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Antibacterial action of doped $CoFe_2O_4$ nanocrystals on multidrug resistant bacterial strains

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ABSTRACT

The bactericidal effect of pristine and doped cobalt ferrite nanoparticles has been evaluated against multiple drug resistant clinical strains by assessing the number of colony-forming units (CFU). Monophasic polycrystalline ferrites have been prepared by the malate–glycolate sol–gel autocombustion method as confirmed by the X-ray diffraction study. Various changes occurring during the preparative stages have been demonstrated using TG–DTA analysis which is well complemented by the FTIR spectroscopy. The antibacterial studies carried out demonstrate a bactericidal effect of the nanoparticles wherein the number of CFU has been found to decrease with doping. Cellular distortions have been revealed through SEM. Variation in the number of CFU with dopant type has also been reported herein.

1. Introduction

Indiscriminate usage of antibiotics for chemotherapy in hospitals and in environmental settings such as prawn hatcheries, aquaculture and farms housing livestock has amounted to an increased antibiotic resistance of bacterial pathogens [1,2].

There are prodigious efforts underway for the exploration of novel antimicrobials to combat this very menace of antibiotic resistance. This quest for the search of a wonder drug has seen the extensive exploration of natural and synthetic sources [3–5]. It is only in the recent years, with the advent of nanotechnology that researchers across the globe are frantically looking into the exploitation of nanomaterials, primarily because of their unique properties such as high surface to volume ratio, attributed to their small size which in turn facilitates greater interaction and faster chemical actions [6–8]. The ease of preparation, stability and the variation that is brought about in the physico-chemical, optical and the electromagnetic properties due to a change in the particle dimension and composition has encouraged many researchers to synthesize these materials with novel properties [9–17]. The attractive properties of mixed-metal oxides namely antibacterial, chemical and catalytic activity make them a material of choice for usage in sterile coatings for biomedical devices such as catheters, dental implants, adhesives, biosensors, biomaterials, tissue engineering, DNA modification, drug-delivery systems and packaging [18–21]. There are tremendous and extensive reports on antibacterial effects of silver [22–24] and copper nanoparticles [25]. Magnetic spinel ferrites have captured the global market and grabbed the attention of many researchers due to their fascinating and exotic electromagnetic properties. The magnetic, electrical and magneto-optical [9–15] properties of pristine and doped cobalt ferrites have prioritized them to be the most widely used ferrite systems in the manufacturing of magnetic recording devices and magnetic fluids [17,18]. However, the bactericidal property of cobalt ferrite nanoparticles has hardly been delved with. There is only a single report of the activity of its antibacterial properties against a single Gram negative (*Escherichia coli*) and a single Gram positive bacterium (*Staphylococcus aureus*) [26]. Motivated by these considerations, the mixed metal oxides have been prepared and characterized by utilizing various characterization techniques and their antibacterial effect has been explored against the Gram negative bacterium *E. coli* ATCC 8739 and Gram positive bacterium *Staphylococcus epidermidis* ATCC 1228.

2. Experimental

2.1. Medium, chemicals and bacterial strains

Mueller Hinton agar [300 g/l beef infusion, 17.5 g/l casein hydrolysate, 1.5 g/l starch, 17 g/l agar with pH adjusted to 7.4 at 25 °C] served as a growth medium for the maintenance of bacterial isolates and also as a medium for assaying the antibacterial effects of chemically synthesized nanoparticles. Various nanoparticles used in this study were pristine and doped cobalt ferrite $CoFe_{2-x}M_xO_4$ [x = 0.12 for

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M = Bi, Gd and Nd]. *S. epidermidis* ATCC 1228 and *E. coli* ATCC 8739 were purchased from American Type Culture Collection (ATCC).

2.2. Preparation of doped $CoFe_2O_4$ nanoparticles

The preparation of $CoFe_{2-x}M_xO_4$ [x = 0.12 for M = Bi, Gd and Nd] was carried out using stoichiometric amounts of analytical grade $Co(NO_3)_2 \cdot 6H_2O$ (Sigma-Aldrich), $Fe(NO_3)_3 \cdot 9H_2O$ (Sigma-Aldrich), $Bi(NO_3)_3$ (Sigma-Aldrich), Gd_2O_3 (Sigma-Aldrich) and Nd_2O_3 (Molychem). To prepare Bi^{3+} doped cobalt ferrite, the water insoluble $Bi(NO_3)_3$ was dissolved in concentrated AR-grade HNO_3. After obtaining a clear solution, $Co(NO_3)_2 \cdot 6H_2O$ and $Fe(NO_3)_3 \cdot 9H_2O$ were added. The solution was maintained at around 100 °C with continuous stirring. Malic acid in the ratio of 1:3 (with respect to metal ion concentration) was then added. pH of the solution was increased (around neutral) with the slow addition of 30% ammonia solution. A prominent change in the color of the solution from light pink to dark wine red was observed which confirmed the chelating action of malic acid. After confirming the pH, ethylene glycol in the ratio 1:4 (with respect to malic acid) was added. The solution was then allowed to concentrate with continuous stirring and the gel so obtained was heated in an oven at 200 °C for 3 h. Formation of a voluminous foamy precursor was observed which was then crushed into fine powder with the help of an agate mortar and pestle. The precursor was than calcined at 400 °C for 4 h. It was again ground with acetone and sintered at 600 °C for 6 h. The same procedure was utilized for the preparation of pristine, Gd^{3+} and Nd^{3+} doped cobalt ferrite. The preparative method is similar to the one reported in the earlier publications [7,8]. The nanoparticles obtained in the powder form were then subjected to various characterization techniques.

2.3. Characterization

The crystallinity, crystal structure and phase purity of the powders were investigated by the X-ray diffraction (XRD) technique using Cu-K_α radiations of wavelength 1.5418 Å (filtered through Ni), in steps of 0.02 degrees on a RIGAKU ULTIMA IV X-ray diffractometer. Thermal behavior of a representative malate–glycolate gel was studied in dry air utilizing a NETZCH STA 409 PC TG/DTA instrument at a step rate of 10 °C min^{-1}. A SHIMADZU FTIR PRESTIGE-21 spectrophotometer was used to record the FTIR spectra of the compound at various preparative stages. Transmission Electron Microscopy (TEM) images were recorded on a PHILIPS CM 200 transmission electron microscope operating at an accelerating voltage of 200 kV and providing a resolution of 2.4 Å.

2.4. Antibacterial activity of nanoparticles

The bactericidal potency of pristine and doped nanoparticles was evaluated against *E. coli* ATCC 8739 (Gram negative) and *S. epidermidis* ATCC 1228 (Gram positive) by assessing the number of colony forming units (CFU).

Pristine and doped nanoparticles were individually dispersed by ultrasonication [Loba Life-Digital Ultrasonic Cleaner (2120-00)] in Mueller Hinton broth (20 μg/ml). This was followed by the inoculation of 0.1 ml of suspension of bacterial cells (10^5 CFU/ml) and incubation on an orbital shaker [Scigenics Biotech-Orbitek (LT)] at 150 rpm, 37 °C for 24 h. Following incubation, 100 μl was spread plated onto a solid agar medium for the visualization of viable colonies. The percentage of antibacterial activity of nanoparticles was calculated using the following formula:

$$\text{Antibacterial activity } (\%) = \frac{CFU_0 - CFU_{(np)}}{CFU_0} \times 100$$

CFU_o colony forming units (without nanoparticles)
$CFU_{(np)}$ colony forming units (with nanoparticles).

The effect of nanoparticles on *E. coli* ATCC 8739 cells was evaluated using Scanning electron microscopy (JOEL JSM 6360 LV) wherein unexposed cells served as a control.

The minimum inhibitory concentration (MIC) of pristine and rare earth doped cobalt ferrite nanoparticles was determined using the tube method. The nanoparticles were weighed aseptically and sterilized using UV radiation for 1 h. Mueller Hinton broth containing 10^5 CFU/ml of bacterial cells was used. The final concentrations of nanoparticles were 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 μg/ml.

The medium was incubated on an orbital shaker at 37 °C for 24 h [Scigenics Biotech- Orbitek (LT)]. Absorbance was recorded at 600 nm using a UV–VIS Spectrophotometer (Mecasys-Optizen 3220-UV). The experiments were carried out in triplicates and appropriate controls maintained. The lowest concentration of the pristine and doped nanoparticles that inhibited the growth of the organisms served as the minimum inhibitory concentration (MIC).

3. Results and discussion

3.1. Structural analysis

Fig. 1 shows the X-ray diffractogram of doped $CoFe_2O_4$ sintered at 600 °C for 6 h. All the diffraction peaks observed for the oxides correspond to the cubic spinel ferrite structure. The phase analysis is carried out by matching the obtained diffractogram with the standard ICDD card number 22-1086. The XRD patterns reveal monophasic formation of the polycrystalline compounds along with greater crystallinity. High peak width signifies finer particle size along with a decrease in density and an increase in the surface to volume ratio of the doped $CoFe_2O_4$ compounds. Unit cell parameter (a) is calculated for the oxides as a Voigt function for the (311) peak. Crystallite size and lattice strain are calculated from the X-ray diffractograms. Williamson–Hall extrapolations as a Lorentzian function are utilized in calculating these parameters. The results are shown in Table 1. Cation migration induced with doping of bigger rare earth ions has already been reported earlier [7]. This phenomenon is observed in nanopowders and is also reported for pristine $CoFe_2O_4$ [8]. The outcome of this, is exchange of ions between the lattice sites thereby bringing about variation in the lattice parameters and therefore a change in magnetic properties [7,8]. No change in the structure can be seen with doping which is evident from the absence of foreign impurity peaks in the XRD pattern of the doped compounds. XRD patterns of the rare earth doped compounds have been reported earlier [7].

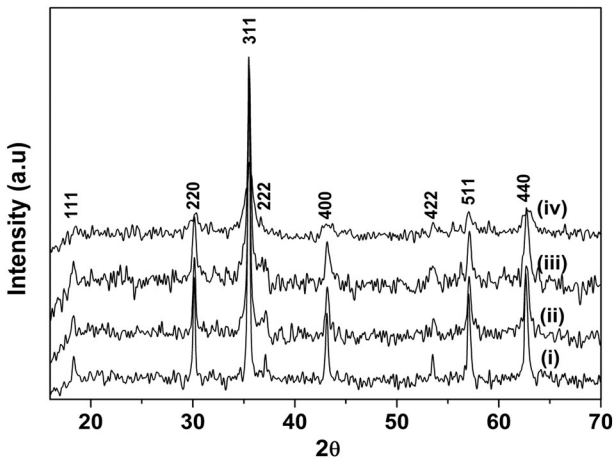

Fig. 1. X-ray diffraction patterns of (i) $CoFe_2O_4$, (ii) $CoFe_{1.88}Bi_{0.12}O_4$, (iii) $CoFe_{1.88}Gd_{0.12}O_4$, and (iv) $CoFe_{1.88}Nd_{0.12}O_4$ sintered at 600 °C.