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Comparison of antibacterial and antifungal activities of 5-amino-2-mercaptobenzimidazole and functionalized NiO nanoparticles

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Abstract

5-Amino-2-mercaptobenzimidazole (AMB), non-functionalized nickel oxide (n-NiO), and functionalized nickel oxide (f-NiO) nanoparticles were studied for antibacterial and antifungal activities, where the functionalization of nickel oxide nanoparticles was carried out by AMB. The particle sizes of synthesized n-NiO and f-NiO nanoparticles were measured to be 16.07 and 20.86 nm, respectively. The XRD results of n-NiO and f-NiO nanoparticles were in perfect match with the diffraction pattern of NiO published in the JCPDS File No. 89-5881, which indicates that there is no effect on the crystal structure due to functionalization. FT-IR spectral studies show that f-NiO nanoparticles effectively bind with AMB by azomethine nitrogen. Furthermore, HR-SEM and EDAX results confirm the surface morphology and functionalization, respectively. The antimicrobial activity of AMB, n-NiO, and f-NiO nanoparticles dispersed in water was investigated. The antibacterial activity was evaluated against the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while the antifungal activity was evaluated against the fungi *Aspergillus niger* by using the agar well-diffusion method. The f-NiO nanoparticles exhibit excellent antibacterial and antifungal activities compared with n-NiO nanoparticles and AMB. The increased effect of f-NiO nanoparticles might be due to enhanced dispersibility and interaction of NiO nanoparticles with membrane and intracellular proteins of bacteria and fungi.

Keywords: Nickel oxide; Benzimidazole; Antibacterial activity; Antifungal activity

1. Introduction

The engineered inorganic nanoparticles display unique physical and chemical properties, which can be used in numerous physical, biological, biomedical, and pharmaceutical applications [1,2]. Recent research achievements offer the possibility of generating new types of surface-coated nanoparticles for enhanced applications [3]. The current interest in the development of new antimicrobial agents can be partially ascribed to the increasing emergence of bacterial

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resistance against antibiotic therapy and to newly emerging pathogens [4]. Many problems remain unsolved for most available antimicrobial drugs, in spite of advancement in antibacterial therapy. Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications. The ring system of benzimidazole was present in numerous antiparasitic, fungicidal, antioxidant, and anti-inflammatory drugs [5,6]. However, the general antimicrobial activity of benzimidazole derivatives has not been extensively investigated. Zhang et al. studied about the photogeneration of reactive oxygen species on uncoated silver, gold, nickel, and silicon nanoparticles to estimate their antibacterial efficacy. The research findings show that nickel possesses excellent antimicrobial effect compared with other metal nanoparticles in the same condition. Another study shows that nickel nanoparticles possess excellent antibacterial activity against *E. coli*, *Lactobacillus casie*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* [7]. In this investigation, we report the synthesis of highly stable nickel oxide (NiO) nanoparticles and nickel oxide nanoparticles surface functionalized with 5-amino-2-mercaptobenzimidazole (AMB) to study the influence on antibacterial and antifungal effects. Efforts were made to understand the underlying molecular mechanism of such antimicrobial actions. The effect of the nanoparticles was found to be significantly more pronounced on the gram-negative strains, irrespective of whether the strains are resistant or not, than on the gram-positive organisms. For discussion, non-functionalized nickel oxide nanoparticles are referred to as n-NiO, functionalized nickel oxide nanoparticles as f-NiO and 5-amino-2-mercaptobenzimidazole as AMB.

2. Materials and methods

2.1. Raw materials

The nickel oxide (NiO) nanoparticles were synthesized using nickel nitrate (Sigma−Aldrich) and sodium hydroxide pellets (Merck). The nickel oxide nanoparticles were surface functionalized with 5-amino-2-mercaptobenzimidazole (Sigma−Aldrich). All the chemicals were of analytical grade and used without further purification.

2.2. Synthesis of NiO nanoparticles

The NiO nanoparticles were synthesized by chemical precipitation method. Nickel nitrate ($Ni(NO_3)_2 \cdot 6H_2O$) and sodium hydroxide (NaOH) were taken in 1:4 molar ratios and dissolved completely in deionized water separately. The salt solution was stirred well using a magnetic stirrer and sodium hydroxide solution was added in drops to obtain a pale green precipitate. The solution was continuously stirred to obtain a homogeneous medium. Furthermore, the precipitated solution was ultrasonicated for 1 h to obtain fine particle sizes. Later, the precipitate was washed several times with deionized water and annealed at 400 °C for 4 h to remove moisture from nickel hydroxide to obtain nickel monoxide (NiO) nanoparticle.

2.3. Surface functionalization of NiO nanoparticles

The surface functionalization of NiO nanoparticles was carried out by mixing 2.0 g of NiO nanoparticles, 2 g of AMB, and 40 ml ethanol in a beaker. The solution was stirred vigorously using magnetic stirrer for 3 h. Then, the amine-functionalized NiO nanoparticles were collected by filtration using Whatman filter paper (No. 1) and rinsed with acetone; later the sample was dried under vacuum for 12 h.

2.4. Test microorganism and maintenance

Test pathogenic bacteria, such as *P. aeruginosa*, *S. aureus*, and fungi *Aspergillus niger*, were used for *in vitro* antimicrobial activity. These selected pathogenic strains were obtained from microbiological division (Jayagen Biologics Analytical Laboratory, Chennai). The pathogenic bacteria was maintained on nutrient agar slants and stored at 4 °C with regular transfers at monthly intervals. For long preservation, 25% glycerol nitrate agar was added to the slants.

2.5. Measurements

The synthesized n-NiO and f-NiO nanoparticles were investigated by X-ray diffraction analysis (XRD-XPert Pro Philips) for structural confirmation and particle sizes were calculated using Debye Scherrer's formula. The functionalization of AMB on nickel oxide nanoparticles was analyzed by FT-IR spectroscopy (Nicolet Magna 550 FT-IR spectrometer) and confirmed with EDAX results. The morphological changes were evaluated using HR-SEM (Modelno.S3400, Hitachi).

2.6. Liquid suspension

The liquid suspensions of the samples AMB, n-NiO and f-NiO nanoparticles were prepared by dispersing

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