



Development of a Novel CMC/f-GO-Glu Composite for Heavy Metal Remediation: Antioxidant, Antimicrobial and Adsorption Studies

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ABSTRACT

The present study focuses on the development of a carboxymethyl chitosan-functionalized graphene oxide (CMC/f-GO-Glu) composite for the enhanced removal of heavy metals like copper and chromium from aqueous solutions. Graphene oxide (GO) was synthesized using a modified Hummers' method and further functionalized with carboxylic acid groups. The CMC/f-GO-Glu composite was prepared by crosslinking carboxymethyl chitosan with functionalized graphene oxide using glutaraldehyde. The synthesized composite was characterized using FT-IR, XRD, and SEM techniques. The adsorption capacity of the CMC/f-GO-Glu composite towards copper and chromium was investigated through batch adsorption studies under optimized conditions. The composite exhibited excellent antioxidant and antimicrobial properties. Batch adsorption experiments revealed that the adsorption of both metals was highly dependent on pH, adsorbent dosage, contact time, and initial metal ion concentration. The Freundlich isotherm model fit the equilibrium data quite well, indicating multilayer adsorption. The Freundlich isotherm model fit the equilibrium data quite well. The adsorption mechanism was found to be dominated by chemical interactions such as electrostatic forces and coordinate bonding, along with the influence of the composite's chemical structure and intermolecular interactions. Desorption studies confirmed the reusability of the CMC/f-GO-Glu composite without significant loss in adsorption efficiency. The results demonstrate the potential



of the CMC/f-GO-Glu composite as an excellent adsorbent to extract heavy metals from aqueous solutions, with promising applications in environmental remediation.

Keywords: Carboxymethyl chitosan, Graphene oxide, Composite, Heavy metals, Adsorption

INTRODUCTION

Water is vital to the existence and well-being of all life on Earth. However, many people worldwide suffer from a lack of potable water due to human activity-induced contamination¹. When released directly into natural rivers and sewage systems, industrial effluent containing heavy metals as Ag, Sb, Cr, Cu, Pb, Zn, Co, and Ni has a detrimental effect on aquatic ecosystems². According to Koester *et al.*³, these heavy metals harm humans, animals, and plants in several ways. Because heavy metals are found throughout the food chain, removing them from water bodies is essential because they can harm both humans and the environment.

As a result, removing heavy metals from water that has been contaminated has been the subject of numerous studies⁴. Numerous techniques have been used in the in-depth research on heavy metal removal technology in aqueous solutions to date. Among the modern methods are coagulation, adsorption, membrane filtration, electrodialysis, oxidation, biological treatment, ion exchange, and photocatalysis⁵. Adsorption is regarded as one of the most prominent and extensively applied heavy metal removal strategies out of all of them. This method's benefits include its low cost, simplicity, convenience of use, and neutrality to toxic contaminants and dangerous substances⁶. High adsorption capacity, reusability, affordability, and porosity adjustment would be the material's ideal characteristics. One of the prospective options created from various polymeric materials for purification of water is bio-based materials, which have the capacity to optimize various properties like dispersibility, hydrophobicity, porosity, surface area, and mechanical strength.

Materials based on graphene are now among the most sought-after materials in recent years because of their special qualities. The substance known as graphene is made up of a single-atom-thick carbon sheet that is organized hexagonally and is sp^2 -bonded. Another way to define graphene

is as a single, sp^2 -hybridized, hexagonally organized layer of graphite⁷. In contrast, the highly oxygenated monolayer graphite oxide is called graphene oxide (GO). that is produced when graphene undergoes oxidation and exfoliation⁸. Chemically modified with functional groups comprising nitrogen and oxygen, graphene is the thinnest nanomaterial, consisting of a layer of graphite that is only one atom thick⁹. For this investigation, graphene underwent chemical alteration, such as oxidation, to become graphene oxide.

Strong acids like H_2SO_4 and HNO_3 can be used to oxidize graphite powerfully, adding various oxygenated functional groups, including carboxylic, hydroxyl, and epoxy groups, to create graphene oxide (GO)¹⁰. These groups decorate the basal plane and the margins of graphene oxide layers in the graphite structure, which increases the gap between the layers and renders the material hydrophilic (i.e., soluble in water). In addition, GO is well recognized for being very hydrophilic, meaning that it dissolves readily in aqueous solutions¹¹. Heavy metal ions exhibit surface complexation with graphene oxide, which improves their adsorption. Graphene oxide-chitosan composites exhibit exceptional mechanical properties and stability when utilized in wastewater adsorption treatment¹².

Although the maximum interaction between the heavy metals and the aqueous solution is ensured by GO's hydrophilicity, it also makes it challenging to separate the adsorbents based on GO from the treated water following adsorption. Furthermore, GO's adsorption capacity is primarily limited by its small number of functional groupings on the surface¹³ and propensity to aggregate during adsorption, both of which degrade GO's performance¹⁴. To overcome its shortcomings, GO was modified utilizing a variety of other materials, and in recent years, the approach has been gaining interest. During oxidation, every carbon atom in graphene is changed into -COOH, -C-O-C- (epoxy), and -OH at the edges¹⁵. It is also responsible for

its high thermal stability and conductivity, unusual optical performance, extraordinary electronic¹⁶, electron transport capabilities¹⁷, and higher chemical reactivity with higher interplanar distance¹⁸. Hybridization of carbon changes from sp² to sp³, resulting in numerous negatively charged oxygen-oriented groups on the monolayer carbon by preventing them from aggregating¹⁹.

According to Huang *et al.*²⁰, chitin, the second most prevalent naturally occurring biopolymer found in the exoskeleton of crustaceans, undergoes incomplete alkaline N-deacetylation to produce chitosan, a natural polysaccharide. Made from naturally occurring polysaccharide chitosan, carboxymethyl chitosan (CMC) has primary amino, hydroxyl, and carboxyl groups, which is a substitute for chitosan since it is insoluble in water².

Zhang *et al.*²¹ described a porous GO/ carboxymethyl cellulose adsorbent that was effective in adsorbing Ni²⁺ and was made by a unidirectional freeze-drying technique. Zaman *et al.*²² created a GO/ MCC nanocomposite adsorbent by combining GO with microcrystalline cellulose (MCC) produced from leftover jute by modifying Hummers' method. Thus, it is anticipated that cellulose and hyperbranched polymers may enhance GO's adsorption capabilities by surface grafting²³.

The primary objective of the project is to prepare functionalized graphene oxide-based composites for the examination of their potential applications in the environmental and biomedical domains. The primary focus of the current work is the improved Hummers' method for synthesizing graphene oxide. Using potent acids, a carboxylic acid group functionalized the produced graphene oxide. Therefore, the f-GO-based composite, which consists of functionalized graphene oxide made with carboxymethyl chitosan and crosslinked with glutaraldehyde (GA), was employed as a material that can be used to remove heavy metals like copper and chromium through adsorption. FT-IR, XRD, and SEM with EDX studies were used to characterize the produced composites. Through batch adsorption tests conducted under ideal conditions, the adsorption capacity of the produced CMC/f-GO-Glu composite towards heavy metals such as copper and chromium from the aqueous solutions was investigated.

MATERIALS AND METHOD

Materials

India Sea Foods, located in Cochin, Kerala, India, provided the carboxymethyl chitosan. Graphite, glutaraldehyde, potassium dichromate, copper sulphate from MPM Scientific Company, Vellore. Analytical grade compounds were all that were used.

Methodology

Synthesis of functionalized Graphene Oxide

GO was developed by modifying Hummer's method with graphite powder²⁴. In an ice bath, five milliliters of cold 98% sulfuric acid were used to dissolve one gram of graphite powder. After stirring the liquid below 20°C for 5 minutes, 4 grams of KMnO₄ were added progressively over the next 20 minutes. After an hour, the reaction was maintained at 35 °C for 30 minutes. 50 milliliters of deionized water were then progressively added to the reaction mixture. After 20 minutes of reaction time at 98 °C, the solution was further diluted to 200 mL with deionized water. Five milliliters of 30% H₂O₂ were added to get rid of any remaining KMnO₄ and MnO₂. The final product was then continuously cleaned four times using deionized water and a 5% HCl solution. For 10 hours at 70°C, the resultant GO (yield 89%) was vacuum-dried in an oven.

Functionalization of Graphene Oxide

Ten milligrams of graphene oxide were utilized for functionalization. Seven of the ten milligrams were added to thirty milliliters of sulfuric acid, and the remaining three milligrams were added to ten milliliters of HNO₃ (3:1 ratio, thirty milliliters of sulfuric acid and ten milliliters of nitric acid). After properly mixing the mixture and letting it sit for ten minutes, the supernatant was centrifuged and decanted. When the pH reached 7, the residue was continually washed with distilled water and used as f-GO.

Preparation of Carboxymethyl Chitosan/f-Graphene Oxide-Glu Composite

The carboxymethyl chitosan was dissolved in water to a minimum of one gram. Additionally, 0.5 g of functionalized graphene oxide was dissolved in water at the same interval. The solutions were thoroughly mixed separately for around 20 minutes

using a magnetic stirrer. The carboxymethyl chitosan was placed in a beaker and gradually mixed with the stirred functionalized graphene oxide solution. After adding 5 mL of glutaraldehyde as the crosslinking agent, the resultant mixture was once again magnetically agitated for roughly two hours before being allowed to air dry in a Petri dish. Numerous characterization investigations were conducted on the dehydrated composite.

Characterisation

Fourier Transform Infra-red Spectroscopy

The SHIMADZU FT-IR Spectrometer, which has a range of 400–4000 cm^{-1} , was used to record the Fourier transform infrared spectrophotometer (FT-IR) spectrum.

X-ray Diffraction Analysis

The Ni filter Cu $K\alpha$ radiation source ($\lambda=0.154\text{nm}$) was used to capture the X-ray diffraction pattern. The D8 ADVANCE Diffractometer was operated with a voltage of 30 kV and a current of 40 mA.

Scanning Electron Microscopy with EDX

To confirm that the polymers were compatible, the surface morphology of the composites was examined using scanning electron microscopy. The topography was studied with a TESCAN, VEGA3-SBH, with a tungsten filament.

Antimicrobial Activity

The resultant composites' antibacterial effectiveness was evaluated in comparison to two gram-negative and one gram-positive bacterial strains namely *E. Coli*, *Klebsiella pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus*, and *Pseudomonas aeruginosa*, utilizing the Muller Hinton Agar (MHA) medium and the disc diffusion method, along with antifungal activity against *Aspergillus niger*, *Rhizopus sp.*, *Mucor sp.*, and *Candida albicans* using Sabouraud Dextrose Agar in the present research work.

Antioxidant Activity

Antioxidants have an important role in preventing diseases. Therefore, the DPPH radical scavenging method was used in the current work to assess the antioxidant property of the composite.

DPPH Scavenging Activity

The antioxidant activity of the composite was examined using the DPPH scavenging experiment. The absorbance of DPPH at 517 nm was used to determine the developed composite's ability to scavenge radicals. The absorbance decreases as a result of the sample scavenging the DPPH by donating hydrogen atoms to develop a stable DPPH complex, when the color changes. A control is a DPPH solution devoid of a sample.

The following formula determined the percentage of inhibition (%) of free radical generation from DPPH.

$$\% \text{ of inhibition} = \frac{A_c - A_s}{A_c} \times 100 \quad \dots(1)$$

Where,

Ac – Absorbance of the control

As – Absorbance of the sample solution

Heavy metal chromium and copper removal from an aqueous solution using CMC/f-GO-Glu Composite by Batch Adsorption studies

Batch experiments utilizing CMC/f-GO with crosslinking agent glutaraldehyde composite were conducted using solutions of potassium dichromate and copper sulphate with predetermined starting metal concentrations. The metal solutions were placed in stoppered bottles and shaken at a steady 210 rpm at 30°C in an orbital shaker. By altering the initial concentration of metal ions, contact time, pH, and adsorbent dose, the extent of metal removal was examined individually. Following each treatment step, the adsorption of the heavy metals was investigated using Varian AAA 220 FS Atomic Absorption Spectroscopy.

The metal's adsorption process under ideal Initial metal ion concentration, adsorbent dose, pH, and contact time were all investigated. The data was fitted to both the Langmuir and the Freundlich isotherms. The formula for the Langmuir equation is

$$\frac{C_{eq}}{C_{ads}} = \frac{bC_{eq}}{K_L} + \frac{1}{K_L} \quad \dots(2)$$

$$C_{max} = \frac{K_L}{b} \quad \dots(3)$$

where

C_{ads} are the metal ion adsorbed quantity (mg/g).

C_{eq} is the metal ion's equilibrium concentration in solution (mg/dm³). The Langmuir constant, or K_L , is represented in dm³/g.

b = Langmuir constant (dm³/mg)

The highest amount of metal ions that can bind to an adsorbent is expressed as C_{max} (mg/g). The Freundlich equation used is expressed as:

$$C_{ads} = KC_{eq}^{1/n} \quad \dots(4)$$

The Freundlich equation was expressed in a linearized form.

$$\log C_{ads} = \log K + 1/n \log C_{eq} \quad \dots(5)$$

where

C_{ads} are the amount of metal ions adsorbed (mg/g). Freundlich constant (mg.g⁻¹) is represented by $1/n$, and C_{eq} is the equilibrium concentration in solution (mg/dm³).

K = Freundlich constant (g.dm⁻³)

By conducting a different set of the adsorption at constant temperatures to monitor the adsorption over time, the kinetics of the adsorption process could be investigated. The pseudo-first and pseudo-second-order models can be used to quantitatively calculate and assess the adsorption rate.

Pseudo-first-order (PFO) equation

$$\log(q_e - q_t) = \log q_e - k_1 t / 2.303 \quad \dots(6)$$

Pseudo-Second-order (PSO) equation

$$t / q_t = 1 / k_2 q_e^2 + t / q_e \quad \dots(7)$$

The adsorption of pseudo-first order and pseudo-second order kinetics are represented by the expressions q_e and q_t , respectively, and K_1 (min⁻¹) and K_2 (g mg⁻¹ min⁻¹) are the amounts of metal adsorbed (mg/g) at equilibrium at time t (min).

The PFO and PSO models are represented

in the linear charts by tracing $\log(q_e - q_t)$ versus t and (t/q_t) versus t , respectively. The visualization of the experimental data yields the rate constants k_1 and k_2 .

Intraparticle diffusion (IPD) Kinetics

The equation for intraparticle diffusion is expressed as follows.

$$q_t = K_{id} t^{1/2} + C$$

where K_{id} is the IPD rate constant (mg/g min^{1/2}) and C is the intercept. Plotting q_t vs $t^{1/2}$ allowed for getting K_{id} and C values based on the slope and intercept.

Desorption Studies

Following the completion of the adsorption process, a desorption research was also conducted for the composites loaded with copper and chromium. 1N HCl was used as the desorbing agent in the desorption experiments. The 0.5 g of the metal-loaded adsorbent was weighed and treated with 1N HCl, shaken well for 1,2,3,4, and 5 hours, and filtered. AAS experiments were then used to determine the metal concentration in the filtrate.

RESULTS AND DISCUSSION

The effectiveness of carboxymethyl chitosan (CMC) and functionalized graphene oxide crosslinked with glutaraldehyde in removing copper and chromium heavy metals was investigated in this work using batch adsorption techniques.

Fourier Transform Infrared spectroscopy

The infrared (IR) spectroscopy data, **Figures 1 and 2** for both Carboxymethyl Chitosan (CMC) and modified adsorbent material CMC/f-GO-Glu composite, confirmed their respective structures. Figure 1: FTIR spectrum of Carboxymethyl Chitosan (CMC)

A broad band in the 3000–3600 cm⁻¹ range revealed the presence of hydroxyl groups in the case of unmodified carboxymethyl chitosan. It was discovered that the O-H group buried the N-H stretching vibration group, which is also found as a strong peak but shifted due to the interaction between the constituent materials, such as f-GO and glutaraldehyde²⁵. Additionally, the carbonyl (C=O) stretches of a carboxyl group (R-COO-) typically

Table 1: X-ray diffraction results of a) CMC, b) CMC/f-GO/Glu composite

Polymer blend	2-theta	(Xc %)
Carboxymethyl chitosan	18°, 22°	22.3
CMC/f-GO/Glu composite	29°, 42°	7.6

appear as a very sharp band in the range of 1760-1690 cm⁻¹. Following cross-linking, all functionalized GO plots show the modest new C-O stretch that occurs in the 1000–1300 cm⁻¹ range, indicating the functionality of esters. These patterns are explained by the effective control of the functionalized GO's physico-chemical characteristics²⁶.

Table 2: Langmuir and Freundlich isotherm constants

Metal ions	Langmuir constants			Freundlich constants			
	KL (dm ³ /g)	b	C _{max}	R ²	KF	n	R ²
Cr (VI)	2.404424	0.00795	302.4804	0.8582	4.7544	1.40885	0.9514
Cu (II)	3.018412	0.01148	262.8812	0.8254	6.4165	1.52906	0.953

Table 3: PFO, PSO, and IPD kinetic parameters

Metal ions	Pseudo-first-order kinetic model			Pseudo-second-order kinetic model			Intraparticle diffusion model		
	k ₁ (min ⁻¹)	q _e (mg/g)	R ²	k ² (g mg ⁻¹ min ⁻¹)	q _e (mg/g)	R ²	K _{id}	I	R ²
Cr (VI)	-0.01049	107.15	0.7927	0.0001509	105.09722	0.9927	2.435	42.18	0.9908
Cu (II)	-0.00920	92.897	0.884	0.0001341	111.0124	0.9895	2.591	43.18	0.974

XRD Studies

The crystalline and amorphous properties of the samples, as well as the particle size, are confirmed by X-ray diffraction investigations.

The X Ray diffractograms and details of plain CMC and CMC/f-GO-GLU composite are presented in Figures 3a and b, and Table 1, respectively. According to Nara and Komiya²⁷, the resultant composite, crosslinked with glutaraldehyde, was found to be exceedingly amorphous when compared to plain carboxymethyl chitosan when the percentage of crystallinity was measured for both samples (Table 1). This may be because the typical ordered semi-crystalline structure is disrupted by the high binding ability of CMC and f-GO to the crosslinking agent glutaraldehyde²⁸.

BET Analysis

A material's surface area and pore volume are crucial factors to take into account when assessing it for adsorption applications. Using the BET method, a N₂ sorption isotherm

was performed at 25 °C for both plain CMC and the CMC/f-GO-Glu composite. The surface area of the CMC/f-GO-Glu composite was 98.4 m² g⁻¹, while that of the carboxymethyl chitosan was 21.20 m²g⁻¹. The pores' size reduced from larger to smaller (34.5 to 17.20 nm) due to the increased surface area of the CMC/f-GO-Glu composite. The pore volume increased from 1.022 cc/g to 3.12 cc/g in comparison to pure CMC, suggesting that the CMC/f-GO-Glu composite exhibited favorable surface properties²⁹. This demonstrates that a reduced pore size and increased surface area result in greater sorption efficacy. Consequently, this CMC/f-GO-Glu composite is an appropriate adsorbent for the removal of copper and chromium heavy metals.

SEM analysis

An extremely rough surface and an uneven porous morphology with irregular forms and numerous fissures and holes were visible in the CMC/f-GO/Glu composite's SEM picture (**Figure 4**). The surface's suitability for adsorption is demonstrated by its roughness. A homogeneous

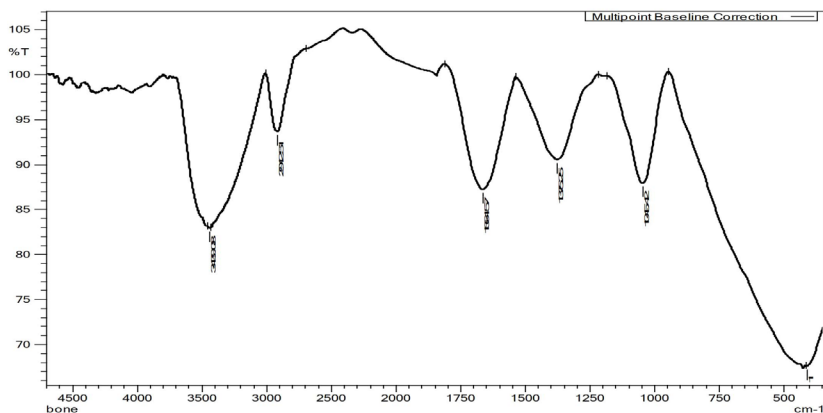


Fig. 1: FTIR spectrum of Carboxymethyl Chitosan (CMC)

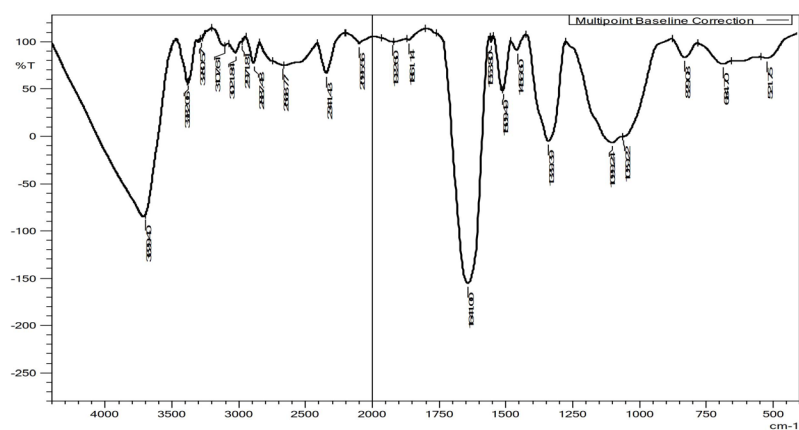
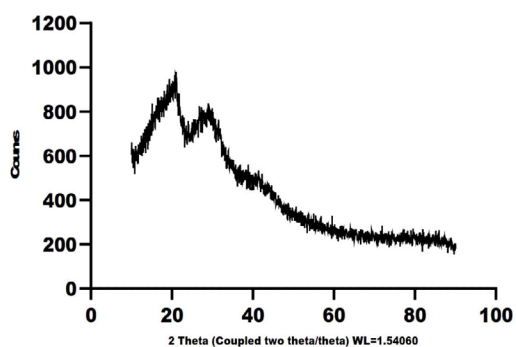
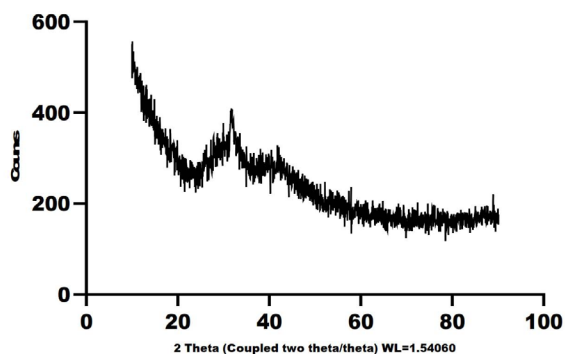


Fig. 2: FTIR spectrum of CMC/f-GO-Glu composite



(a) X Ray diffractogram of CMC



(b) X Ray diffractogram of CMC/f-GO-Glu

Fig. 3: X Ray diffractograms of a) CMC, b) CMC/f-GO/Glu composite

distribution of f-GO in CMC, which is extensively crosslinked by glutaraldehyde, is indicated by the particles' compact packing and lack of obvious agglomeration³⁰.

Antioxidant Activity (AOA)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used to assess the glutaraldehyde crosslinked CMC/f-GO-Glu composite's capacity to scavenge free radicals. The prepared composite's

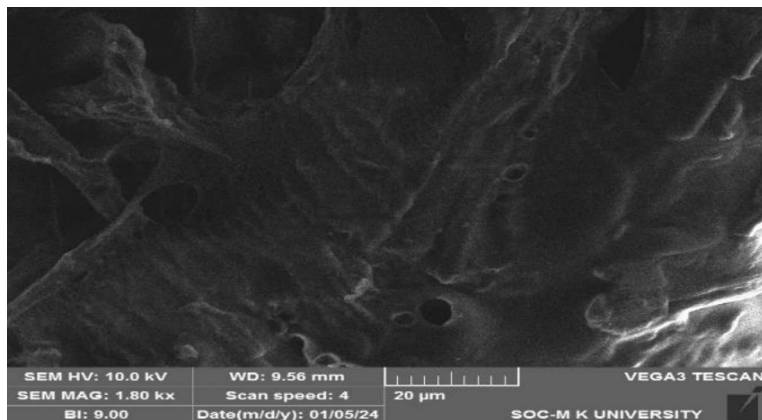


Fig. 4: SEM micrograph of CMC/f-GO/Glu composite

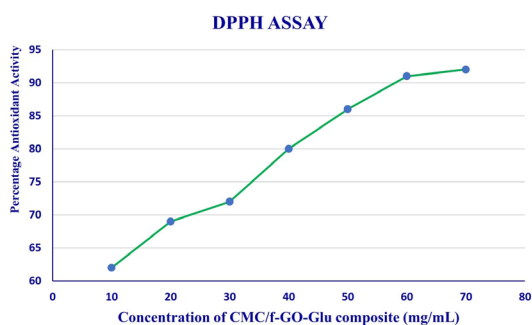


Fig. 5: Antioxidant assay of CMC/f-GO/Glu composite

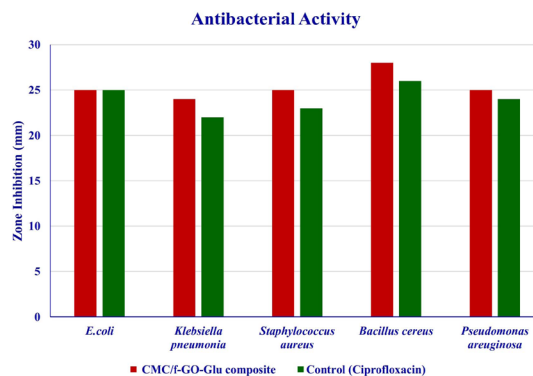


Fig. 6: Antibacterial assay of CMC/f-GO/Glu composite

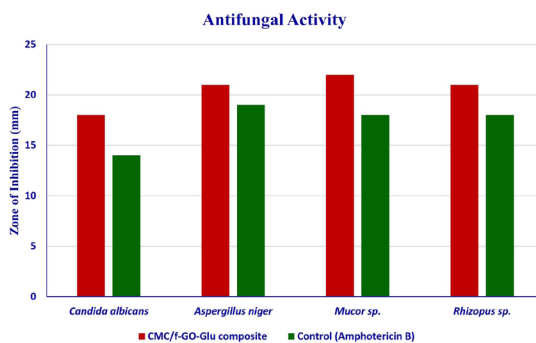


Fig. 7: Antifungal assay of CMC/f-GO/Glu composite

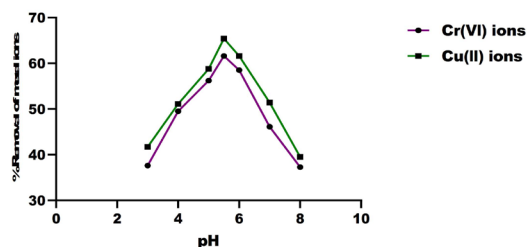


Fig. 8: Effect of pH on the adsorption of Cr and Cu

various concentrations were plotted against the percentage AOA (**Figure 5**).

Figure 5 indicates that the antioxidant activity rose from 62% to 90% when the composite's concentration increased from 10 mg/mL to 70 mg/

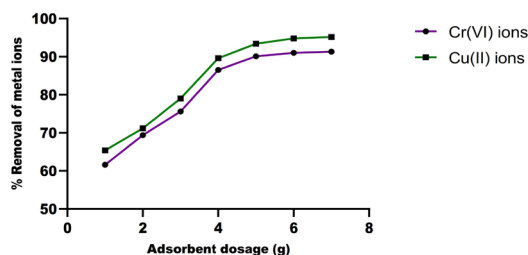


Fig. 9: Effect of adsorbent dose on the adsorption of Cr and Cu

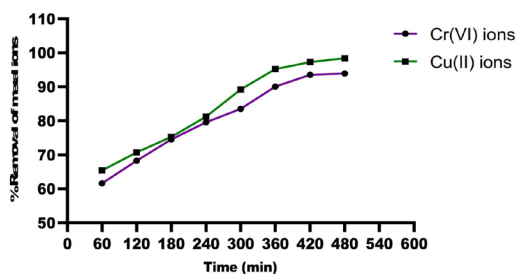


Fig. 10: Effect of contact time on the adsorption of Cr and Cu

mL. A hydrogen transfer mechanism powers the DPPH scavenging process. Strong and promising antioxidants are chitosan and its derivatives³¹. Metal chelation and free radical scavenging are accomplished by this polymer's NH₂ and OH functional groups³². Chitosan's potent ability to donate hydrogen has demonstrated its antioxidant properties. By stopping the oxidation chain reaction, chitosan's antioxidant effect shields the target organism from damage brought on by oxidative stress^{33,34}.

Antimicrobial Activity

Antibacterial Activity

Five different bacteria, including *E. Coli*, *Klebsiella pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus*, and *Pseudomonas aeruginosa*, were

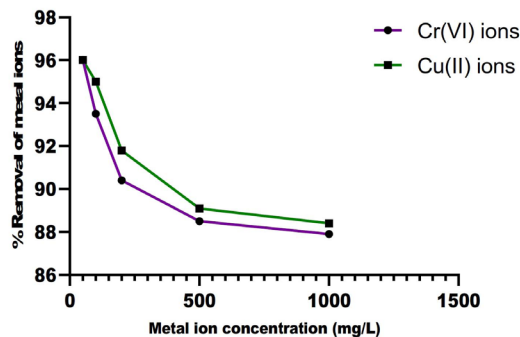


Fig. 11: Effect of initial metal ion concentration on the adsorption of Cr and Cu

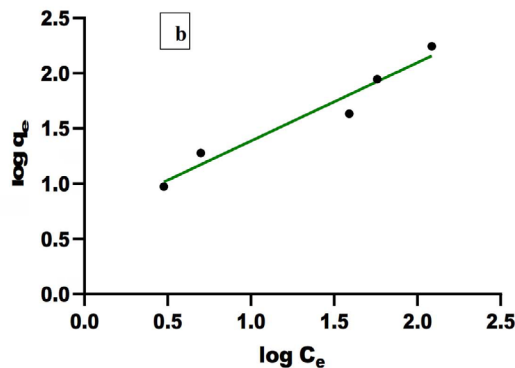
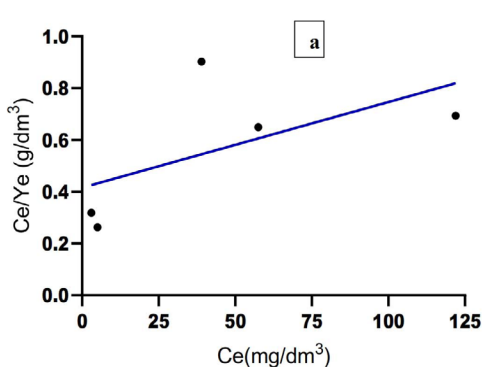


Fig. 12: (a & b): Langmuir isotherm plots of Cr and Cu adsorption

used to investigate the antibacterial activity of the produced CMC/f-GO-Glu composite. The common antibacterial drug ciprofloxacin was chosen as a standard. The degree of antibacterial activity was measured as a zone of diameter and plotted as Figure 6.

It is clear from Figure 6's antibacterial activity that the developed composite outperformed the drug ciprofloxacin in terms of antibacterial activity, demonstrating the composite's potent biological qualities. This efficacy is probably caused by the protonation of chitosan's amino groups in

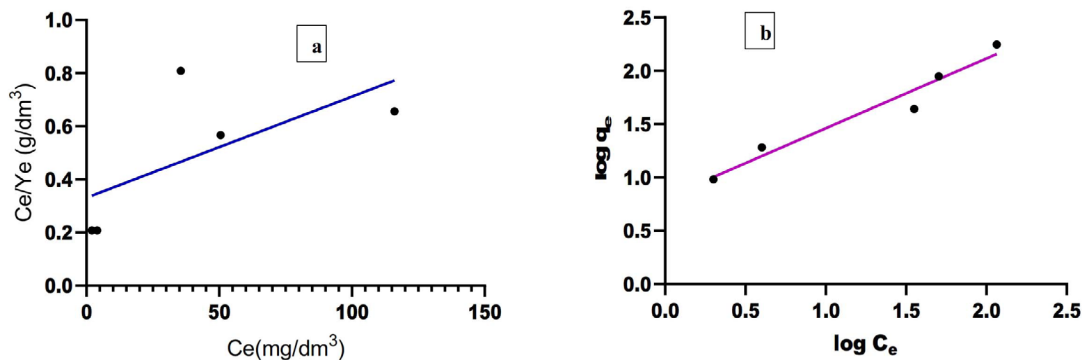


Fig. 13 (a & b): Freundlich isotherm plots of Cr and Cu adsorption

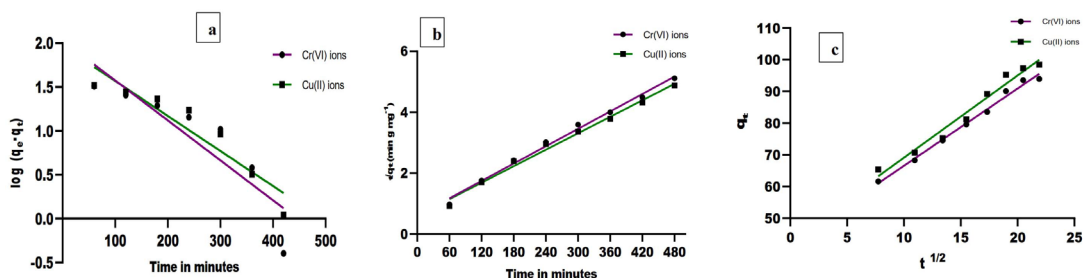


Fig. 13 (a, b & c): PFO, PSO, and IPD plots of Cr and Cu

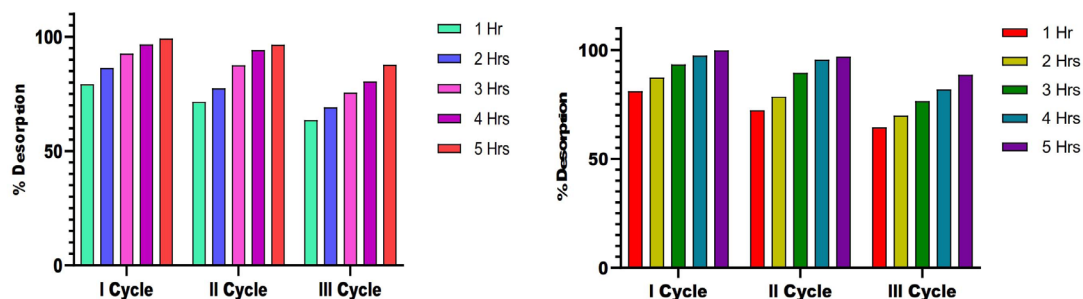


Fig. 14: Desorption of Cr and Cu from CMC/f-GO-Glu composite

an acidic environment, which raises the amount of positively charged chitosan molecules present. Microbial growth is inhibited, and cell wall rupture occurs as a result of these positively charged chains' interaction with the negatively charged surfaces of microbial cells³⁵. The bacterial cell wall may be harmed by the protonation of the amino groups in chitosan³⁶.

It is well known that a negatively charged cell membrane is attracted to positively charged chitosan, which interferes with the movement of materials within the cell. Additionally, oligomeric chitosan may be able to enter cells and prevent DNA transcription³⁷. Chitosan diffuses directly into the bacterial cell wall of Gram-positive bacteria due to the lack of an outer membrane^{38,39}. This may help to explain the notion that chitosan works better against Gram-positive bacteria than Gram-negative ones.

Antifungal Activity

According to several studies, chitosan has remarkable antifungal qualities against a wide variety of molds and yeasts⁴⁰. Hence, the antifungal activity of the prepared composite of carboxymethyl chitosan with f-graphene oxide was evaluated, and the results are plotted in **Figure 7**. The amphotericin B drug was chosen as the standard and compared for its activity the composite against different species.

The findings demonstrate that the CMC/f-GO/Glu composite's antifungal activity was significantly greater than that of amphotericin B, the conventional drug. This demonstrates the strong antifungal activity of the CMC compound against *Aspergillus niger*, *Rhizopus sp.*, *Mucor sp.*, and *Candida albicans*. The theory states that the fungal cell membrane's negatively charged phospholipids and positively charged chitosan can interact, damaging the membrane and allowing the chitosan to enter the cytoplasm. The literature has demonstrated that chitosan exhibits potent antifungal activity against fungi that are susceptible to it⁴¹. According to Qin et al.⁴², chitosan's antifungal properties are generally thought to be fungistatic rather than fungicidal, and they are very successful in preventing spore germination, radial expansion, and germ tube elongation.

Adsorption Studies

To adsorb heavy metal ions, the composite

adsorbent has a variety of functional groups, such as carboxyl, hydroxyl, and amino groups, which can offer an active binding site. The diverse architectures of the adsorbents make the adsorption mechanism extremely complex. The quantity of available active binding sites, the pollutant's affinity for the adsorbent surface, and the existence of a range of functional groups that can interact acceptor-donor with the heavy metal ions are the variables that influence the effective adsorption onto the surface of biosorbents. To determine the equilibrium conditions, which are detailed below, adjustments are made to the initial metal ion concentration, contact time, adsorbent dosage, and pH.

Effect of pH

An essential factor in the adsorption process is pH. pH has a significant impact on the adsorption of Cr(VI) and Cu(II) ions. The percentage removal of metal ions with respect to the change in pH from 3 to 8 has been recorded in **Figure 8**.

It is observed that as the pH increases from 3 to 5.5, it starts lowering from 6 to 8. Thus, in the acid medium, the adsorption capacity of the sorbent increased up to 5.5, which reduced up to 8. Lower pH causes the protonation of imine groups on the adsorbent surface, or active sites. As a result, the protonated groups and the metal cations experience electrostatic repulsions that prevent the metal ions from adhering and, as a result, lowers the uptake of metal ions⁴³. Depending on the ionic concentration and pH of the solution, the several anionic forms of Cr (VI), specifically for chromium, manifest in the aqueous phase as $\text{Cr}_2\text{O}_7^{2-}$, HCrO_4^- , CrO_4^{2-} , and HCr_2O_7^- . The primary fraction is HCrO_4^- at low concentrations in the pH range of 2–4, whereas the concentration of the CrO_4^{2-} moiety increases as the pH value rises and becomes the predominant form at $\text{pH} > 7.0$ ⁴⁴. Superior chromium removals at low pH are supported by HCrO_4^- ions, and the protonated sites of the sorbent interact electrostatically. However, the adsorption capability diminishes at higher pH values because the OH^- ion competes for the protonated sites with the oxyanions of Cr(VI) (HCrO_4^- and CrO_4^{2-}). Free imine groups are accessible on the adsorbent for ion adsorption at higher pH values. Additionally, free carboxylic acid groups are transformed into carboxylate ions at higher pH values, making them accessible for metal ion adsorption⁴⁵. Similarly, at high pH, copper

precipitates as $\text{Cu}(\text{OH})_2$, which lowers the ability of both metal ions to adsorb.

Effect of adsorbent dose

The adsorption of chromium and copper was tested with various adsorbent dosages, and the adsorption percentage of metals is plotted against the dosages and presented in **Figure 9**.

The percent of adsorption is very high, to the extent of above 60%, for both the metals with the adsorbent dose of 1gm of CMC/f-GO/Glu composite. This further increases with the rise of the adsorbent dosage of up to 5 gms. From there onwards, there is only a very shallow increase, showing that the equilibrium has been reached (**Figure 9**). Thus, the figure demonstrates that increasing the adsorbent dose increases the total surface area provided for an example adsorption procedure, which is an essential feature for this type of method. Both the number of active sites and the percentage of adsorption that may be accomplished are improved by increasing the amount of adsorbent. The adsorption capacity further decreases when the driving power decreases because some of the adsorbent's active sites fill up. This is because adsorption sites become saturated when the dose of adsorbent is increased, while the concentration of Cr and Cu and solution volume remain unchanged⁴⁶. Zhan *et al.*⁴⁸ and Senturk *et al.*⁴⁷ reported similar findings.

Effect of contact time

One of the factors influencing batch adsorption tests is adsorption time. In order to ascertain the composite's effectiveness in removing Cr(VI) and Cu(II) ions from the aqueous solutions, the study varied the contact duration between the adsorbent and adsorbate from 60 to 480 minutes, as shown in **Figure 10**.

The percentage removal of both copper and chromium grew rapidly at the start of adsorption, but after 180 minutes, the trend slowed. As a result, the copper and chromium ions quickly attached to CMC/f-GO-Glu. The adsorbed quantity of the two metal ions showed almost no change after around 360 minutes, indicating that equilibrium had been established⁴⁹.

Effect of Initial Metal Ion Concentration

Metal aggregation at the boundary through

the solid/liquid segments is a feature of the mass relocation process called "metal ion removal." As a result, while maintaining other parameters constant, the effect of the start metal quantity has been examined by varying its concentration from 50, 100, 200, 500, and 1000 mg L^{-1} . (**Figure 11**).

The percentage elimination of both metal ions decreased as the starting metal concentration rose from 50 mg/L to 1000 mg/L . (**Figure 11**). This decrease is caused by the fact that all adsorbents have a set number of active sites that become saturated at larger doses⁵⁰. Since the majority of contaminated wastewaters often contain varying quantities of metal ions, determining the impact of the starting concentration of metal ions is essential for a comprehensive adsorption study⁵¹.

Adsorption Isotherms

The quantity required to remove a pollutant mass under the system setting is known as the adsorbent capacity, which is revealed by the equilibrium data, sometimes referred to as adsorption isotherms, which are fundamental parameters for designing the adsorption systems⁵². The Freundlich and Langmuir isotherm models were used to assess the equilibrium sorption data. A monolayer adsorption onto an adsorbent surface that has a limited quantity of identical sorption sites is described by the Langmuir isotherm⁵³. The Freundlich model is used on heterogeneous surfaces for non-ideal sorption⁵⁴.

From the Table 2 and Figures 12 & 13 it is very clear that the adsorption of both chromium and copper followed Freundlich adsorption isotherm confirming the multilayer adsorption and the nature of linkages are influenced more by physisorption than by chemisorption. Hence, among various linkages like π - π interaction, H bonding, Van der Waals forces, etc, are more pronounced than the coordinate type of bond and electrostatic interactions.

Adsorption Kinetics

Adsorbate-adsorbent interactions have been described using a variety of kinetic models. The Lagergren pseudo-first-order rate equation is typically used when adsorption occurs before diffusion via a boundary layer⁵. Therefore, these adsorbents' adsorption mechanism cannot be described by pseudo-first-order kinetics. Several authors⁵⁶ have

shown that the kinetics of adsorption onto solids are pseudo-second-order. The development of chemisorptive bonds, which the pseudo-second-order model considers to be the rate-limiting stage, involves the electron sharing or exchange between the adsorbent and the adsorbate.

The CMC/f-GO-Glu composite's kinetic investigation of metal ion uptake was carried out utilizing feed solutions for both metals with starting ion concentrations of 200 ppm. The experimental adsorption rate and the linear fits of the different kinetic models and the different kinetic parameters derived from these fits are shown in Figure 13 and Table 3, respectively. The PSO kinetic model effectively represented the adsorptive behavior of both Cu^{2+} and Cr^{6+} metal ions onto the CMC/f-GO-Glu composite, according to the R^2 value determined for the kinetic model fits.

Mechanism of adsorption

The existence of functional groups that contain nitrogen and oxygen on CMC/f-GO-Glu composite offers more metal ion binding sites by means of complexation or coordination processes⁵⁷. By stabilizing the adsorbed species, hydrogen bonding interactions between metal ions and functional groups on the composite can further aid in the adsorption process. Although the adsorption of heavy metals onto the composite seems to be dominated by chemical interactions, including coordination bonding and electrostatic forces, the chemical structure and intermolecular interactions of the composite with the metals, including oxygen-

containing functional groups, van der Waals forces, and hydrogen bonding, may also play significant roles⁵⁸. To completely understand the impact of these interactions on the adsorption of heavy metals on the composites and to look into the precise contributions of these interactions, further investigation is required.

Desorption Studies

The reusability of CMC/f-GO-Glu composite was evaluated based on its desorption efficiency. The experiment on desorption was conducted during different periods of time and the results are displayed in **Figure 14**.

The findings show that the developed CMC/f-GO-Glu composite is highly regenerable and should be used frequently in metal ion adsorption investigations with minimal adsorption efficiency loss⁹. As an extension of the recently reported study, the CMC/f-GO-Glu composite might be employed in a recirculating fluidized bed column that continuously extracts heavy metal ions from their aqueous streams. Hydrodynamics studies could be conducted as needed, and operating parameters might be optimized using response surface methodology.

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Conflict of Interest

All the authors declare no conflict of interest

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