from the water by developing a cytosine polyethyleneamine absorbent using surfactant emulsion techniques and modifying the target host group. This is a very efficient experience, time, cost, and effectiveness [20].

Remu and Rahman (2020) analyzed the mechanisms and variables of the process of removing chromium leaked into the water by using chitosan nanocomposites and focusing on their absorption capacity and the stability of adsorption temperature to remove toxic chromium from solution and wastewater [21].

3. PROPERTIES OF CHITOSAN

Chitin and chitosan (C56H103N9O39/ 1526.5 g/mol) are the normally happening polysaccharides (see Figure 1). They have special features that incorporate polyxysalt arrangement, capacity to frame films, optical underlying qualities, and chelates metal particles [22]. Chitin is exceptionally
hydrophobic and it is insoluble in water and most natural solvents. Chitosan is solvent in hexafluoro-isopropanol, hexafluoroacetone, chloroalcohols information with fluid arrangements [23].

C. Rouget initially found the chitosan in 1859. A typical strategy for the union of chitosan is the deacetylation of chitin utilizing sodium hydroxide in abundance as a reagent and water as a dissolvable [24]. Chitosan has restricted utilization of water treatment because of its low mechanical strength and adaptable conduct. Chitosan has disadvantages like acidic solvency, low warmth, and mechanical steadiness. To defeat these disadvantages, chitosan can be adjusted actually and synthetically additionally [25]. In chitosan, the amino gathering is free and contains 8.7% of nitrogen. In contrast to chitin, the dominant part of the acetyl bunches has been eliminated in chitosan (by and large over 70%). It has the same β-(1,4)-D-glucopyranose units’ spine that of cellulose, except for the 2-hydroxy is supplanted by an acetamide gathering. Chitin and chitosan are high sub-atomic weight biopolymers of glucosamine and N-acetylg glucosamine [26].

![Figure 1: Structure of chitin and chitosan.](image)

The biological and chemical properties are illustrated in Figure 2 [27].

![Figure 2: The biological and chemical properties of chitosan [27].](image)

4. HEAVY METALS ENVIRONMENTAL IMPACT

In the treatment of industrial wastewaters, heavy toxic metals such as zinc, copper, nickel, arsenic, cadmium, and chroma are especially relevant. Zinc is an important trace factor for the
wellbeing of humans. This is essential as it controls many biochemical processes for the physiological functions of living tissue. However, too much zinc can cause significant problems in health such as pressures on the stomach, skin irritations, vomiting, nausea, and anemia [28]. In animals, metabolism copper does an important function. However, the heavy consumption of copper causes severe toxics, like vomiting, cramps, seizures, or even death [29]. Apart from gastrointestinal pain, pulmonary fibrosis, and skin dermatitis, and it is understood that nickel is a human carcinogen, nickel that reaches its essential level may cause significant lung or kidney problems [30]. Mercury, which can destroy the central nervous system, is a neurotoxin. The typical example of mercury intoxication is Minamata Bay, with its high mercury concentrations causing pulmonary and renal dysfunction, chest ache, and dyspnoea [31]. The U.S. designation of cadmium was possibly human carcinogenic, the Environmental Protection Agency. The vulnerability to cadmium to significant threats for human wellbeing. Chronic cadmium toxicity can lead to kidney dystrophy and mortality in elevated doses. Lead can lead to damage to the central nervous system. The kidney, liver, and reproductive system, essentially biological processes, and brain functions may also be affected by lead. Anemia, insomnia, vomiting, dizziness, irritability, muscle weakness, hallucination, and renal damage are the harmful signs [32]. Cr (III) and Cr (VI) are primarily generated in two states in the aquatic environment. Cr (VI) is typically more toxic than Cr (III). The illness causes significant health issues, ranging from basic skin irritations to lung carcinoma, which causes human physiology [33].

Results on metal ions adsorption onto cross-linked chitosan using a different cross-linking agent are shown in Table I. It is important to note that the adsorption capacities of the adsorbents cited in this paper depend on the experiment conditions such as pH, initial concentration, contact time, adsorbent dosage, presence of competitive ions, etc.

<table>
<thead>
<tr>
<th>Crosslinking Agent</th>
<th>Metal Ion</th>
<th>Qmax (mg g⁻¹)</th>
<th>pH</th>
<th>Isotherm</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPP</td>
<td>Cu(II)</td>
<td>22</td>
<td>4-5</td>
<td>Langmuir</td>
<td>[69]</td>
</tr>
<tr>
<td>GA</td>
<td>Pb(II)</td>
<td>24.2</td>
<td>5-7</td>
<td>Langmuir</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td>Cd(II)</td>
<td>23.9</td>
<td></td>
<td>Langmuir</td>
<td></td>
</tr>
<tr>
<td>GA crown ether</td>
<td>Cd(II)</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td>Hg(II)</td>
<td>126.4</td>
<td>6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pb(II)</td>
<td>96.8</td>
<td>6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>GA 2-aminopyridine</td>
<td>Pb(II)</td>
<td>166.7</td>
<td>8</td>
<td>Langmuir</td>
<td></td>
</tr>
<tr>
<td>Schiff's base</td>
<td>Cu(II)</td>
<td>124</td>
<td>5</td>
<td>Langmuir</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cd(II)</td>
<td>84</td>
<td>5</td>
<td>Langmuir</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ni(II)</td>
<td>67</td>
<td>5</td>
<td>Langmuir</td>
<td></td>
</tr>
<tr>
<td>Adipic acid dihydrazid</td>
<td>Cu(II)</td>
<td>200.1</td>
<td>5</td>
<td>Langmuir</td>
<td></td>
</tr>
</tbody>
</table>

On the other hand, crosslinked chitosan was successfully grafted by using the coordination bonds. The beads have shown a good adsorption capacity towards several metal ions such as Pb (II), Cu(II), and Cd(II). The interaction between adsorbent and Cu (II) ions is also proposed according to XPS analysis (Scheme 1).
5. POLYMER HYDROGEL

Hydrogels are crosslinked structures of hydrophilic polymers that can capture vast volumes of water or biofluids. Hydrogels are one of the next groups of polymer solutions spanning a wide variety of medicinal and pharmaceutical areas [34]. They provide strong prospects for protein delivery systems or tissue engineering scaffolds due to their intrinsic biocompatibility characteristics. Its smooth hydrophilic design guarantees minimum tissue inflammation and a low propensity on the hydrogel surface for the cells and proteins [34].

Hydrogels have considerable potential uses, including soil/water stabilization layers for agricultural and civil-engineered soil conditioners [35], controlled release of drug [36] fiber and metal cable [37] in water technology due to their flexible and unprecedented characteristics [38].

Hydrogels are synthesized using various methods of polymerization, both chemical and physical crosslinks. It is possible to cross-link all-natural polymers as proteins or synthetic polymers like PVA with a high-water affinity. For the construction of a hydrogel, various interconnection approaches may be applied. The following methods illustrate hydrogel synthesis, chemical and physical [39].

The chain-growth polymerization, the incorporation, and condensation polymerization, the gaming, and electron beam polymerization are synthesized by chemically interlinked hydrogels. The polymerization of chain growth encompasses free radical polymerization, free radical regulated polymerization, anionic and cationic polymerization [40]. It is carried out through three phases, beginning, propagating, and finishing. An open, radical, active website is produced after the initiation, which adds monomers in a chain-like manner [40].

The advantages of longer-lasting chain life relative to free radical polymerizations in macromolecular engineering are controlled living radical polymerization. Anionic and cationic methods of polymerization are very susceptible to water conditions and therefore not used in polymeric hydrogels synthesis [9]. Poly-functional cross-connecting agents with the single function groups are supplemented and polymerized in condensation steps.

Using crosslinkers including tetramethylene diamine, water-soluble monomers can be converted into hydrogels (TEMED) [41]. In the presence of water, polymer chains may be joined to form a hydrogel [42]. The hydrogel has distinctive surface properties and the waters fill voids within the network [43]. High energy microwave irradiation as a cross-linking agent requires gamma and electron beam polymerization. These energy-sensitive radiations will cross-link water-soluble monomer or polymer ends with no cross-linker [44]. Aqueous solutions of monomers are polymerized to form a hydrogel during irradiation using a gamma or electron beam [18].

The initiation, spreading, and termination of gamma and electron beam polymerization, as in the radical polymerization, are also involved. Hydroxyl radicals are formed and the vinyl monomers propagate in a simple chain-additive way free radical polymerization [43]. This procedure benefits from other linking processes as it can be carried out at room temperature and physiological pH without toxicity and without trouble extracting linking agents such as potassium persulfate [43].

The crosslinks that form between the lactic acid oligomers of the opposite chirality from a hydrogel form the formation of stereo complexes (see Figure 3). Hydrophobic interactions contribute to the polymer swell and absorb water, which forms the hydrogel [45]. In literature polysaccharides such as chitosan, dextran, pollen, and carboxymethyl curdlan are stated to be used for preparing hydrophobically intertwined hydrogels. The association of proteins comprises blocks of copolymers containing the repetition of blocks identical to silk and elastin called ProLastin [40]. The ProLastins are fluid water solutions that can undergo a physiological transition from solution to gel leading to the crystallization of soil-like regions [41].

Figure 3: Cross-linking in polymer [41].
6. Modification of Chitosan for Use in Sorption of Ionic Species

Chitosan from multiple organism exoskeletons has a great number of uses. Chitosans have some amazing promise because of their poly-cationic properties. Chitosan is a possible candidate for heavy metals and dyes sorption because of the inclusion of both amino and hydroxy groups [46].

Chitosan’s structure enables excellent chelation with metal ions, particularly metal transitions and inner transition. Chitosan has major problems in the native state, including its acidic (pH < 3) and mechanical strength solubility. Despite these limits, chitosan is interlinked with an effective crosslinker, not only stabilizing it in acidic conditions but also increasing its mechanical properties [47].

Chitosan has the highest metal adsorption potential, as the amino and/or hydroxy groups on chitosan chains are used in coordination [48] for many biopolymers. Chitosan is the most important biopolymer for metals [49]. Chitosan Metal ions can be taken on chitosan by various processes, including chelation of metal ions on an electronic free doubling of amine groups or mechanisms of ion exchange.

The amino group nitrogen atom has a single lone, while the hydroxyl group oxygen atom has 2 lone electrons and can bind a proton or metal ion through a lone pair of electrons creating a complex. The nitrogen atom has a larger ability to donate the lone pair of electrons to be exchanged with metallic ions to form a metal complex than oxygen atoms, because of their high electronegativity as compared to that of atomic nitrogen. Thus, amine groups are responsible for taking metal cations through the following chelation process [50].

\[
\begin{align*}
R-\text{NH}_2 + \text{H}^+ &\rightarrow R-\text{NH}_3^+ \\
R-\text{NH}_2 + \text{M}^{n+} &\rightarrow R-\text{NH}_2\text{M}^{n+} \\
R-\text{NH}_3^+ + \text{M}^{n+} &\rightarrow R-\text{NH}_2\text{M}^{n+} + \text{H}^+ 
\end{align*}
\]

Often closely regulated are the absorption properties of polymers by chemical modifications in polymers. Chitosan membranes were synthesized using the solvent casting process [51]. These chitosan membranes were compounded with various metallic ions such as Cu (II), Fe (III), La (III), Mo (VI), and Zr (IV) for as (V) sorption. The sorption of Fe (III) chitosan (91±2%) from seawater as(V) was higher than the sorption of La (III) chitosan (80±2%). These components were also used in aqueous media like tap water, groundwater, and seawater for preconcentrations of As (V).

During its selection of coefficients, Vold et al. [52] reported quantification of the binding of various ions to chitosan and studied the effect on the selection coefficients of pH, ionic strength and fraction of acetylated units. The fraction of acetylated units was measured with a 1H NMR spectroscopy. Chitosan was shown to be unselective in chloride and nitrate ion binding while high selectivity in molybdenum polyoxyanions was observed with a selectivity factor of about 100 [52].

Similarly, chitosan exhibited high copper selectivity (Cu²⁺) with selectivity coefficients of 10 to 1,000 relative to metal ions Zinc (Zn²⁺), cadmium (Cd²⁺), and nickel (Ni²⁺) with minimal to none ionic strength and acidity [52].

Tabakci et al [53] reported the synthesis and evaluation of the sorption characteristics of Cr₂O₇²⁻ /HCr₂O₇⁻+/orphic Cr³⁺, Pb⁴⁺, and dichromate chelating polymers for heavy metal ion sorption, Co³⁺, Ni²⁺, and Cu²⁺.

Sorption experiments have shown that calyx is associated with chitosan aren as an outstanding sorbent, although it is observed that pristine chitosan is worse in the sorbent. Sorption tests of anions from dichromates revealed a highly successful sorbent of chitosan [9] aren at pH 1.5.

The effect of chitosan membrane morphology on copper ion adsorption was investigated by Niassar et al. [54]. Macroporous chitosan membranes have been synthesized with varying polymer amounts and silica-chitosan proportions. Scanning electron microscope (SEM) and atomic force microscopy (AFM) characterized the membrane morphologies and surfaces. Batch processes conducted adsorption tests. Increased polymer concentration was found to contribute to increased copper adsorption. Growing the silica to chitosan ratio resulted in higher membrane porosity and surface ruggedness, which resulted in more copper-ion-accessible amino groups [54].

The use of novel Cr³⁺ beads for aqueous solutions has been documented by Balasubramanian et al [55]. These beads have been tested for the elimination of Cr³⁺ ions with varying amounts of poly (vinyl alcohol)/citric acid/chitosan. It was observed that the highest adsorption for Cr³⁺ ions was observed in poly (vinyl alcohol) / citric acid/cytosan mass ratios of 1/5/1.
At low pH, i.e. pH 4, the degree of adsorption was maximal [56]. The highest amount of adsorption of activated carbon and chitosan has been found among these four adsorbents. The high quality was due to the highly functional and porous nature of the activated carbon and to the protonated amines of chitosan. Guibal et al [57] have investigated the influence of organic ligands on the sorption of metal ions in a solution. In the presence of citrate in various metal/ligand ratios copper sorption has been investigated by chitosan.

The findings show that the acidic solution was absorbed by copper by electrostatic attraction between the chitosan-anionic amine group and copper-citrate anionic complexes. There has been very little or no sorption of the acid used for pH regulation below pH 3 due to rivalry between dissociated anionic ligand and anti-ionic ion. When the fraction of the anionic copper complexes is greater than that of anionically copper-free ligand, copper sorption was important. A novel natural polymer chitosan/cellulose mixture of N-methyl morpholine- N-oxide was synthesized by a homogeneous chitosan/cellulose dissolution [58].

The SEM micrographs show raw and folded morphology of the surface and inner pores. Cu [II], Fe (III), and Ni (II) metal ion sorption mean that these beads should be used as adsorption of metal ions. Ghaei et al [59] prepared a macroporous chitosan membrane by particulate leaching out the method for competitive adsorption of copper and nickel ions. Adsorption studies were carried out by batch experiments with mono and binary component solutions on chitosan membrane. In the mono-component, Cu$^{2+}$ adsorption was $19.87\, \text{mg/g}$ which was higher than those of the nickel (i.e. $5.21\, \text{mg/g}$), while the initial concentrations in both cases were the same [59].

For the use of Pb$^{2+}$, Cd$^{2+}$, and Cu$^{2+}$ ions in aqueous solutions, Paulino et al. [60] have added chitosan-based, greased copolymerized methylenebisacrylamide, and poly (acrylic acid). The pH of the initial metallurgical concentration of $300\, \text{mg dm}^{-3}$ was observed between 4.5 and 5.5. Peterson's adsorption isotherms showed a loss in adsorption ability in the presence of magnetite. For the reduction of Cu$^{2+}$ and Pb$^{2+}$ ions in water, chitosan-coated sand has been used.

Sorption from the solution of Cu$^{2+}$ to chitosan at two pH levels was examined [61]. The adsorption data were evaluated using the equations of Langmuir, nice, and Redlich–Peterson. The Langmuir equation is the optimal balance formula for the sorption of copper ions on chitosan from three models.

Ngah et al. [62], using batch adsorption experiments as a pH, agitation duration, agitation intensity, and concentration of Cu$^{2+}$ ions, investigated the adsorption of Cu$^{2+}$ ions in chitosan and interconnected chitosan pearls. The aqueous acidic and simple solution was insoluble in chitosan crystals, cross-related with glutaraldehyde, epichlorohydrin (ECH), and ethylene glycol diglycidyl ether. Sorption Maximal Cu$^{2+}$ was found at 6.0 pH.

The retrieval of Zn$^{2+}$ ions from an aqueous solution was investigated in Milosavljevic et al [63], using new pH-controlled hydrogels based on chitosan, itaconic acid, and methacrylic acid. The impacts on adsorption were examined by pH, contact time, initial metal ion concentration, and temperature. The adsorption follows the pseudo kinetics of the second order. Spontaneous and exothermic adsorption was demonstrated by the negative values of energy free and enthalpy. The hydrogels are increased with increased temperature, swelling, and adsorption power [63].

A graft copolymer synthesized by free radical polymerization using the bromate/ cyclohexanone redox, characterized by Infra-red spectroscopy, thermogravimetric study and swelling tests, N atomic acid and O atomic carbonyl group was found to be responsible for coordination with Zn$^{2+}$ ions [64] studies has been investigated for the sorption of Cu$^{2+}$, Ni$^{2+}$, Zn$^{2+}$, Pb$^{2+}$ and Hg$^{2+}$ [65].

For adsorption of copper ions in water solution, chitosan transparent thin membrane prepared using solutions casting method has been tested. SEM analyzes and Fourier transform infrared (FTIR) analysis are the characteristic arrangement and morphology of the membrane. The findings from the analysis showed that the adsorption could be represented as chemical adsorption by the model of pseudo-second-order [66].

Initial ion concentrations and sorption tests of certain metal ions with varying pH values have been performed. In Langmuir–Freundlich isotherm, the results were well matched. Sorption process thermodynamic parameters were also determined. In four series cycles of the sorbent regeneration tests, no substantial loss in sorbent capability was shown [67]. Chitosan was used in the adsorption of copper (II) and nickel (II) ions, covering polyvinyl chloride beads, and distinguished by the Fourier transform infrared spectrum, porosity, and surface area analyses. Detailed studies were carried out on adsorption variables, including pH, chaos, adsorbate concentration, and adsorbent number. The experimental results fit well with the adsorption isotherms of Langmuir and Freundlich. The
experimental findings showed that the PVC-coated chitosan beads are efficient sorbents for removal from the water medium of Cu (II) and Ni (II) ions [68].

7. CONCLUSIONS

One of the most basic demands at the level of researchers around the world is the problem of recovering and removing heavy metals from industrial wastes. Various techniques were applied, which inspired researchers to develop new and bio-absorbent materials and others derived from biopolymers such as chitosan due to their effectiveness in absorbing ions and being green, environmentally friendly materials with low cost.

Chitosan acts influenced by the surrounding environment, such as the temperature and the pH of the solution, while there are other internal factors such as crystallization and the degree of acetylation. The absorption of metal ions occurs by removing heavy metals on the amine and hydroxyl group from chitosan and works continuously in certain cases by electrostatic attraction in the acid solution. Some methods increase the efficiency, selectivity, and quality of chitosan: the first is through chemical modification using grafting and crosslinking, and the second by physical modification, such as manufacturing gel granules and reducing crystallization.

In this article, the utilization of chitosan in wastewater refinement as featured. An enormous number of studies were performed, and the outcomes for the most part indicated phenomenal exercises without toxicity. In any case, there are as yet a few rules that should be tended to later on account of the functional utilization of chitosan in certain ventures. Accordingly, these difficulties ought to be centered around additional examination to consolidate chitosan in these enterprises.

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