Immobilization of GOx Enzyme on SiO₂-Coated Ni–Co Ferrite Nanocomposites as Magnetic Support and Their Antimicrobial and Photocatalytic Activities

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ABSTRACT: The present study used a sol–gel auto-combustion approach to make silica (SiO₂)-coated Ni–Co ferrite nanocomposites that would be used as a platform for enzyme immobilization. Using glutaraldehyde as a coupling agent, glucose oxidase (GOx) was covalently immobilized on this magnetic substrate. X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), high-resolution transmission electron microscopy (HRTEM), and fourier transform infrared spectroscopy (FTIR) was used to determine the structural analysis and morphology of Ni–Co ferrite/SiO₂ nanocomposites. FTIR spectra confirmed the binding of GOx to Ni–Co ferrite/SiO₂ nanocomposites, with a loading efficiency of around 85%. At alkaline pH and higher temperature, the immobilized GOx enzyme exhibited increased catalytic activity. After 10 times of reuses, it still had 69% catalytic activity. Overall, the immobilized GOx displayed higher operational stability than the free enzyme under severe circumstances and was easily recovered by magnetic separation. With increased doping concentration of the nanocomposites, the photocatalytic activity was assessed using a degradation process in the presence of methylene blue dye under UV light irradiation, which revealed that the surface area of the nanocomposites with increased doping concentration played a significant role in improving photocatalytic activity. The antibacterial activity of Ni–Co ferrite/SiO₂ nanocomposites was assessed using the agar well diffusion method against Escherichia coli, a gram-negative bacteria (ATCC 25922). Consequently, it was revealed that doping of Ni²⁺ and Co²⁺ in Fe₂O₄/SiO₂ nanocomposites at varied concentrations improved their antibacterial properties.

INTRODUCTION

Nanocrystalline ferrites have attracted a lot of attention in the last decade because of their uniqueness and remarkable properties such as small size, high surface area to volume ratio, low toxicity, and strong magnetic effect. They have been successfully used in intracellular uptake and separation,¹,² drug delivery, hyperthermia, magnetic resonance imaging contrast enhancement, enzyme and protein immobilization, and protein purification.³–⁶ For the synthesis of nanoferrites, many synthetic approaches have been used, including mechanical milling, co-precipitation, thermal decomposition, sol–gel auto-combustion, and hydrothermal methods.⁷ Because it does not require pH control or a subsequent annealing step in the synthesis of phase-pure and highly crystalline ferrite nanoparticles, auto-combustion synthesis offers several distinct benefits over co-precipitation and ceramic approaches.⁸

Enzymes are very efficient and highly specific biocatalyst that catalyzes simple as well as very complex reactions at ambient environmental conditions and produces very specific products. Nowadays various industries are based on the production of specific products catalyzed by enzymes such as pharmaceuticals, washing powders, food industry, tannery, bioethanol process, leather processing, etc.⁹ Enzymes are proteinaceous in general, and their thermal stability is a critical concern in industrial applications.¹⁰,¹¹ As a result, immobilizing industrially significant enzymes on a solid support is an important technique for improving operational stability, product recovery, and reusability.¹²–¹⁷ Several immobilization strategies (physical adsorption, covalent bonding, trapping, and cross-linking) have been developed to keep the enzyme structure and spatial orientation, as well as overall catalysis, stable under various conditions. In most cases, during or after the immobilization procedure, the enzyme structure slightly distorts, which hampers the overall catalytic efficiency of the enzyme.¹⁶,¹⁷ These modifications will be essentially uncontrollable, but making a large library of biocatalysts organized using various immobilization procedures to cover a wide range of conditions could lead to solutions that improve enzyme characteristics.¹₃,¹⁹,²¹ So, for maximum residual catalytic
efficiency of an enzyme after immobilization, we need efficient support as well as immobilization strategies.\textsuperscript{12} GOx (E.C. 1.1.3.4) is an oxidoreductase that catalyzes the oxidation of $\beta$-$\text{D}$-glucose to $\delta$-glucono-$\delta$-lactone with molecular oxygen, and the intermediate lactone extemporaneously hydrolyzes into gluconic acid and hydrogen peroxide by lowering the reaction’s activation energy (Figure 1).\textsuperscript{22} GOx is used in a variety of industries, including food, beverage, textiles, clinical research, and biotechnology.\textsuperscript{22,23} Baking, dairy products, starch conversion, and beverage processing (beer, wine, fruit, and vegetable juices) are various applications of the GOx enzyme in the food industry.\textsuperscript{24} The use of GOx enzyme in increasingly advanced sectors such as biosensors, is fast growing due to the specificity of enzymes, which is of a key position in biosensors.\textsuperscript{25−27} They are used to detect, transmit, and record data that is then processed into an analytical signal, allowing them to be employed in a variety of applications.\textsuperscript{25−27} Many other key sectors, such as health care, pharmaceuticals, and chemical manufacturing, are taking advantage of GOx’s remarkable properties.\textsuperscript{30,31} Carbon felt, gold nanostars, cellulose nanocrystals, carbon nanotubes, nanofibers, and other materials have been used to immobilize GOx due to its industrial value.\textsuperscript{22−24} Although, among the various supports, magnetic supports for biological and biomedical materials are of specific attention due to their intrinsic characteristics, such as non-toxicity, large surface area, and capacity to generate required magnetic properties, as well as their capability to be separated with magnets and be recycled.\textsuperscript{35,36} Immobilization of enzymes, however, has several drawbacks, including inadequate immobilization, mass transfer, and diffusion limitation. The surface chemical characteristic of the support material, i.e., functional groups present on the surface, has a significant impact on the performance of immobilized enzymes. The presence of valuable functional groups on the surface can help enzymes integrate better, resulting in more active enzyme loading and less leaching during applications.\textsuperscript{37} In recent decades, photocatalytic degradation of organic contaminants in wastewater using semiconductor nanoparticles has received a lot of interest. Among the numerous photocatalysts (metal oxide semiconductor photocatalysts), TiO$_2$ is the most widely used and best suited for environmental applications. In practice, however, separating and recovering nanosized Ti-based photocatalysts is challenging and expensive. Conventional separation procedures, such as centrifugation and filtration, can result in significant catalyst losses. As a result, it is necessary to develop a non-TiO$_2$ based photocatalyst that incorporates magnetic nano- or micro-particles that have overcome the abovementioned challenges by readily separating them from solution under an externally applied magnetic field.\textsuperscript{38} Because they combine flexible surface functionalization, non-toxicity, and sensitive magnetic response, iron oxides have long been a target for usage in composite materials. SiO$_2$ is especially appealing because of its anticidal characteristics, which may kill bacteria, molds, viruses, and even cancer cells.\textsuperscript{39,40} SiO$_2$ has no photocatalytic activity, which is a significant issue. Researchers have sought to build a visible-light SiO$_2$ based photocatalyst and an antibacterial agent to address these issues. There are three basic methods for preparing such agents: (1) noble metal ion or transition metal ion doping such as Pt, Au, Ag, V, Cr, Mn, Fe, Co, and Ni; (2) nitrogen doping; and (3) coupling with a small-band-gap semiconductor that extends light absorption into the

![Figure 1. GOx reaction is depicted here.](https://doi.org/10.1021/acsomega.1c04360)

![Figure 2. XRD patterns of Ni−Co ferrite nanocomposites coated with SiO$_2$.](https://doi.org/10.1021/acsomega.1c04360)
visible region. Metal doping (particularly Ni and Co) has received a lot of attention among these alternatives. Ni and Co are thought to improve photocatalytic activity by facilitating electron–hole separation and/or providing more adsorption surface area. It is thought that visible light absorption by Ni and Co surface plasmons induces electron transfer to Fe₂O₄, resulting in charge separation and thus activation by visible light.⁴¹–⁴⁴

Herein, we present the synthesis of SiO₂-coated Ni–Co ferrite nanocomposites and their characterization using several spectroscopic approaches as a follow-up to our previous work on nanoparticles.⁴⁵–⁴⁸ Further, we investigated the biological applications of these Ni–Co ferrite/SiO₂ nanocomposites by covalent immobilization of GOx through GA activation and their antimicrobial and photocatalytic properties. By comparing the results of FTIR spectroscopy and HRTEM with those of unbounded NCs, the covalent binding of GOx to Ni–Co ferrite/SiO₂ nanocomposites was confirmed. The relative evaluation of operating factors such as kinetic constants, variable thermal and pH stability, and the reusability of unbound and immobilized GOx helped to determine the ideal operating conditions. To the best of our knowledge, no article has yet described a method for immobilizing GOx on Ni–Co ferrite nanocomposites coated with SiO₂.

### RESULTS AND DISCUSSION

#### Structural Analysis

**Figure 2** shows XRD patterns for Ni₅Coₓ−₁Fe₂O₄/SiO₂ (x = 0.0, 0.5, 1.0) nanocomposites. A hump around 2θ ≈ 23° can be seen in all of the spectra, which corresponds to the amorphous matrix of SiO₂. The powder samples of Ni₅Coₓ−₁Fe₂O₄/SiO₂ nanocomposites show diffraction peaks at 2θ ≈ 30.10 (220), 35.57 (311), 43.16 (400), 54.10 (511), and 62.51° (440), which could be easily indexed to the face-centered cubic (FCC) spinel structure of Ni–Co ferrite by comparing with JCPDS File No. 03-0864. The discrepancy in FWHM is in agreement with the crystallite size calculated by Debye–Scherrer’s formula:

$$D = \frac{0.9 \lambda}{\beta \cos \theta}$$

where λ is the wavelength of Cu Kα radiation, D is the particle size, β is the full width half maximum intensity, and θ is the position of the peak. The main diffraction peak (311) was used to calculate the crystallite size, and the results are given in Table 1.

#### FTIR Spectroscopic Studies

The FTIR spectra are shown in Figure 3, aided in determining the pattern of the spinel structure in Ni–Co ferrite/SiO₂ nanocomposites. Strong absorption bands were observed in the prepared sample at 467, 799, 1090, and 1631 cm⁻¹, which can be attributed to Si−O−Si symmetric bond stretching vibrations, vibration mode of the ring structure of SiO₂ tetrahedra,⁴⁹ Si−O−Si asymmetric bond stretching vibrations,⁵⁰ and bending vibrations of H−O−H absorbed in silica,⁵¹ respectively. The generation of a silica network is shown by the typical absorption bands at 1090, 799, and 467 cm⁻¹.⁵²−⁵⁴ The shoulder at 954 cm⁻¹ was most likely attributable to Si−O−Fe vibrations as well Si−O−H stretching vibrations. The occurrence of Si−O−Fe vibrations indicated some interactions between the Fe³⁺ ions and the surrounding silica network. Additionally, a band at 590 cm⁻¹ may be accredited to the existence of cobalt ferrite.⁵³,⁵⁴ These outcomes are consistent with the formation of well-crystallized Ni–Co ferrite/SiO₂ nanocomposites. Figure 4 also shows FTIR spectra of pure GOx and immobilized Ni–Co ferrite/SiO₂ nanocomposites. The stretching vibrations of −OH adsorbed on the surface of the NCs are accountable for the absorption peaks at 3421, 3423, and 3425 cm⁻¹ in curves b, c, and d, respectively, (Figure 4). APTES is adsorbed on the surfaces of magnetite NPs by Fe−O−Si bands, and the coating of APTES is recognized by the presence of stretching vibrations of CH₁ bonds on aminopropyl groups appearing around 2922 and 2850 cm⁻¹, which showed the binding of APTES molecules at the magnetite surface (Scheme 1).

The preservation of enzyme activity on supporting materials is critical because secondary structural alterations in the enzyme can have a significant impact on its activity. Herein, the secondary conformation changes of the polypeptide chain of GOx on the Ni–Co ferrite/SiO₂ nanocomposites are investigated using FTIR. For the native GOx (Figure 4a) and the GOx-encapsulated Ni–Co ferrite/SiO₂ nanocomposites (Figure 4b–d), the two typical protein bands⁵⁶–⁵⁸ can be detected at approximately 1675 and 1515 cm⁻¹. These spectral features show that GOx has been successfully integrated into Ni–Co ferrite/SiO₂ nanocomposites and that the secondary structure of the immobilized GOx molecules has been well preserved.

#### Analysis of Surface Morphology and Composition

The SEM images and EDS spectra were collected at a magnification of 4000 (Figure 5). The particles are not evenly distributed and show diffused patterns of particles in the form of aggregates in the SEM images of Ni₅Coₓ−₁Fe₂O₄/SiO₂ (x = 0.0, 0.5, 1.0) nanocomposites. EDS was used to evaluate the compositions of several samples, Ni₅Coₓ−₁Fe₂O₄/SiO₂ (x = 0.0, 0.5, 1.0) nanocomposites for the different elements in relation to weight % and atomic % (Table 2), indicating that Si, Ni, Co, Fe, and O are present in almost the predicted stoichiometric proportions.

#### Transmission Electron Microscopy Study

HRTEM images of Ni–Co ferrite/SiO₂ nanocomposites without (Figure 6A–C) and with (Figure 6D–F) immobilized GOx enzymes are shown with their size distributions. Unimmobilized nanocomposites appeared to be very well and uniformly dispersed, with diameters ranging from 20 to 30 nm and an overall mean diameter of 22.90 ± 7.11 nm. However, after immobilization, the nanocomposites stay isolated and have a mean diameter of 20.65 ± 5.46 nm, which is similar to that of unimmobilized ones. The discrepancy in size between bare nanocomposites and nanocomposites containing the GOx complex was determined using statistical analysis of both images, indicating that the binding process did not affect the size of nanocomposites. Due to the immobilization of GOx on nanocomposites, this is obvious from the physical assessment of images without exhibiting extra aggregation.

#### Properties of Immobilized GOx: Loading Efficiency of GOx

To test the industrial applicability of Ni–Co ferrite/SiO₂ nanocomposites, GOx was covalently immobilized on the
surface of Ni−Co ferrite/SiO₂ nanocomposites via GA, which acted as a linker between GOx and nanocomposites (Scheme 1). The loading efficiency of GOx was 85.184% on the GA activated surface of Ni−Co ferrite/SiO₂ nanocomposites.

**pH Effect on Immobilized GOx Activity.** Enzymes are very sensitive to the pH alteration in the reaction mixture, which directly affects the overall catalytic performance of enzymes, so for industrial applications, we need an enzyme that is more stable and gives maximum catalytic activity for the broader pH range. Therefore, we observed the effect of pH on the catalytic activity of the soluble and immobilized GOx on Ni₄₊ₓCoₓFe₂O₄/SiO₂ (x = 0.0, 0.5, 1.0) nanocomposites (Figure 7). It has been observed that there is a slight shift in the pH optima for maximum catalytic activity after immobilization; soluble GOx gave a maximum activity at pH 5.5, whereas it shifted to pH 6.0 in the case of immobilized GOx. In general, alteration of pH in the reaction mixture directly affects the ionizable groups of amino acids present in the enzyme and its active sites, which leads to the unfolding of enzyme and subsequent loss of catalytic activities. The immobilized GOx showed better catalytic activity at both the acidic pH 3.0 and alkaline pH 10.0 as compared to a soluble fraction, which signifies that the soluble GOx is very sensitive to the pH alteration in the reaction mixture. We also observed that when we increased the Ni²⁺ concentration in nanocomposites, immobilized GOx on NiFe₂O₄/SiO₂ nanocomposites retained 44.17 and 59.84% of the initial activity at pH 3.0 and pH 10.0, although soluble enzyme retained 19.6 and 7.71% of the initial activity at these pH values, respectively. These retained residual catalytic activity might be attributed to the presence of the ionizable group on the support and the strong covalent interaction between the enzyme and the support.

**Temperature Effect on Immobilized GOx Activity.** The catalytic activities of the biocatalyst are greatly influenced by the temperature of the reaction mixture or ambient temperature where the reaction takes place, so all the biocatalysts have a particular optimum temperature at which they give maximum catalytic activity. Therefore, we analyzed relative catalytic activities of soluble and immobilized GOx at different temperature ranges from 25−65 °C (Figure 8). It was observed that after immobilization, GOx retained significantly higher catalytic activity (60.09%), whereas soluble GOx retained only 26.23% of catalytic activity at 65 °C. One possible explanation of higher activity at higher temperatures is the formation of a covalent linkage between GOx and nanocomposites, which stabilizes the native structure of GOx at a higher temperature. Apart from that, GA also provides point attachment of GOx to the nanocomposites, which further strengthens its three-dimensional structure at higher temperatures as compared to soluble GOx. It was also observed that
there is a slight shift (5 °C) in the optimum temperature for maximum catalytic activity in the case of immobilized GOx, so it gave maximum activity at 45 °C (Figure 8). The same increment profile was also observed in our previous report.60
Thermal Stability of Immobilized GOx. The thermal stability of GOx holds significant importance in those industries where this enzyme is used as a key biocatalyst.

The comparative thermal stability profile of both the soluble and immobilized GOx after incubation at 50 °C for different time intervals is shown in Figure 9. It was observed that all the 

Table 2. EDS Analysis of \(\text{Ni}_{x}\text{Co}_{1-x}\text{Fe}_2\text{O}_4/\text{SiO}_2\) Nanocomposites at Various Compositions (Weight % and Atomic %)

| elements | composition | \(x = 0.0\) | atomic % | \(x = 0.5\) | atomic % | \(x = 1.0\) | atomic % |
|----------|-------------|-------------|----------|-------------|----------|-------------|----------|
| O        | weight %    | 43.83       | 69.40    | 36.57       | 63.77    | 50.29       | 74.46    |
|          | Si          | 12.55       | 11.32    | 11.09       | 11.02    | 13.27       | 10.86    |
|          | Fe          | 22.04       | 10.00    | 15.29       | 7.64     | 19.14       | 7.88     |
| Ni       |             |             |          |             |          |             |          |
| Co       |             |             |          |             |          |             |          |

Figure 6. TEM images and their statistical analysis of (A) \(\text{CoFe}_2\text{O}_4/\text{SiO}_2\) NCS, (B) \(\text{Ni}_{0.5}\text{Co}_{0.5}\text{Fe}_2\text{O}_4/\text{SiO}_2\) NCS, (C) \(\text{NiFe}_2\text{O}_4/\text{SiO}_2\) NCS, (D) immobilized \(\text{CoFe}_2\text{O}_4/\text{SiO}_2\) NCS, (E) immobilized \(\text{Ni}_{0.5}\text{Co}_{0.5}\text{Fe}_2\text{O}_4/\text{SiO}_2\) NCS, (F) immobilized \(\text{NiFe}_2\text{O}_4/\text{SiO}_2\) NCS.

Figure 7. pH effect on the activity of the soluble enzyme (sol) and immobilized GOx on Ni–Co ferrite/SiO\textsubscript{2} nanocomposites.

Figure 8. Temperature effect on the activity of the soluble enzyme (sol) and immobilized GOx on Ni–Co ferrite/SiO\textsubscript{2} nanocomposites.
immobilized GOx preparation retained considerably higher catalytic activity after 240 min of incubation at 50 °C, whereas, at similar conditions, soluble GOXs do not retain any catalytic activity. When comparing with different formulations of NCs, we found that GOX immobilized on NiFe₂O₄/SiO₂ nanocomposites retained 59% of residual catalytic activity after 240 min of incubation at 50 °C. In this scenario, the increased thermal stability of immobilized GOXs may be due to nanocomposites provided by an additional shield to the enzyme that absorbed some heat from the system.62,63 It is also believed that after immobilization, GOX becomes more rigid and retains its native structure at a higher temperature. A similar type of results we also reported in our previous study.64

**Determination of Kinetic Parameters of Immobilized GOX on Ni–Co Ferrite Nanocomposites Coated with SiO₂.** For the determination of kinetic parameters, steady-state kinetics were performed for both the formulations (soluble and immobilized GOX) by varying the concentration of glucose at standard assay conditions. The Michaelis–Menten parameters (Kₘ and Vₘₐₓ) were calculated by converting the steady-state plot to a double reciprocal plot (Lineweaver Burk plot) as shown in Figure 10.

It has been observed from Table 3 that the calculated value of Kₘ was slightly increased (45.28 vs 58.29 mM) whereas Vₘₐₓ (1745.22 vs 969.93 μmol min⁻¹) decreases after immobilization. In comparison with soluble enzymes, immobilized GOX has a lower affinity toward glucose; it might be due to the diffusional limitation of the substrate to the active sites of immobilized GOXs because of the nanocomposites. The maximum rate of immobilized GOX is also lower than that of soluble GOX. The decreased Vₘₐₓ of GOX might be due to some conformational changes in the immobilized GOX due to conjugation with coated Ni—Fe ferrite/SiO₂ nanocomposites, which affect either formation of an enzyme—substrate complex or the release of a product from the enzyme.

**Immobilized GOX Reusability.** One of the most important goals in the immobilization of enzymes for industrial applications is to make them reusable. The main apprehension for the immobilized enzyme during several cycles of reusability is leakage from support; hence, tremendous efforts have been made to prevent enzyme leakage.62,63 Figure 11 depicts the residual activity of the immobilized GOX enzyme on various supports after several rounds of reuse. The residual activity of immobilized GOXs declines as the number of cycles increases, and after the 10th repeated cycle, NiFe₂O₄/SiO₂ nanocomposites kept roughly 69% of residual activity, but the other two groups retained less activity. During the reusability assay, we also calculated enzyme leakage from support. Table S1 (see Supporting Information) shows that over 10 consecutive uses, 18.35% of immobilized GOX on CoFe₂O₄/SiO₂ nanocomposites leaks out, but only 7.45% leaks out on NiFe₂O₄/SiO₂ nanocomposites. As a result, GOX immobilized on NiFe₂O₄/SiO₂ nanocomposites had the highest residual activity. The percentage of leakage reduces as the doping of Ni²⁺ to the NCs increases. This could be because, in addition to the covalent conjugation of GOX to nanocomposites, Ni²⁺ ions give extra support via interacting with the histidine moieties of GOX. The findings indicated that immobilized GOXs on Ni—Co ferrite/SiO₂ nanocomposites has good durability and recovery, making it ideal for industrial applications.

**Photocatalytic Studies.** In this work, we used NiₓCo₁₋ₓFe₂O₄/SiO₂ (x = 0.0, 0.5, 1.0) nanocomposites to examine the photodegradation of organic pollutant model dye (i.e., MB dye) under UV light. As indicated, before the photocatalytic reaction, the samples were mixed with the solutions and stirred in the dark for 120 min to attain adsorption equilibrium. UV spectral changes of MB dye after different times of irradiation with NiₓCo₁₋ₓFe₂O₄/SiO₂ (x = 0.0, 0.5, 1.0) nanocomposites. Figure 12, depicts time-dependent UV–vis spectra of an aqueous MB solution in the vicinity of NiₓCo₁₋ₓFe₂O₄/SiO₂ (x = 0.0, 0.5, 1.0) nanocomposites exposed to UV light at short intervals.

Figure 12 shows a continuous decrease in intensity without the presence of new absorption peaks, implying that no resultant intermediate was formed during the photocatalytic reaction (up to 200–800 nm). Under the UV light illumination, NiₓCo₁₋ₓFe₂O₄/SiO₂ nanocomposites demonstrated significantly higher photocatalytic activity than CoFe₂O₄/SiO₂ nanocomposites and NiFe₂O₄/SiO₂ nanocomposites, indicating homogeneous doping of Ni and Co.
According to Figure 13, Ni_{0.5}Co_{0.5}Fe_{2}O_{4}/SiO_{2} nanocomposites demonstrated the highest photocatalytic activity among these samples.

Furthermore, the experimental data demonstrated that the MB dye is resistant to self-photocatalysis; when the same experiment was conducted in the dark, a very small decrease in concentration due to adsorption of dye on the catalyst was detected (see Figure S1). Precisely, under UV irradiation, Ni_{0.5}Co_{0.5}Fe_{2}O_{4}/SiO_{2} nanocomposites showed the maximum photocatalytic degradation of MB dye within 120 min, i.e., ~89% (Figure 14). Whereas, under UV light irradiation for 120 min, the lowest photocatalytic degradation of the parental CoFe_{2}O_{4}/SiO_{2} nanocomposites can be seen in Figure 14.

Our study verifies the prior reports of the photocatalyst;\textsuperscript{46,64,65} the photocatalytic degradation of MB dye may be explained using the Langmuir–Hinshelwood model\textsuperscript{66,67} i.e., which is pseudo-first-order, where the initial concentration C_{0} is very small (nearly negligible), and ln(C_{0}/C) = kt, where k is the first-order rate constant. As shown in Figure 13, under UV light irradiation, Ni_{0.5}Co_{0.5}Fe_{2}O_{4}/SiO_{2} nanocomposites have the highest rate constant (ca. 0.01825 min^{-1}), which is 2.13 times higher than CoFe_{2}O_{4}/SiO_{2}.
nanocomposites. We observed that the photocatalytic and kinetic rate constants were of the following order, Ni0.5Co0.5Fe2O4/SiO2 NCs > NiFe2O4/SiO2 NCs > CoFe2O4/SiO2 NCs, when we paralleled all samples, i.e., Ni1−xCoxFe2O4/SiO2 (x = 0.0, 0.5, 1.0) nanocomposites. Due to a lack of trapping sites, CoFe2O4/SiO2 nanocomposites have the lowest photocatalytic activity when exposed to UV light. The formation of trapping sites, efficient trapping, and a lower recombination rate enhance interfacial charge transfer and increase the photocatalytic reaction rate, as the doping content of Co2+ and Ni2+ within Fe2O4/SiO2 gradually increases.

Antimicrobial Activity of SiO2 Coated Ni–Co Ferrite Nanocomposites. Researchers are significantly making their awareness of NCs and looking for a new way to evolve improved nano-ranged antibacterial drugs against E. coli (ATCC 25922). Markedly, we investigated the antibacterial activity of the CoFe2O4/SiO2 NCs, NiFe2O4/SiO2 NCs, and Ni0.5Co0.5Fe2O4/SiO2 NCs, which were observed morphometrically qualitatively by the disk diffusion assay. All the cases (i.e., CoFe2O4/SiO2 NCs, NiFe2O4/SiO2 NCs, and Ni0.5Co0.5Fe2O4/SiO2 NCs) have shown effective antibacterial behavior, whereas Ni0.5Co0.5Fe2O4/SiO2 NCs have shown excellent antibacterial activity (Figure 15). The synergistic effect between the surfaces of Ni1−xCoxFe2O4 (x = 0.0, 0.5, 1.0)/SiO2 nanocomposites and bacteria because of adsorption—desorption chemical—physical characteristics toward the bacteria is the foremost reason in different antibacterial activities. The CoFe2O4/SiO2 nanocomposites have revealed the lowest antibacterial activity that may be because of the low concentration of doped metal in the matrix of Fe2O3/SiO2 nanocomposites and its intricacy to diffuse into an agar gel plate. Nonetheless, zones of inhibition become visible as metal doping concentrations (Ni2+ and Co2+) increase in Fe2O3/SiO2 nanocomposites. The antibacterial activity as a function of metal doping is shown in Figure 15; as the doping concentration of both metal ions gradually increases into Fe2O3/SiO2 nanocomposites, the zone of inhibition also increases.

The electron microscopic analysis demonstrates the consequence of the Fe2O3/SiO2-based NC treatment method on microorganism cells. Typical rod-shaped morphology is shown through control E. coli. The cell was unwrinkled without the ratification of impairment on the cell surface morphology (Figure 16). Despite the fact that the bacteria with distorted fragments appeared in the Fe2O3/SiO2-based nanocomposites, they were rather than normal rod-shaped cells, the bacterial cell puffs up to become larger and more aggregated. It should be noted that treating bacteria with Fe2O3/SiO2-based nanocomposites caused significant damage to E. coli cells, which eventually resulted in bacterial cell wall disruption.

Furthermore, the results designate that the Ni1−xCo0.5Fe2O4/SiO2 nanocomposites exhibited higher antibacterial activity than NiFe2O4/SiO2 nanocomposites. This is because doping of Ni2+ and Co2+ on the surface of SiO2 lowers the charge carrier’s recombination rate and raises charge separation, which increases the charge carrier’s lifetime by splitting the arrival time of photogenerated electrons and holes to reach the surface of the photocatalyst. As a result, electrons and holes can easily participate in the formation of highly oxidizing (hydroxyl radical) and reducing (superoxide radical anion) agents, enhancing the antibacterial activity of Ni2+– and Co2+–doped nanocomposites. The percent area of inhibition by Ni0.5Co0.5Fe2O4 (x = 0.0, 0.5, 1.0)/SiO2 nanocomposites against all bacteria was plotted (Figure 15).

CONCLUSIONS

Ni–Co ferrite/SiO2 nanocomposites were made using a sol–gel auto-combustion synthetic way that used inexpensive and environmentally friendly reactants followed by characterization using various techniques. GOx enzyme via GA activation was bound to the Ni–Co ferrite/SiO2 nanocomposites. FTIR spectroscopy was used to confirm the binding and its feasible mechanism, and HRTEM was used to characterize the particle sizes based on their mean diameter of 20.65 ± 5.46 nm. There was no discernible difference between immobilized GOx on Ni–Co ferrite/SiO2 nanocomposites and unimmobilized GOx in terms of the average diameter. At a pH range of 7.0–9.0 and a temperature range of 45–60 °C, enzyme activity was increased to some extent after immobilization. The enzyme’s affinity for substrate decreased after immobilization due to conformational changes and restricted accessibility. Immobilized GOx is found to be more stable than free GOx, according to research. The novel and effective immobilization method developed in this study has a wide range of industrial applications, not just for GOx but also for other enzymes and biomolecules. The photocatalytic study revealed that nanocomposites made of Fe3O4/SiO2 can degrade MB dye. Fe2O3/SiO2 nanocomposites can be doped/implanted with Ni2+ and Co2+ at different concentrations to improve photocatalytic activity. The observed data shows that doping of Ni2+ and Co2+ in Fe3O4/SiO2 nanocomposites improves antimicrobial properties in the following order: Ni0.5Co0.5Fe2O4/SiO2 NCs > NiFe2O4/SiO2 NCs > CoFe2O4/SiO2 NCs.

MATERIALS AND METHODS

Chemicals. All reagents or chemicals used were of analytical grade. Iron nitrate nonahydrate (Fe(NO3)3·9H2O), nickel nitrate hexahydrate (Ni(NO3)2·6H2O), cobalt nitrate hexahydrate (Co(NO3)2·6H2O), tetraethoxysilane (TEOS), sodium hydroxide (NaOH), sodium chloride (NaCl), glycine (C2H5NO2), sodium acetate (CH3COONa), sodium phos-
phosphate (Na₃PO₄), ethanol (EtOH), hydrochloric acid (HCl), 3-amino propyl triethoxysilane (APTES), dimethylformamide (DMF), 2-hydroxy-3,5-dinitrobenzoic acid (DNSA), glutaraldehyde (GA), and glucose oxidase (GOx) isolated from Aspergillus niger were procured from E. Merck (Germany) and Sigma Aldrich (St. Louis, MO, USA).

**Preparation of Ni–Co Ferrite Nanocomposites Coated with Silica (NiₓCo₁₋ₓFe₂O₄/SiO₂ NCs).** The sol–gel auto-combustion method was used to make nanocrystalline SiO₂-coated NiₓCo₁₋ₓFe₂O₄ (x = 0.0, 0.5, 1.0) nanocomposites. The molar ratio was fixed at 1:4:7 (TEOS:EtOH:H₂O), and TEOS diluted in EtOH was slowly added into deionized water (DI). To maintain an acidic pH of 2, an appropriate amount of acetic acid was added to the solution. When the precursor solution was stirred at 50 °C for 2 h, TEOS hydrolysis occurred, yielding a transparent viscous sol (Sol A). Ni(NO₃)₂, Co(NO₃)₂, Fe(NO₃)₃, and citric acid were dissolved in deionized water (DI) to make Solution B. The citric acid to metal ions molar ratio was fixed at 1:1. To adjust the pH to ~7, an appropriate amount of ammonia was added to the solution. After that, Sol A and Sol B was mixed to form a homogeneous transparent aqueous solution. The solution was evaporated at around 70 °C till a transparent sol was obtained, and then the resultant sol was heated at 110 °C for 2 h to allow the dried gel formation. When the dried gel was ignited in the air at 250 °C, it self-propagated. The as-burnt Ni–Co ferrite/SiO₂ nanocomposites were reddish-brown and voluminous.

**Characterization of Ni–Co Ferrite Nanocomposites Coated with SiO₂.** FTIR was used to characterize the size, structure, and magnetic properties of the Ni–Co ferrite/SiO₂ nanocomposites without and with immobilized Gox. Spectra were recorded using the Perkin Elmer FT-IR Spectrometer Spectrum Two, and values are given in cm⁻¹. SEM and HRTEM micrographs were taken with LEO 435-VF and JEOL TEM (JEM 2100F) instruments, respectively, to obtain the surface morphology and particle size. XRD data were collected using a PHILIPS PW1710 diffractometer with Cu Kα radiation at 1.540 Å in the range of 5° ≤ 2θ ≤ 70° at 40 kV.

**GOx Immobilization on Ni–Co Ferrite Nanocomposites Coated with SiO₂.** Activation of NCs. To activate Ni–Co ferrite/SiO₂ NCs, they are first silanized with APTES and then activated with the coupling agent GA. For salinization, 30 mg of each series of Ni–Co ferrite/SiO₂ nanocomposites were ultrasonically dispersed in 5 mL of 70% EtOH solution for 30 min, and then equal volumes of APTES and DMF (0.25 mL each) were added to the dispersed nanocomposite solution. The solutions were stirred for 4 h at 300 rpm, and then salinized NCs were collected by applying a small magnetic field to them. To get GA activated NCs, 10 mL (2 mg mL⁻¹) of salinized NCs were dispersed into 10 mM PBS (pH 7.3), then 400 μL of 0.5% GA solution was added to the dispersed solution, and the entire reaction mixture was kept stirring (300 rpm) at 30 °C for 1 h and then activated GOx on the Ni–Co ferrite/SiO₂ NCs were stored at 4 °C until used.

**GOx Immobilization with Activated Ni–Co Ferrite Nanocomposite-Coated SiO₂.** For the immobilization of GOx, activated Ni–Co ferrite/SiO₂ nanocomposites were resuspended into 100 mM phosphate buffer of pH 6 to make a final concentration of 3 mg mL⁻¹ and then 4 mg of GOx was mixed with 6 mL of resuspended nano ferrite composites and kept stirring for 2 h at 4 °C. After stirring, the reaction mixture was washed several times with the same buffer and the whole solution was placed under the influence of a permanent magnet to remove the unbound GOx. Finally, stock solutions of immobilized GOx on nanocomposites were stored at 4 °C until used.

**Determination of GOx Loading Efficiency.** The loading efficiency of GOx on the Ni–Co ferrite/SiO₂ NCs was calculated by using the following equation:

\[
\text{loading efficiency} = \left( \frac{C_b V_b - C_f V_f}{C_b V_i} \right) \times 100\%
\]
where $C_0$ represents the initial concentration of GOx stock and $V_f$ represents the volume of the GOx solution prior to immobilization. $C_i$ stands for the concentration of GOx in washed fraction, and $V_i$ stands for the volume of the filtrate, which was left after immobilization.

**Standard Activity Assay of GOx.** The catalytic activities of both soluble and immobilized GOx were analyzed by a UV/vis spectrophotometer using DNSA as a chromogenic substrate. The standard reaction mixture in a total volume of 2 mL contained 8 μg of GOx and 5 mM glucose in 100 mM phosphate buffer, pH 6.0. The reaction was initiated by the addition of glucose, followed by incubation at room temperature with continuous stirring for 10 min. After incubation, the reaction was stopped by the addition of 2 mL DNSA solution and then the whole solution was boiled for 10 min at 100 °C, a bright orange color appeared and finally absorbance was measured at 576 nm. Appropriate reaction blanks were used for both the soluble and immobilized GOx, i.e., for the soluble enzyme, all components of the reaction mixture were present in the blank except the enzyme, and for the immobilized enzyme, all components of the reaction mixture including Ni−Co ferrite/SiO$_2$ nanocomposites were present in the blank except the immobilized enzyme.

**Optimization of pH and Temperature for Immobilized GOx.** The optimization of pH for the enzymatic activities of soluble and immobilized GOx was performed in 100 mM of different buffers of pH ranging from 3.0 to 10.0. The buffers used were glycine-HCl (pH 3.0), sodium acetate (pH 5.0), sodium phosphate (6.0, 7.0), and Tris–HCl (pH 8.0–10). For calculating the percent residual catalytic activity at different pH ranges, the catalytic activity at pH 5.5 and 6.0 was chosen as the maximum activity (100%) for the soluble and immobilized GOx, respectively. The optimization of temperature for catalytic activities for soluble and immobilized GOx was performed under standard reaction conditions in 100 mM of sodium phosphate buffer of pH 6.0 by varying temperatures ranging from 25–65 °C. For calculating the residual percent catalytic activity at different temperatures, the activities at 40 and 45 °C were chosen as the maximum residual activity (100%) for soluble and immobilized GOx, respectively. All the assays were performed in triplicates, and results were reported as a mean value.

**Thermal Stability of GOx.** For the determination of thermal stability, free and immobilized GOx enzyme formulations were simultaneously incubated at 50 °C in 100 mM of phosphate buffer of pH 7.4 for 4 h. After every 30 min, an appropriate amount of soluble and immobilized GOx enzyme was withdrawn and put in crushed ice for 3 min. Both the samples were brought to room temperature till the enzyme assay was done, and then catalytic activity was performed by using the standard assay procedure. The residual activity was expressed relative to the reference control and considered to be 100%, which was the original activity assessed at 0 h before heating.

**Kinetic Constants Determination.** We used varying concentrations of glucose (10−150 mM) as a substrate in 100 mM PBS buffer of pH 6.0 at standard reaction conditions to determine kinetic parameters such as the maximum velocity ($V_{\text{max}}$) and Michaelis–Menten constant ($K_m$) of soluble and immobilized GOx. The apparent values of $V_{\text{max}}$ and $K_m$ of both the formulations were calculated by using the Lineweaver Burk plot (double reciprocal plot).

The Analysis of Reusability. To explore the reusability and recovery of immobilized GOx from the reaction mixture, we assessed the activity of immobilized GOx for repetitive cycles. After each repetitive cycle, immobilized GOx was washed 2–3 times with assay buffer and recovered from the solution by centrifugation at 4000 rpm for 20 min. After centrifugation, the supernatant was discarded and the pellet was resuspended in the activity buffer and stored at 4 °C for another cycle, so these procedures were recurring for 10 days in a row. The activity of immobilized GOx determined on the first day is considered as the maximum residual activity (100%) for the evaluation of the remaining percent residual activity after repetitive cycles.

**Photocatalytic Measurements.** Under UV light irradiation against MB dye, the photocatalytic activity of nanocrystalline Ni$_x$Co$_{1-x}$Fe$_2$O$_4$ ($x = 0.0, 0.5, 1.0$)/SiO$_2$ nanocomposites was measured. A total of 30 μg mL$^{-1}$ of each CoFe$_2$O$_4$/SiO$_2$ NCs, Ni$_x$Co$_{1-x}$Fe$_2$O$_4$/SiO$_2$ NCs, and NiFe$_2$O$_4$/SiO$_2$ NCs was added to 100 mL MB dye solution (25 μg mL$^{-1}$) in the photocatalytic photodegradation experiment. Before irradiation, the suspensions containing MB dye and Ni$_x$Co$_{1-x}$Fe$_2$O$_4$ ($x = 0.0, 0.5, 1.0$)/SiO$_2$ nanocomposites were magnetically stirred in the dark for 120 min to ensure the establishment of an adsorption/desorption equilibrium, respectively. Aliquots (5 mL) were sampled at a fixed time interval (up to 120 min) and then magnetically separated to remove essentially all of the Ni$_x$Co$_{1-x}$Fe$_2$O$_4$ ($x = 0.0, 0.5, 1.0$)/SiO$_2$ nanocomposites. A UV−vis spectrophotometer (Perkin Elmer Life and Analytical Sciences, CT, USA) was used to record variations in the maximum absorption band ($A_{668}$ nm) of the filtrate in the wavelength range of $A_{320}$−$800$ nm. The following formula was used to calculate the photodegradation of the MB dye via the photocatalytic activity of Ni−Co ferrite/SiO$_2$ nanocomposites:

\[
\text{photodegradation(%) = } \frac{C_0 - C}{C_0}
\]

where $C_0$ represents the initial concentration of the MB dye before photodegradation and $C$ represents the absorbance at various time intervals.

**Antibacterial Activity.** Using the agar well diffusion method, the antibacterial activity of Ni$_x$Co$_{1-x}$Fe$_2$O$_4$ ($x = 0.0, 0.5, 1.0$)/SiO$_2$ nanocomposites was determined and analyzed against Gram-negative bacteria *E. coli* (ATCC 25922). On Luria-Bertani (LB) agar plates, approximately 106 CFU mL$^{-1}$ was inoculated. Filter papers with a diameter of 1.5 cm were sucked with 100, 200, 300, and 400 μg mL$^{-1}$ of Ni$_x$Co$_{1-x}$Fe$_2$O$_4$/SiO$_2$ ($x = 0.0, 0.5, 1.0$)-based nanocomposites that were placed on the surface of a seeded agar plate. The diameters of the inhibition zones were measured after 24 h of incubation at 37 °C.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://doi.org/10.1021/acsomega.1c04360.

Figure of the photocatalytic degradation of MB dye in the dark condition in the presence of Ni$_x$Co$_{1-x}$Fe$_2$O$_4$ ($x = 0.0, 0.5, 1.0$)/SiO$_2$ NCs (Figure S1); table of the leakage percentage of GOx from the nanocomposites after 10 cycles of reusability (Table S1) (PDF).
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Notes

The authors declare no competing financial interest.

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