Synthesis of Cu(OH)$_2$ nanowires modified by Fe$_3$O$_4$@SiO$_2$ nanocomposite via green and innovative method with antibacterial activity and investigation of magnetic behaviours

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In this study, green synthesis of modified Cu(OH)$_2$ nanowires by Fe$_3$O$_4$@SiO$_2$ core–shell nanospheres was easily performed via chemical reduction. In other words, the direct coating of Cu(OH)$_2$ on Fe$_3$O$_4$@SiO$_2$ was successfully realized without the extra complicated procedures. Various concentrations of synthesized nanocomposites were tested on pathogenic and nosocomial bacteria. In this study, the structural information and characterization of Fe$_3$O$_4$@SiO$_2$/Cu(OH)$_2$ nanowires (FSCNWs) were obtained using FE-SEM, FT-IR, EDX and X-ray diffraction. This nanocomposite can effectively kill important infectious bacteria, including Staphylococcus aureus, Escherichia coli, Staphylococcus saprophyticus, Pseudomonas aeruginosa and Klebsiella pneumoniae. Studies have shown that FSCNW nanocomposites affect common antibiotic-resistant bacteria. This result confirms the function of FSCNW as an effective, beneficial and environmentally friendly antibacterial agent that can used in a wide range of applications in medicine. FSCNWs can be separated conveniently from bacteria-containing solutions using a magnet. Compared with nanocomposites based on other metals such as silver and gold, the use of FSCNWs in water treatment has been recommended because of the precursor of copper for its low price and less toxicity. In addition to its special properties such as mild reaction conditions, green
1. Introduction

In recent years, water pollution has become a global concern, and harmful microorganisms such as viruses, fungi and bacteria in drinking water or water sources can cause many diseases, putting human health at risk. *Staphylococcus aureus* and *Escherichia coli* are among the bacteria that cause diarrhoeal diseases in humans through contaminated water. Therefore, a safe drinking water supply is vital for human health [1,2]. Chlorine, ozone and chloramine are examples of traditional disinfectants that can effectively suppress microbial infections. However, in practical applications, these disinfectants cause may carcinogenic by products that are detrimental to human health [3,4].

Nanotechnology has led to safer, more cost-effective and more environmentally friendly methods than traditional chemical disinfectants for removing microbial contaminants worldwide [5]. One of the most pressing topics for scientists in the field of nanotechnology is the development of biocompatible antibacterial agents [6]. Antibacterial agents have been used in various fields such as water purification, medicine, textiles and food packaging. Efficient and beneficial antibacterial agents are being developed to prevent pathogenic bacterial damage and emergence of antibiotic-resistant strains [7].

*Klebsiella pneumoniae*, *Staphylococcus saprophytic* and *Pseudomonas aeruginosa* are samples of pathogenic bacteria, and successful eradication of these bacteria has emerged as a clinically vital issue [8–11].

*Klebsiella pneumoniae* is a Gram-negative pathogen found extensively in the skin, mouth and intestine. In addition, it is responsible for a wide variety of nosocomial infections in immunodeficient patients and respiratory, urinary and gastrointestinal tract infections in humans [9,12].

Gram-positive bacterium *Staphylococcus saprophytic* is the second most common cause of acute urinary tract infection [11].

*Pseudomonas aeruginosa* is a pernicious opportunistic pathogen and a Gram-negative bacillus [10,13]. Owing to the extraordinary resistance of *P. aeruginosa* to antibacterial medicines, especially β-lactam antibiotics, including cephalosporins and carbapenem, their eradication has become increasingly challenging [10,14,15]. Thus, growing concerns regarding to evolution of bacteria resistant to common antibiotics have led to the widespread use of nanostructured materials with antibacterial properties such as copper, silver, gold and oxidized titanium [16,17]. Recently, copper nanoparticles (NPs) have received increasing attention among metal NPs and metal oxides. Copper-based nanomaterials with excellent antibacterial properties are suitable alternatives to antibacterial materials because of their abundance and low cost rather than silver [18–24].

In this study, to inhibit oxidation or dissolution in an aqueous solution and retain the initial magnetic activity of Fe3O4 (core), Fe3O4@SiO2 nanocomposite microspheres were first prepared by coating the Fe3O4 surface with a thin layer of SiO2 [25–31]. The magnetic cores ensured quick separation [32]. Furthermore, Cu(OH)2 nanowire-decorated Fe3O4@SiO2 core–shell nanospheres (FSCNWs) with high-performance antibacterial properties were synthesized by efficient procedures. Through combining materials with potential attributes, a composite material with higher effectiveness was created than its constituents. Therefore, Cu(OH)2 NPs were placed on the surfaces of FSCNWs. Eventually, owing to the unique properties of each component, low-cost synthesis and excellent antimicrobial activity, this nanocomposite is appropriate for water treatment.

In addition, the presence of magnetic NPs allows a magnet to save the nanocomposite from the reaction media. Also, high chemical stability has made it possible to re-use nanocomposites with a stable function in removing bacterial contaminations from drinking water.

Moreover, considering the effectiveness of the nanocomposite on antibiotic-resistant human pathogenic bacteria can be mentioned as a great advantage (scheme 1).

2. Material and methods

2.1. General

Pure materials such as solvents, metallic salts and chemical compounds were procured from Merck companies. The FE-SEM images were taken by using a Hitachi S-4160 microscope. The FT-IR spectrum was acquired using a Shimadzu IR-470 spectrometer with a KBr pellet. X-ray diffraction (XRD) was performed by using a D8 Advance model made via Bruker. Numerix DXP-X10P reported the EDX analysis of the nanocatalyst.
2.2. Preparation of Fe₃O₄@SiO₂@Cu(OH)₂

First, FeCl₃.6H₂O (20 mmol) and FeCl₂.4H₂O (20 mmol) were dissolved in 200 ml of distilled water to synthesize Fe₃O₄ via co-deposition. The mixture was agitated in a closed vessel at 80°C for 2 h. Then, by adding fresh NH₃.H₂O ammonia (25%) solution to the resultant solution, iron oxide NPs were obtained at pH = 12. Finally, the dark magnetic precipitate of Fe₃O₄ was produced and amassed through an external magnetic field and dried after three rinses with distilled water.

Second, 45 mg magnetic NPs were dispersed in water (16 ml) by ultrasonic waves for 15 min and mixed in fresh NH₃.H₂O (25 wt%) (2 ml) then ethanol (80 ml). In the next step, tetraethoxysilane (TEOS) (0.8 ml) was added to the system, and the pre-hydrolysis of TEOS lasted for 24 h under stirring. Then Fe₃O₄@SiO₂ was collected at the bottom of the container using a magnet and dried at 70°C after rinsing with distilled water.

Finally, 0.1 g Fe₃O₄@SiO₂ was added to 10 ml deionized water, the mixture was ultrasonicated for 15 min, and eventually 0.85 g CuCl₂.H₂O was added to reaction mixture. The mixture then adjusted to pH = 13 using 1.0 mol.l⁻¹ NaOH aqueous solution so that the colour of the reaction solution changed to dark green at the end of the reaction [33–37].

2.3. General procedure for the antibacterial test

Before each microbiological test, samples and glassware were sterilized by autoclaving at 121°C for 15 min. Gram-negative and Gram-positive microorganisms such as *E. coli* and *S. aureus* were used for the antibacterial experiment. For this purpose, Mueller–Hinton agar containing plates, as a microorganism growth medium, with McFarland turbidity as a standard of 0.5, and the inhibition zone method were used for colony counting tests. For that, 3.8 g of Muller Hinton Agar powder was dissolved in 100 ml of distilled water and autoclave sterilized. The pH of the medium was generally maintained around the physiological pH of 7.4. Then, cooled agar solution (approximately 20 ml) was poured aseptically into each sterilized Petri dish. Finally, bacteria impregnated with a glass hockey stick were cultured on a solid agar medium in all directions and incubated at 37°C for 24 h. In addition, 0.5 McFarland turbidity standard, DMSO (0.1 ml) and NPs (0.1 g) were added to the nutrient broth culture medium. After incubation of the mixtures at 37°C, 0.1 ml of this mixture was preserved in an agar medium for the colony counting experiment [38,39].

3. Results and discussion

Fe₃O₄@SiO₂/Cu(OH)₂ was synthesized via a simple method with water as an eco-friendly and green solvent in the present investigative study. The synthesized FSCNWs were characterized using FE-SEM, EDX, XRD and vibrating sample magnetometer (VSM). Furthermore, antibacterial activities were examined using an agar disc diffusion and colony counter method.

3.1. Characterization of Fe₃O₄@SiO₂/Cu(OH)₂

The EDX analysis was applied as a typical technique to ensure the presence of copper, iron, silicon and oxygen in the synthesis Fe₃O₄@SiO₂/Cu(OH)₂, as presented in figure 1.
Figure 1. EDX spectrum (a) and mapping analysis of Fe$_3$O$_4$@SiO$_2$/Cu(OH)$_2$ (b) Si, (c) O, (d) Cu and (e) Fe.
3.1.1. Microscopic imaging study

The FE-SEM spectrum was used to investigate the morphology of the synthesized Fe₃O₄@SiO₂/Cu(OH)₂ and its highly uniform distribution (figure 2a). This image confirms the synthesis of Cu(OH)₂ nanowires modified by Fe₃O₄@SiO₂. The surface morphology of nanowires was observed with an average length and diameter of 1 µm and 55 nm, respectively. The FE-SEM images show that the aggregation of the NWs led to the formation of a wicker texture structure. Moreover, the results obtained from the particle size distribution diagram (figure 2b) showed that a large percentage of the nanowire diameter was smaller than 78 nm.

Figure 2. (a) FE-SEM images and (b) the particle size distribution curve.

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The crystal structure of Fe₃O₄@SiO₂/Cu(OH)₂ was examined by XRD analysis (figure 3). The XRD patterns of FSCNWs synthesis showed the diffraction angle of 16.76°, 24.01°, 34.13°, 35.80° and 53.41° (figure 3a). Also, figure 3b is observed in the peak list of FSCNWs, and figure 3c is the Cu(OH)₂ orthorhombic structure reference (JCPDS card no. 01-072-0140). Figure 3d is Fe₃O₄ cubic structure reference (JCPDS card no. 01-075-0449), and figure 3e is SiO₂ hexagonal structure reference (JCPDS card no. 01-077-1414), and patterns were quite consistent with the characteristic data of FSCNWs. Based on this information, the average crystallite size of FSCNWs was approximately 30 nm. Their crystallite size was computed by Debye–Scherrer using equation (3.1) as follows:

\[ D_p = \frac{0.94\lambda}{\beta \cos \theta} \] (3.1)

According to the above equation, the average crystal size \( D_p \) is given by the X-ray wavelength \( \lambda \), the peak width at half the maximum height on the horizontal axis of the diffraction pattern \( \beta \) and the Bragg angle size \( \theta \) [40–44].
3.1.2. Vibrating sample magnetometer analysis

The VSM method evaluated the magnetic behaviour of the Fe$_3$O$_4$, Fe$_3$O$_4$@SiO$_2$ and Fe$_3$O$_4$@SiO$_2$/Cu(OH)$_2$ nanocomposite at room temperature. Consequently, the magnetization ($M_s$) of Fe$_3$O$_4$ (56.5 emu g$^{-1}$) and Fe$_3$O$_4$@SiO$_2$ NPs (46.4 emu g$^{-1}$) was higher than the Fe$_3$O$_4$@SiO$_2$/Cu(OH)$_2$ nanocomposite (7.2 emu g$^{-1}$); also, data regarding remanence ($M_r$) and coercive force ($H_c$) are shown in table 1. As a result, the efficient linking of the Cu(OH)$_2$ to the Fe$_3$O$_4$@SiO$_2$ NPs is established.

Heterogeneous paramagnetic nanocomposites are produced due to their significant advantages: unique surface properties, frequent use and the ability to simply segregate from the reaction mixture, which is essential due to the economical cost and time at an industrial scale (figure 4) [44,45].

3.1.3. FT-IR spectrum of the Fe$_3$O$_4$@SiO$_2$/Cu(OH)$_2$ in the range of 400–4500 cm$^{-1}$

FT-IR spectroscopy was carried out to investigate purity and the presence of compounds in the prepared FSCNWs. In the FT-IR spectra of Fe$_3$O$_4$@SiO$_2$, a wide peak has appeared at approximately 1090 cm$^{-1}$ according to the O-Si-O stretching vibration, which confirms the favoured synthesis of Fe$_3$O$_4$@SiO$_2$. The features of peaks at 587, 1000 and 3576 cm$^{-1}$ are related to the Cu-O stretching vibration and Cu-O-H, as well as O-H stretching vibration proving the successful synthesis of coated Cu(OH)$_2$ with Fe$_3$O$_4$@SiO$_2$. In addition, 1500 cm$^{-1}$ belongs to H-O-H bending vibrations of H$_2$O adsorbed onto the FSCNWs lattice (figure 5) [35,46,47].

3.2. Antibacterial properties of Fe$_3$O$_4$@SiO$_2$/Cu(OH)$_2$, Fe$_3$O$_4$@SiO$_2$ and Fe$_3$O$_4$

The antibacterial test of Fe$_3$O$_4$@SiO$_2@Cu(OH)_2$ was examined as a practical application. Agar disc diffusion as the most practical method was chosen to peruse the antibacterial activity of FSCNWs against *Staphylococcus aureus* (S. aureus) (ATCC 12600) and *Staphylococcus saprophyticus* (S. saprophyticus) (ATCC 1440), a Gram-positive bacterium, and *Escherichia coli* (E. coli) (ATCC 9637), *Klebsiella pneumoniae* (K. pneumoniae) (ATCC 700603) and *Pseudomonas aeruginosa* (P. aeruginosa) (ATCC 27853), a Gram-negative bacterium. As presented in figure 6, in the presence of 0.01 g FSCNWs at 37°C for 24 h, the diameters of the zones of inhibition (ZOI) were 0.5, 0.6, 0.5, 0.6 and 0.7 cm for *E. coli*, *S. aureus*, *S. saprophyticus*, *K. pneumoniae* and *P. aeruginosa*, respectively (figure 6).

The ZOIs around the circle-tested samples were measured to evaluate their comparative antibacterial efficiency against these five essential microorganisms.

For example, these variations in results may be attributed to the distinct bacterial cells. *Staphylococcus aureus* consists of a thick peptidoglycan layer sensitive to intracellular transmission, which causes cell wall

![Figure 4. The room temperature M–H curve of (a) Fe$_3$O$_4$, (b) Fe$_3$O$_4$@SiO$_2$ and (c) Fe$_3$O$_4$@SiO$_2$/Cu(OH)$_2$.](image)

![Table 1. Magnetic parameters of Fe$_3$O$_4$ and Fe$_3$O$_4$@SiO$_2$/Cu(OH)$_2$.](table)

| sample                      | $M_s$ (emu g$^{-1}$) | $M_r$ (emu g$^{-1}$) | $H_c$ (Oe) |
|----------------------------|----------------------|----------------------|------------|
| Fe$_3$O$_4$                 | 56.5                 | 2.1                  | 27.7       |
| Fe$_3$O$_4$@SiO$_2$         | 46.4                 | 3.35                 | 44.03      |
| Fe$_3$O$_4$@SiO$_2$/Cu(OH)$_2$ | 7.2                  | 0.8                  | 52         |
fractures. By contrast, *E. coli* possesses a phospholipid bilayer cell membrane and a thin peptidoglycan layer [48–51].

In order to ensure the accuracy of the results, the antibacterial performances of Fe₃O₄ and Fe₃O₄@SiO₂ nanomaterials rather than Fe₃O₄@SiO₂/Cu(OH)₂ nanocomposite were evaluated on several bacteria such as *S. aureus, E. coli, S. saprophyticus, P. aeruginosa* and *K. pneumoniae*. As shown in figure 7, Fe₃O₄ and Fe₃O₄@SiO₂ components did not exhibit antibacterial properties. The antibacterial effect of the final composite was attributed to the antibacterial properties of Cu(OH)₂ nanowires.

The colony counter method was used to assess the concentration of live bacteria in cultivated samples [52–56].

![Figure 5. FT-IR spectra of Fe₃O₄@SiO₂/Cu(OH)₂.](image)

![Figure 6. Images of Agar disc diffusions of (a) S. aureus, (b) E. coli, (c) S. saprophyticus, (d) P. aeruginosa and (e) K. pneumoniae in the presence of Fe₃O₄@SiO₂/Cu(OH)₂.](image)
Figure 7. Images of Agar disc diffusions of (a) E. coli, (b) S. aureus, (c) S. saprophyticus, (d) P. aeruginosa and (e) K. pneumoniae in the presence of (a) Fe$_3$O$_4$@SiO$_2$/Cu(OH)$_2$, (b) Fe$_3$O$_4$@SiO$_2$ and (c) Fe$_3$O$_4$ after 24 h.

Figure 8. Photographs of (a) E. coli and (b) S. aureus in the absence and presence of Fe$_3$O$_4$@SiO$_2$/Cu(OH)$_2$ after 24 h.
Aiming to evaluate, the antibacterial functions of increasing concentrations of Fe₃O₄@SiO₂/Cu(OH)₂ were compared with the results of the control obtained under identical conditions without Fe₃O₄@SiO₂/Cu(OH)₂.

According to the digital images shown in figure 8, FSCNWs inactivated *E. coli* and *S. aureus* pathogen bacteria after 24 h (figure 8 and table 2).

### 3.3. Antimicrobial activity mechanism

The present study showed that Cu(OH)₂ has antibacterial properties on *S. aureus* and *E. coli*, and the attendance of Cu(OH)₂ has been linked to the production of reactive oxygen species (ROS).

When the particle size shrinks, the effects will be stronger and more pronounced. As a result, Cu(OH)₂ nanostructures with a higher surface-to-volume ratio produced more ROS and were best at penetrating cell membranes. A bactericidal effect has been suggested due to the electrostatic interaction between NPs and the negatively charged bacterial cell membrane and the release of Cu²⁺ ions. Cu²⁺ may interact with DNA and proteins, causing structural changes and disrupting biological procedures [57–60].

*Ksp* is the equilibrium constant for extremely insoluble solids, computed by multiplying the molar concentration of anions and cations dissolved in the saturated aqueous solution, which is $2.2 \times 10^{-20}$ for Cu(OH)₂ by their respective solubility products [51].

### 4. Conclusion

In the present study, a new, practical, low-cost, re-usable, heterogeneous Fe₃O₄@SiO₂/Cu(OH)₂ nanocomposite was produced for the first time and designed by a green and effective method at low temperature for a short time. FSCNWs have been synthesized at low temperatures and atmospheric pressure without hazardous chemicals, indicating facile operation.

The results of the present study suggest that copper, rather than silver, could be used as an antiseptic in the processing of drinking water with lower risk to human health. Furthermore, FSCNWs are far less expensive than silver and superior for commercial uses either in economic or environmental aspects; thus, they are expected to be more widely used.

Furthermore, these Cu(OH)₂ nanowires modified by Fe₃O₄@SiO₂ magnetic nanocomposite as a highly useful re-usable nanomaterial was separated by a magnet for antibacterial activity.

In addition, the FSCNWs revealed excellent antibacterial properties. For this purpose, the antibacterial properties of Fe₃O₄@SiO₂/Cu(OH)₂ against *E. coli* and *S. aureus* were tested using agar disc diffusion and colony counter methods. Nevertheless, *S. aureus* demonstrated a higher ZOI than *E. coli* bacterium. Moreover, the colony method confirmed the inactivation of *S. aureus* and *E. coli* bacteria in the presence of FSCNWs. The results of the present study have suggested that these nanocomposites as cost-effective antibacterial agents could be very helpful in water purification and industrial applications.

Also, bacteria such as *S. saprophytic*, *P. aeruginosa* and *K. pneumoniae* cause serious diseases in humans. Therefore, the effect of FSCNWs on certain antibiotic-resistant and human pathogenic bacteria makes them a suitable option for biotechnology and therapeutic applications. Finally, the FSCNWs were effective against five pathogenic bacterial strains.

In addition, the VSM analysis and easy separation corroborated its magnetic properties, and the obtained EDX, XRD and IR results agreed with the proposed structure; moreover, these magnetic nanocomposites with nanowire structures and diameters average of 75 nm were fully characterized through FE-SEM.
Ethics. We did not need to receive ethical approval due to the fact that no research work was done on animals and no fieldwork conducted in this study. Also, all the authors of the article read it in full and approve it with satisfaction. Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material [61].

Images of repeat colony test on Escherichia coli and Staphylococcus aureus in the presence and absence of Fe₃O₄@SiO₂@Cu(OH)₂ after 24 h are in the electronic supplementary information section. Also, the images resulting from the inhibition zone method in the presence of Fe₃O₄ and Fe₃O₄@SiO₂ after 24 h are available in the article version on the publisher’s website.

Authors’ contributions. E.Z.-B.: formal analysis and investigation; M.R.A.: data curation, formal analysis, investigation, methodology and writing—original draft; A.M.: conceptualization, funding acquisition, project administration, supervision and writing—review and editing; H.G.: validation.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein. Conflict of interest declaration. The authors declared no conflicts of interest promulgated by the authors. Funding. The authors received no specific funding for this work.

Acknowledgements. The authors sincerely thank the Iran University of Science and Technology for their assistance and partial financial support in conducting this research.

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