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Synthesis, characterization and antibacterial activity of a graphene oxide based NiO and starch composite material

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ABSTRACT

This work reports a novel, facile and hydrothermal approach to prepare graphene oxide/NiO/ Starch composite materials (GO/NiO/Starch). The composite materials were fully characterized by Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray analysis (EDS), X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Raman spectroscopy and X-ray photo-electron spectroscopy (XPS), and Thermo gravimetric analysis (TGA-DTA). Furthermore, the antibacterial properties of NiO and starch decorated graphene oxide were tested against *Staphylococcus aureus* by using the well diffusion method. The obtained results show that the GO/NiO/Starch composite material has a substantial antibacterial activity against *Staphylococcus aureus*.

GRAPHICAL ABSTRACT

This is the flowchart of synthesis, characterization and biological activity of graphene oxide based NiO and starch composite materials:



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KEYWORDS

Graphene oxide; graphene oxide/nickel oxide; graphene oxide/nickel oxide/starch; antibacterial activity

1. Introduction

After Geim and Novoselov discovered graphene, other scientists studied different aspects of two-dimentional (2-D) carbon nanostructures. It is known that one layer of graphene, with one atom thickness, shows large thermal and electrical conductivity,^[1] high carrier mobility at room-temperature^[2] and high lateral quantum confinement.^[3] It shows a wide range of applications in super capacitors technology,^[4] photovoltaics devices,^[5] fuel cell systems^[6] and nanofluids,^[7] due to its remarkable mechanical properties, chemical stability electronic capacity and large specific surface area.Thus, graphene can be used as an alternative to make metal and polymer composite materials with competitive multifunctional properties. However, it is well known that the interfacial interaction is crucial to improve the properties of polymers, while as-prepared graphene cannot be dispersed in water or organic solvents. Therefore, graphene oxide (GO) was explored as a promising filler to synthesize GO based metal and polymer composite materials.^[8] In addition, some authors reported that the properties of metals and polymers could be greatly improved by the incorporation of GO nanosheets. Zhou et al. reported that graphene (GR)-based nanocomposites with different mass ratios of NiO and GR can be prepared by a hydrothermal method using Ni(NO₃)₂ as the precursor of nickel, and urea as the

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Table 1. Comparison of antibacterial activity of GO/NiO/Starch composite and other GO-based composites.

S. no.	Material	Method	Bacteria	Zone of inhibition	Results	References
	go-pla/pu	_	Staphylococcus aureus	7–26 mm	The incorporation of 5% GO into PLA/PU composite reduced the growth of <i>Staphylococcus</i> <i>aureus</i> up to 100%.	[23]
2.	GO/gelatine/cellulose nanofibrils (CNF)	Colony count	Staphylococcus aureus	Increased cell growth with $2.67 \times 10^5 \pm 3.2 \times 10^3$ cfu/ml in the medium	Excellent antimicrobial properties	[24]
3.	rGO/TiO ₂ /PVA	Well diffusion	Staphylococcus aureus	$10 \pm 0.41 \text{ mm}$	Larger antimicrobial activity	[22]
4.	Chitosan – Fe oxide coated graphene oxide	Agar diffusion	Staphylococcus aureus	-	Excellent antimicrobial properties	[25]
5.	RGO/Ag	Colony count	Staphylococcus aureus	-	RGO/Ag composites showed large prevention of bacteria growth	[26]
6.	GO/Chloramine-T/N- Bromosuccinimide/ FeCl₃/KIO	Well diffusion	Staphylococcus aureus	18 mm	GONS present maximum toxicity against Staphylococcus aureus	[27]
7.	GO/NiO/Starch	Well diffusion	Staphylococcus aureus	15–26 mm	GO/NiO/Starch nanocomposite has significant antibacterial activity, larger against Staphylococcus aureus.	Present work

hydrolysis-controlling agent. This study found that the GR sheets and NiO particles originate uniform nanocomposites with the NiO particles absorbed on the GR surface, with excellent cycling performance.^[9] Yuan et al. reported the extraordinary properties (excellent conductivity, superior electrochemical capacity) of graphene oxide (GO), with a nickel oxide/glassy carbon (GC) modified electrode being prepared by electrodeposition.^[10] Shahrokhian et al. reported a three-dimensional (3 D) graphene/nickel oxide (ERGO/NiO) composite showing a high charge storage capacity.^[11] Kottegoda et al. reported the high rate capability and good stability of graphene/NiO composites.^[12] Xie et al. reported that a graphene oxide/Bi₂WO₆ (GBW)/starch composite film showed the strongest visible light degradation activity for ethylene.^[13] Ambre et al. reported the hyper branched graphene oxide starch thin film nanocomposite membranes (HGOST) synthesized and it is found that the inclusion of GO-starch composites shows good promise for enhancing the performance of polyamide membranes.^[14] Sattari et al. reported GO based maleate-modified starch hydrogel composite. The results showed the thermal stability, phase transition temperature and internal network crosslinking of hydrogel composite that increases with increasing the GO feed ratio. Also, the swelling/deswelling, hemolysis, and MTT[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay studies confirmed that the hydrogel composites are a fast response, hemocompatible and biocompatible materials.^[15] Based on the above facts, we choose nickel oxide and starch as matrix because water can be used as the common solvent, resulting in well-dispersed GO in polymer matrix. It is well known that starch is one of the best candidates for replacing current synthetic plastics due to its attractive combination of biodegradability, low cost, abundance and it has been applied in the field of agriculture, medicine and packaging industries.^[16] Moreover, various types of nano-fillers are being incorporated to enhance the properties of polymer matrices depending on the interaction and dispersion of the nano-fillers inside. Among various types of nano-fillers, silver nanoparticles

(AgNPs) and GO have extensively been investigated for their biomedical applications. $^{\left[17\right] }$

Antimicrobial activity is defined as the annihilation or inhibition of microorganisms (bacteria, fungi or viruses) growth in water or in air surfaces.^[18] Antimicrobial agents are extremely needed in several industrial applications, like food, packaging, textiles, medical, health care products, etc. Contaminated food, for example, due to microorganisms grows or spoilage is a major concern and a threat to public health, being also an economic problem for the food industry.^[19] Composite materials can help achieving better antimicrobial coverage for food, by the use of photocatalytic nanoparticles and polymer based composites films that will improve packaging and storage.^[20] Metal oxide nanoparticles show remarkable antimicrobial properties and are able to inactivate a large spectrum of dangerous microorganisms (like fungi, algae, virus and bacteria).^[21] The common therapies with antibiotics are losing efficiency due to the development of resistant bacteria, which leads to lack of adequate treatment and increase in healthcare costs. Thus, the need for novel agents with antimicrobial activity to efficiently kill pathogenic bacteria is of vital importance.^[22] Several authors reported the powerful antibacterial properties of GO against several pathogens, including Gram-positive and Gram-negative bacteria and phytopathogens (Table 1). Shao et al. reported a green facile synthesis of silver nanoparticles (AgNPs) decorated graphene oxide composites (GO-Ag). Moreover, GO-Ag composites antibacterial activity was tested on Escherichia coli ATCC 25922 (Gram-negative) and Staphylococcus aureus ATCC 6538 (Gram-positive) by the plate count and disk diffusion methods. GO-Ag showed very good antibacterial activity, demonstrating a large potential for biomedical applications.^[28] Dhanasekar et al. tested copper doped TiO₂ nanoparticles with reduced graphene oxide as a solid support, being introduced as new ambient light antimicrobial agents. The results show that the Cu₂O-TiO₂/rGO composites exhibit better antibacterial properties with higher area of zone of inhibition and lower value of minimum inhibitory concentration for both Gram positive

and Gram negative microorganisms, compared to the bare TiO2.^[25] More recently, a combination of antimicrobial agents with polymers are being used as antimicrobial packaging, able to detain the microorganism activity. This interesting approach is attracting attention, as it preserves food quality and safety, leading to longer shelf life.^[29] Konwar et al. reported a strong antimicrobial bioflim made of chitosan and Fe oxide coated GO nanocomposite hydrogels. Interestingly, this nanocomposite hydrogel showed noteworthy antimicrobial activity against Gram-positive and Gram-negative bacteria, like methicillin-resistant Staphylococcus aureus and Escherichia coli, and the Candida albicans opportunistic dermatophyte. Comparing the antimicrobial activity of chitosan-iron oxide-GO with chitosan-GO and chitosan-iron oxide nanocomposite films, the former showed larger antimicrobial activity in all tests. In vitro hemolysis potentiality tests and MTT [3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays approved the non-cytotoxic nature of films, allowing applications biomedicine their potential in and food industry.^[30]

Nickel oxide (NiO) is an antiferromagnetic semiconductor with a band gap of 3.6 eV.^[31] It has been applied in various fields including biological applications. It was found to possess antibacterial, antifungal^[11] and anticancer activity.^[32] The efficiency of GO-metal system is dependent on the conductivity of the underlying substrate. Higher the conductivity, the better is the germicidal action of the materials. Non-conducting GO-Glass system did not show any germicidal activity. It has been suggested that graphene metal oxide composites can extract electrons from microbial membranes leading to loss of viability.^[33] Therefore, starch and other polymer can form secondary structures to enrich the antibacterial groups that can have medicinal applications. Therefore, they are able to kill bacteria upon contact with durable and sustainable antimicrobial activity when covalently attached to the surfaces of a variety of materials.^[27]

Herein, we report a cost-effective, facile, hydrothermal method to fabricate starch and NiO decorated GO composite materials (GO/NiO/starch). The prepared samples were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), Fourier-transform infrared spectroscopy (FTIR), Raman Spectroscopy, X-ray photoelectron spectroscopy (XPS), thermogravimetric analysis–differential thermal analysis (TGA-DTA) and Zeta potential. Antibacterial tests were also performed for the obtained GO/NiO/Starch composites.

2. Experimental

2.1. Chemicals

Graphite powder was purchased from Sigma Aldrich. Starch (corn starch), nickel chloride (NiCl₂), hydrogen peroxide (H_2O_2) 33%, sulfuric acid concentrated (H_2SO_4) 98% pure, potassium permanganate (KMnO₄), citric acid, disodium hydrogen phosphate (Na₂HPO₄), hydrochloric acid (HCl),

ammonia solution (NH_3) , sodium hydroxide (NaOH) and nitric acid (HNO_3) were purchased from Merck, India, Ltd.

2.2. Methods

2.2.1. Preparation of graphene oxide (GO)

Graphite powder was oxidized by following the Hummers' method (with addition of KMnO4). For the modified Hummer's synthesis, 2.0 g of graphite powder were mixed with 100 mL H₂SO₄ and stirred in an ice bath for 2 h. Next, 8.0 g of KMnO₄ were slowly added so that the temperature of the mixture remained below 10° C. The suspension was then reacted for 2h in an ice bath and stirred for 60 min, before being stirred again in a 40 °C water bath for 60 min. The temperature of the mixture was adjusted to a constant 98 °C value, for 60 min, while water was continuously added. Deionized water was further added so that the volume of the suspension was 400 mL.20 ml of H_2O_2 (33%) were added after 5 min to reduce the unreacted oxidant until the slurry turned golden yellow. The reaction product was filtered and repeatedly washed with deionized (DI) water and 5% HCl solution. Finally, the product was dried at 60 °C for 24 h.^[34]

2.2.2. Preparation of GO/NiO

In a typical synthesis of NiO, a 0.1 M solution of nickel chloride (NiCl₂, 0.58 g) was prepared by dissolution in a 4.0 M HCl solution and stirred at 60 °C for 15 min, then the pH was increased to \sim 7 by addition of 2 M NaOH and stirring was continued for 2 h.The result was a greenish solution, to which 20 mg of GO, previously dispersed in 40 mL of deionized (DI) water by ultrasonication, were added to assure a complete combination of the metal ion and the oxygen-containing functional groups on the edges and surface of GO. After complete dissolution, ammonia (28 wt% in water) was added drop by drop to the GO dispersion with continuous stirring for 2 h. The product formed was washed repeatedly with water and alcohol, finally with acetone, and dried at room temperature. The sediment was collected and dried in an oven at 45 °C for 24 h to improve the crystallinity of GO/NiO composites.

2.2.3. Preparation of cross-linked starch dispersion

3.0 g of starch were dispersed in 100 ml of DI water. In order to obtain cross-linking of the starch molecules, 3.0 g of a cross linking agent (citric acid) and 1.5 g of Na₂HPO₂•H₂O catalyst were dissolved in the starch solution. The obtained suspension was heated to 60 °C for 2 h, with continuous stirring, and then cooled to room temperature.

2.2.4. Preparation of GO/NiO/starch composite material

GO/NiO/starch composites were obtained by mixing the cross-linked starch and GO-NiO composite dispersions with continuous stirring, 60 °C, for 6 h. The resultant solution was allowed to settle overnight at 25 °C. The supernatant liquid was then decanted and the solution was filtered and

washed with HNO_3 1.0 M to remove excess reagents. The excess of acid was removed by washing with DI water and then the composite was dried in a hot air oven, for several hours, at 80 °C. The dry final product was crushed into small granules of similar size, suitable for further applications.

2.2.5. Sample preparation for antibacterial activity tests of synthesized GO/NiO/starch

GO, GO/NiO and GO/NiO/starch composites were dispersed in methanol (10 mg/mL) and DI water (10 mg/mL) a used as controls for the analysis of antibacterial activity. After suspension in the respective solvent, samples were subjected to vortexing for complete homogenization and better results.

2.2.6. Well diffusion method

The sterilized nutrient agar was poured (20 ml/plate) on to the Petri plates and left for a while until the agar solidified. Pathogenic strain *Staphylococcus aureus* was taken and made into cultures. Fresh over night inoculums (100 μ L) of each culture were evenly spread onto nutrient Agar plates surface in three different Petri plates, the wells were casted by pores (3 mm diameter). The sample (GO/NiO/Starch) was loaded in equal volumes (30 μ L) on the plates. Control plates consist of distilled water and antibiotics. The square plates were incubated at room temperature for 24 h. Activity was clearly visible on the plates after 24 h. The zone of inhibition was measured and the sample of the GO/NiO/Starch showing maximum antibacterial activity was noted.

3. Instrumentation

XRD was recorded on a Bruker AXS D8 Advance using Cu radiation at a wavelength of 1.5406 Å, and a detector Si (Li) PSD was used. The temperature was measured by a Anton Paar TTK 450, with a temperature range from -170° C to +450° C. FTIR was recorded on a Thermo Nicolet, Avtar 370 FTIR spectrophotometer. Samples were prepared as KBr disks. The spectral range was around $4000-400 \text{ cm}^{-1}$. A DTGS detector was used. Resolution was set as 4 cm^{-1} . Structural features were obtained by using a JEOL Model JSM-6390LV microscope with a tungsten filament. Particle sizes were determined using ImageJ software. Raman spectra were recorded on a BRUKER RFS 27: stand alone FT-Raman. The scan range was around $50-4000 \text{ cm}^{-1}$ and resolution was around 2 cm⁻¹. XPS was recorded on a PHI 5000 Versa probe III. The average depth of analysis for an XPS measurement is around 5 nm. The curve-fitting was performed using a Gaussian function at high resolution. TGA was recorded on a Perkin Elmer, Diamond TGA/DTA apparatus. TGA temperature range was from ambient to 1200 °C, using 200 mg sample. DTA was recorded on a Perkin Elmer STA 6000. The DTA measurement range was ±1000 mV, sample volume 45 ml or 90 ml, and sensitivity of DTA 0.06 mV.



Figure 1. XRD diffractograms of (a) GO, (b) GO/NiO and(c) GO/NiO/ Starch composites.

4. Results and discussion

4.1. XRD

XRD is mainly useful for the determination of crystalline phases. Here, we discuss the XRD spectrum of GO and GO based composite materials, like GO/NiO and GO/NiO/ Starch (Figure 1). For GO, a diffraction peak is obtained at 10.35° and the crystalline size of particles (D) is obtained at 1.26 nm and d-spacing is around 0.968 Å.^[35] For GO/NiO diffraction peak is around 9.00° and the crystalline size of particles (D) is obtained at 4.9 nm, with d-spacing around 1.878 Å.^[36] GO/NiO/Starch displays diffraction peaks at 15.23°, 19.42°, 23.12°, 26.67°, 42.44°, with d-spacing 1.104 Å. The average crystalline size of GO, GO/NiO, GO/NiO/Starch composite materials is determined using the "Scherrer formula."^[37]

$$D = \frac{k\lambda}{\beta \,\cos\theta} \tag{1}$$

where, D is the average crystalline size (nm), k is 0.9, λ is the wavelength of radiation source used (1.5406 Å), β is the full width of half maximum peak (FWHM), θ is the diffraction angle at maximum peak.^[37] d-spacing is determined using Bragg's equation, i.e.,

$$2d\sin\theta = n\lambda$$
 [2]

Where d is the interplanar distance between lattice planes and n is an integer.

4.2. FTIR

FTIR was used to confirm the presence of GO, GO/NiO in the GO/NiO/Starch composite material. The typical features of GO (Figure 2a), namely the C=O carbonyl stretching at 1633 cm^{-1} ,C=O stretching at 1383 cm^{-1} and C-O stretching at 1062 cm^{-1} are visible. The spectra also has a C=C peak at 1739 cm^{-1} dealing with the remaining sp² character, and a very broad and intense peak at 3445 cm^{-1} , for O-H stretching frequencies. The peaks from 1111 cm⁻¹ to



Figure 2. FTIR spectra of (a) GO, (b) GO/NiO and (c) GO/NiO/Starch composites.



Figure 3. Raman data of (a) GO, (b) GO/NiO and (c) GO/NiO/Starch composites.

1156 cm⁻¹ is also the characteristics peak of C-O-C and C-OH GO stretching frequencies.^[38] For the GO/NiO composite, Figure 2b shows the peak at 3411.53 cm^{-1} shifted to 3361.21 cm^{-1} , while the stretching vibrations of COO⁻ at 1622.36 cm^{-1} and 1425.67 cm^{-1} shifted to 1622.36 cm^{-1} and 1425.67 cm^{-1} . Oxygenated hydrophilic functional groups, present on the surface and edges of GO sheets, improve the compatibility between GO and the polymeric matrix.^[39] Figure 2c shows the characteristics peaks of GO/NiO/starch at 3403, 2931, 2859, 1622, 1421, 1113 and $1020 \text{ cm}^{1.[40]}$ GO,



Binding Energy (eV)



Figure 4. C1s XPS data of (a) GO, (b) GO/NiO and (c) GO/NiO/ Starch composites.





Figure 5. SEM images of (a,b) GO, (c,d) GO/NiO and (e,f) GO/NiO/Starch composites at different magnifications (×1500 and ×500, respectively).

GO/NiO, GO/NiO/Starch show peaks in the region of 2859 cm^{-1} , assigned to asymmetric mode of CH₂ group vibrations. NiO and starch effectively formed a layer on the surface of GO.^[41] The sharp peaks at 585 cm^{-1} , for Ni-O stretching frequency, confirm that the oxide is effectively bound to GO.^[42]

4.3. Raman spectra

The Raman spectra of GO, GO/NiO and GO/NiO/Starch composites material are shown in Figure 3. The peaks around $1385 \,\mathrm{cm}^{-1}$ come for the disordered structure of GO (D mode), and those around $1500 \,\mathrm{cm}^{-1}$ deal with the



Figure 6. Size distribution histograms of (a) GO, (b) GO/NiO and (c) GO/NiO/Starch composites.

graphite structure (G mode). The G band is often related to E_{2g} phonon of sp² atoms, while the D band is the breathing mode of k-point phonons of A_{1g} symmetry. The intensity ratio (I_D/I_G) of the D to G bands in graphitic materials can be used for the determination of the sp² domain size.^[43] An increase in the average size of sp² domains or changes in the properties of the GO sheets are due to interactions with NiO and starch. The I_D/I_G ratios were 0.926 for GO, 0.928 for GO/NiO and 0.929 for GO/NiO/starch. Accordingly, the decrease in ID/IG ratio implies the existence of π - π interactions between the benzene rings of starch and the sp² domains of the GO.

4.4. XPS

The C1s XPS spectrum of GO (Figure 4a) shows four components related to the different types of carbon atoms found with diverse functions: oxygen-free ring (283.3 eV), C-O bond (285.2 eV), carbonyl (287.4 eV) and carboxyl (C-O-O) (288 eV) groups, which are in agreement with the present work.^[46] The oxygen-containing group of GO can make bond with nickel and starch polymer. Figure 4b and 4c displays the C1s XPS spectra of GO/NiO and GO/NiO/ Starch, respectively. The materials have three components, related to the carbon atoms in C-C (284.7 eV), C-OH (286.9 eV) and (O-C-O) (288.7 eV) bonds.^[47] The spectra of GO/NiO and GO/NiO/Starch lacks components related to the C atom of carbonyl (C=O), present in GO. In contrast, the absence of other C-O groups (like epoxy, carboxylic and hydroxyl), compared with pure GO, demonstrates the chemical interaction between GO, GO/NiO and GO/ NiO/Starch.^[48]

4.5. SEM

The surface morphology of GO/NiO/Starch was investigated by SEM. Figure 5a and 5b shows the synthesized wrinkled GO sheets. Figure 5a and 5b shows that the surface of GO is not smooth, and the GO exhibits a layered structure. The GO sheets are stacked and have wrinkles on the surface.^[49] Figure 5c and 5d shows GO/NiO with agglomeration. Those images show that NiO exhibits a relatively smooth surface. However, GO/NiO/Starch composites (Figure 5e and 5f) display a roughness surface with some joints and protuberances

2 -	Element	Wt%	Atomic %
14-	С	59.18	67.11
	0	36.55	31.11
12-	Al	0.05	0.02
	S	0.12	0.06
	Si	2.95	1.12
10- 0	Cl	0.99	0.38
	K	0.17	0.06
	Total	100	100
see - 6- - - - - - - - - - - - - -			
0 - 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1			' 18 keV





Figure 7. EDS spectra of (a) GO, (b) GO/NiO and (c) GO/NiO/Starch composites.



Figure 8. TG curves of (a) GO, (b) GO/NiO and (c) GO/NiO/Starch composites.



Figure 9. DTA thermograms of (a) GO, (b) GO/NiO and (c) GO/NiO/ Starch composites.

on the composites surface, suggesting that GO and GO/NiO nanoparticles are attached to the surface of Starch. Furthermore, these images indicate that the surface of GO nanosheets is loose, curled and entangled, which is probably attributed to the addition of starch in the composite synthesis process, leading to a force unbalance in the planes of GO layers. On the other hand, NiO was not removed from the surface of GO nanosheets in the GO/NiO composite after washing, suggesting that NiO deposited on GO nanosheets is strongly adhered.^[50] The average particle sizes are 5–7 nm, 5–7 nm, and 9–10 nm for GO, GO/NiO and GO/NiO/Starch composites, respectively, as shown in Figure 6.

4.6. EDS

The EDS spectrum of GO sheets (Figure 7a) shows the presence of C and O elements, while the spectra of GO/NiO (Figure 7b) and GO/NiO/Starch (Figure 7c) composites indicate the presence of C, O, Ni and some other elements. Small amounts of impurities, such as Mn, S and Cl present



Figure 10. Zeta potential of (a) GO, (b) GO/NiO and (c) GO/NiO/ Starch composites.

in the purified GO are an unsolved issue for the proposed purification technique. Therefore, we are investigating some modifications for the process to eliminate the manganese impurities.^[51] The filtration method could overcome the presence of other soluble impurities such as P and K within the advantage of relatively short time as compared to that reported in the literature so far. Nevertheless, the peaks in the EDS spectra can be perfectly assigned to the elements found in GO/NiO/Starch composites material, as also found by other authors.^[51,52]

4.7. TGA-DTA

The TGA and DTA plots of GO, GO/NiO and GO/NiO/ Starch are shown in Figures 8 and 9. GO shows around 17.6% mass loss below 162 °C, with maximum decomposition rate at 39.2 °C, coming from evaporation of adsorbed water. There is a rapid mass loss from 166 °C to 400 °C, with maximum decomposition rate at 240 °C, coming from the decomposition of oxygen-rich functional groups.^[53] After 400 °C, GO shows a regular mass loss, due to further removal of more functional groups, and corresponding DTA peak at 165 °C. The TGA of GO/NiO shows only ~0.9% mass loss at 400 °C (the onset decomposition temperature), corresponding to the DTA peak at 160 °C.^[54] The TGA plot of GO/NiO/Starch showed a three step mass loss, with the corresponding thermal events shown by DTA. The first loss was between 39 and 157 °C (17.7%), corresponding to the DTA peak at 163 °C, explained by single step dehydration. After being dehydrated, the compound shows stability up to 161 °C. Above that, it thermally decomposes in consecutive steps. The other mass loss (11.7%) was observed between 160 and 354 °C, and is related with an oxidative process shown by the sharp exothermic peak at 183 °C. The last mass loss is due to the destruction of a large portion of physisorbed and chemisorbed starch. This loss (62%) was between 372 and 487 °C, with the exothermic peak at 227 °C, related to the disruption of the Ni-OH bond (between NiO and starch).^[55]

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Table 2. Zones of inhibition (ZOIs) of antimicrobial activity for GO, GO/NiO, and GO/NiO/Starch, for various concentrations in distilled water and methanol solvent (50, 100, and 150 µg/mL).

		Concentration of GO					
S. no.	Bacterial culture	Distilled water			Methanol		
1	Stanbulococcus aurous	50 μL	100 μL	150 μL	50 μL 14 mm	100 μL 15 mm	150 μL
1. C		011111	011111		1411111	1311111	1011111
S. no.	Bacterial culture	Concentration of GU/NiU/Starch					
		Distilled water		Methanol			
		50 μL	100 μL	150 μL	50 μL	100 μL	150 μL
1.	Staphylococcus aureus	14 mm	15mm	17 mm	0 mm	0 mm	1 mm
S. no.	Bacterial culture	Concentration of GO/NiO/Starch					
		Distilled water			Methanol		
		50 μL	100 μL	150 μL	50 μL	100 μL	150 μL
1.	Staphylococcus aureus	16 mm	19 mm	25 mm	22 mm	24 mm	26 mm





Figure11. Antibacterial activity test against *Staphylococcus aureus* with GO based composites in different concentrations in distilled water and methanol solvent (50, 100, and 150 µg/mL). (a) GO, (b) GO/NiO, (c) GO/NiO/Starch.

4.8. Zeta potential

Zeta potential is used to determine the surface charge (acidity of basicity) of an adsorbent, with measurements carried out as a function of pH. Figure 10 shows that the Zeta potential of GO/NiO/Starch $(-3.82 \pm 1.80 \text{ mV})$ was more negative than those of GO ($2.88 \pm 0.83 \text{ mV}$) and GO/NiO ($-2.42 \pm 0.42 \text{ mV}$), but all showed negative values in the range of pH range tested, as also obtained by other authors.^[56] In the case of the composite material, the significantly more negative zeta potential suggests the higher stability of the mixed solution. Based on all the facts, it can be



Figure 12. Histogram showing the concentrations and zones of inhibition of *Staphylococcus aureus*.

concluded that the dispersion of the composite material is better than that of GO, causing the carbon nanomaterial to fully express its potential either on the membrane surface or in the polymer matrix.^[57,58]

4.9. Antimicrobial activity studies

The antibacterial activity of samples is demonstrated by the formation of zones of inhibition (ZOIs) at 37 °C at 24 h. The presence of such zones confirms the inhibitory antibacterial activity of the materials. The study revealed that GO/ NiO/Starch presents maximum toxicity against Staphylococcus. Aureus (26 mm diameter), as shown in Table 2 and Figure 11, exhibiting good antibacterial activity, when compared to GO and GO/NiO. Table 2 and Figure 10 show the different ZOIs for antibacterial activity obtained for GO, GO/NiO and GO/NiO/Starch, with various concentrations (50, 100, 150 µg/mL) in methanol and distilled water. GO and GO/NiO produce a minimum ZOI, while comparatively GO/NiO/Starch gives a good ZOI. GO/NiO/Starch also shows better response for *Staphylococcus aureus*. The area around the sample was clear, showing complete inhibition. The space that surrounds the ZOI is called the partial zone of inhibition, where the antibacterial activity is smaller than in the complete zone of inhibition. The size of ZOIs of Staphylococcus aureus are shown in the histogram of Figure 12. A complete inhibition is observed for Staphylococcus aureus by GO/NiO/Starch composite material that has negative charge and allows the penetration of a larger number of negatively charged anions and hydroxyl radicals inside the cell membrane, causing several damage and death of the cell. It is known that hydroxyl radicals produced by immunological response kill invading microorganisms by oxidation. Besides the immune system, it has been demonstrated that hydroxyl radicals play an important role in the bactericide action of antibiotics.^[59]

Heavy metals are toxic and reactive with proteins; therefore, they bind protein molecules; as a result, the cellular metabolism is inhibited causing death of microorganisms. It is believed that nickel nanoparticles, after penetration into bacteria, inactive their enzymes, generate hydrogen peroxide and cause bacterial cell death. After adherence to the surface of the cell membrane, they are distributed as Ni²⁺, interacting with enzymes of the respiratory chains of bacteria. Therefore, it can be concluded that complete bacterial inhibition depends upon the concentration of nanoparticles and on the number of bacterial cells. This reflects that Ni has a significant biocide effect in reducing bacterial growth, with practical applications.^[60] Similarly, corn starch can act as reducing and stabilizing agent and also as a template for synthesizing graphene metal oxide based composites with excellent antibacterial activity.^[61]

5. Conclusions

The chemical structure of different graphene based metal polymer composites were analyzed by FTIR, XRD and Raman spectroscopy. This demonstrated the strong chemical interaction between GO/NiO and starch. SEM images showed improvement of the interfacial adhesion of the composites. Under comparable conditions, interaction of GO with NiO and starch was beneficial to the increase of the thermal properties of the composite materials. Interestingly, the composites with NiO and polymer showed excellent antibacterial properties against Gram-positive bacteria. Therefore, the present work encourages the use of a graphene based metal oxide into a natural renewable starch matrix, as an alternative to synthesized packaging materials for several industrial applications. The GO/NiO/Starch composite shows a large potential as antibacterial material to be used in a wide range of biomedical applications.

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