Synthesis of CoFe₂O₄/BiOI Nanocomposite: Effects on Staphylococcus aureus, Escherichia coli, Pseudomonas Aeruginosa and Bacillus Cereus

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Abstract

With the increase of general knowledge and the advancement of science and technology, antibacterial substances were used more than antibiotics. In our current study, the antibacterial virtues of CFO/BiOI nanocomposite were investigated due to its high importance on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus*. MIC, MBC, Disk Diffusion and IC50 tests Cefalotin (CF), Amoxicillin (AMX), Gentamicin (GM), Trimethoprim-sulphamethoxazole (SXT) and Ceftriaxone (CRO) antibiotics in concentration 30W, 10i, 10t, 25h and 30 were used to find the antibacterial properties of the synthesized nanocomposite, respectively. For the synthesis of nanocomposites polyethylene glycol (PEG) and sulfonic acid was used as a solvent. It is noteworthy that the synthesis was performed by heat dissolution method without the presence of surfactant. Also, various techniques such as X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM), High resolution mapping and Energy Dispersive X-ray spectroscopy (EDAX) have been used to determine the properties of produced nanocomposites. SEM test results showed that the formed nanoparticles were globular and their size was limited area of 22 to 34 nm. The results showed CFO / BiOI nanocomposite exhibits strong significant biological activity against *Bacillus cereus*. The results of MBC (Minimum Bactericidal Concentration) and MIC (Minimum Inhibition Concentration) tests for CFO/BiOI nanocomposites on bacteria were examined in the range of 0.12-0.48 mg/ml and 0.06 to 0.24 mg/ml respectively. According to the results, the minimum IC50 value was determined at a concentration of 0.061 mg/ml. On the other hand, the most resisting and susceptible bacteria in this method were *Pseudomonas aeruginosa* and *Bacillus cereus*, respectively. These findings are identical to those of a prior study on CoFe₂O₄ nanoparticles antibacterial properties. MBC of the nanocomposites, 50 µl from all the tubes that showed no obvious bacterial growth were distributed on BHI agar plates and incubated for 24 h at 37 °C. The MBC endpoint is defined as the lowest concentration which killed 98% of the bacterial population.

1. Introduction

Nanomaterials are receiving growing interest for his or her promise as engineering and biomedical miracles. In fact, nanomaterials help overcome length restrictions and have the potential to change the scientific landscape. Magnetic nanoparticles (MNPs) are a subset of nanomaterials that are widely used in biomedical applications. Cobalt ferrite (CoFe₂O₄) MNPs are widely used in Imaging (MRI), cancer treatment, fluids, drug delivery, hyperthermia [1, 2] diagnosis [2, 3] and in electronic devices due to high permeability, by force, medium magnetization finding, high saturation magnification [4]. Physiochemical stability [5] and antibacterial properties.

BiOI is a V-VI-VII ternary compound with an open layer structure made up of [Bi₂O₂] slabs interrupted by binary I atom slabs [6]. BiOI has been selected as one of the most important and new semiconductors for visible light catalysts due to its high structural diversity, easy synthesis and narrow bandwidth (1.78eV) [7–8]. BiOI magnetic nanocomposites have been extensively studied due to their convenient assembly by an exterior magnetic field [9]. One of the methods of synthesis of BiOI magnetic composites is the

deposition of magnetic nanoparticles on the structure of BiOI by exothermic methods. Recently, Fe_3O_4 core / shell nanoparticles with BiOI coating have been reported by thermal and sequential ion layer ion adsorption and reaction (SILAR) [10]. These BiOI nano magnetic powders, however, have a restricted application due to they are readily disposed of and collected. Therefore, the search for suitable carriers for BiOI immobilization is desirable for the fabrication of new BiOI magnetic nanostructures. CFO/BiOI nanocomposite has recently been found to have extremely significant biological activity against *Bacillus cereus*.

In our current study, a CoFe_2O_4 /BiOI (CFO/BiOI) composite nanofiber with high magnetic strength and visible light photocatalytic performance for color degradation was introduced. The synthesis of CFO/BiOI nanofibers was obtained by heating and growing CFO/BiOI on the surface of CoFe_2O_4 fiber. The important point is that the strong magnetic response of CoFe_2O_4 can be used to estimate the need for BiOI deposition and magnetic separation. First, inorganic CoFe_2O_4 has better thermal register than organic fiber support [11]. Second, the structure of magnetic fibers is easily collectible and the accumulation of BiOI on the surface of the fibers is not easy [12, 13]. Furthermore, pure BiOI with a strong response to visible light for the photocatalysis process can be applied to the fiber surface using several synthetic techniques without attaching to metallic nanoparticles.

2. Synthesis Of Bioi

To begin, 10 mL ethylene glycol was mixed with 1/51 g bismuth trinitrate and swirled for 45 minutes at high speed until thoroughly dispersed and simultaneously 0.5 g of potassium iodide was dissolved in 5 ml of distilled water. Using an injection syringe, potassium iodide solution was injected dropwise to the original solution under vigorous agitation. The reaction was continued for 2 hours after mixing the initial solution with potassium iodide solution, and the resultant red solution was transferred to a steel autoclave. The autoclave was heated for 12 hours at 180°C , then cooled to ambient temperature before the precipitate was centrifuged and filtered through a $0.22\ \mu\text{m}$ millipurper membrane. To remove possible impurities, the product was washed 3 times with water, distilled twice and ethanol, then dried at 75°C oven for 6 hours [14, 15].

3. Synthesis Of Cofe2o4

In the present study, a heating bribe was used to synthesize CoFe_2O_4 magnetic nanoparticles. In this method, we dissolve 2 grams of PEG in 30 ml of diameter water, the resulting solution is eaten by a magnetic stirrer for 10 minutes. To complete the process, a certain amount of dehydrating iron (III) chloride, cobalt chloride, sodium acetate and sulfonic acid were added to the solution and stirred for about 8 hours with a magnetic stirrer to homogenize the mixture. The use of sulfonic acid makes it easier to reuse nanoparticles. The final solution was dried for 15 hours at 190°C in a furnace. To remove impurities from the precipitate, it is rinsed several times with 96% ethanol for further purification. The final

precipitate was thoroughly dried in a free medium for one day before being heated at 450°C for 2 hours to yield CoFe₂O₄ magnetic nanoparticles [16].

4. Synthesis Of Cfo/bioi Hybrid Nanofiber By Solvothermal Method

As mentioned in the previous section, the BiOI thermal growth method on the CoFe₂O₄ fiber surface was used to synthesize CFO/BiOI nanofiber. For the synthesis of CFO/BiOI nanofibers, equal concentrations of bismuth nitrate and potassium iodide were dissolved in 40 ml of ethylene glycol. Then 30 mg of CoFe₂O₄ nanofibers were prepared. The resulting mixture was transferred to an autoclave of 50 ml and heated at 160°C for 10 hours. After 10 hours to reach free space temperature was placed in the environment. The final product was washed several times with ethanol to remove impurities [17].

5. Bacterial Strains And Culture Conditions

The bacterial strains utilized to investigate BiOI's biological activity and CoFe₂O₄/BiOI have been provided from the Persian Type Culture Collection. The strains *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* were cultured on appropriate nutrient media. The standard bacterial inoculum was made using a spectrophotometer calibrated to 620 nm and 0.5 McFarland's standard (1.5108CFU/ml) and cultured for 24 hours at 37°C.

The sensitivity of each strain to nanoparticles were assessed with the Kirby-Bauer agar disk diffusion method. For this test, 100 µl of each above bacterial inoculum streaked on Mueller Hinton Agar surface by the swab, the impregnated discs with various nanoparticles were then placed on the agar plate's surface. The blank disc with no nanoparticle and antibiotic discs including Cefalotin (CF), Amoxicillin (AMX), Gentamicin (GM), Trimethoprim-Sulphamethoxazole (SXT) and Ceftriaxone (CRO) were utilized as negative and positive control, respectively. Finally, the heated plates were incubated for 24 hours at 37°C under white light at 18 volts at a distance of 33 cm, and the breadth of the growth inhibition zone was determined.

For Minimum Inhibitory Concentration (MIC), the standard broth dilution procedure was utilized to assay the biological activity of nanoparticles by evaluating the visible growth of microorganisms in the agar broth. Serial two-fold dilutions of nanoparticles in concentrations ranging from 0.03, 0.06, 0.12, 0.24 and 0.48 mg/ml with adjusted bacterial concentration (1.5×10⁸ CFU/ml, 0.5 McFarland's standard) were used to determine MIC in BHI broth. The control contained only inoculated broth and medium contained only nanoparticles incubated for 24 h at 37°C. The MIC endpoint is the lowest concentration of nanoparticles where no visible growth is seen in the tubes. The visual turbidity of the tubes was noted, both before and after incubation to confirm the MIC value. For Minimum Bactericidal Concentration (MBC) of the nanoparticles, 50 µl from all the tubes which showed no visible bacterial growth were distributed on BHI agar plates and incubated for 24 h at 37 °C. The lowest concentration that killed 99% of the bacterial population is termed as MBC endpoint.

6. Result And Discussion

According Table 1 *S. aureus* showed higher sensitivity to both nanoparticles than other strains. As well as, a moderate sensitivity was also observed for the strains *P. aeruginosa* and *E. coli*. The MIC and MBC endpoints for strains *aeruginosa*, *aureus* and *E. coli* were observed to be the same and 0.24 and 0.48 mg/ml respectively (Table 2). The BiOI and CFO/BiOI can have inhibitory and lethal effects on *B. cereus* in lower amounts. In general, BiOI alone and in combination with CFO (CFO/BiOI) show the same antimicrobial effect.

6.1 Analysis of XRD

Using X'Pert Pro system from the Panalytical company, X-ray diffraction patterns of CoFe_2O_4 BiOI and CFO/BiOI nanofiber were reported under the following conditions: 40 mA, 40 kV, step size: 0.02, and timer per step 1 s/step. The XRD analysis of CoFe_2O_4 NPs is displayed in **Figure 1**. As seen in **Figure 1a**, the peaks at 2θ values of 30.19° , 35.53° , 43.17° , 53.54° , 57.087° , 62.68° and 74.20° were respectively correspond to the (220), (311), (222), (400), (422), (511) and (440) planes with a cubic spinel structure (EX PAPER). In **Figure 1b**, the peaks observed of BiOI in the 2θ angles of 29.71° , 31.75° , 37.26° , 39.30° , 45.51° , 46.62° and 51.51° related to (312), (004), (204), (020), (604), (316) and (912) reflections plane.

6.2 Analysis of FE-SEM

The Field Emission Scanning Electron Microscope (FE-SEM) is similar to the SEM microscope and generally retains the basic characteristics of SEM such as resolution, depth of field, signal diversity, and processing, as well, there is no need to conduct the sample in FE-SEM and it is possible to create a liquid injection system and it is also possible to observe the dynamics of the gas-liquid-solid system.

FE-SEM tests of sample BiOI and CFO/BiOI were performed using the device according SIGMA VP model system from ZEISS Company and one of the important results obtained from FE-SEM test was the uniform distribution of spherical nanoparticles. **Figure2** displays the morphology of FE-SEM and also showed that the particle size is in nanometers.

The results of SEM test showed that particle BiOI had a size of 76-34 and particle CFO/BiOI had a particle size of 51-51 nm, which corresponds to the size given in the XRD test. The result of SEM test showed non-uniform accumulation due to magnetic force in the morphology of CFO / BiOI particles.

EDAX test was utilized to determine the elements present on the surface of CFO/BiOI nanocomposite. EDX analysis indicated pure CoFe_2O_4 /BiOI nanoparticles formation; **Figure 3a** depicts the three elements that make up synthesized BiOI, whereas **Figure 3b** depicts the purity of CoFe_2O_4 /BiOI nanoparticles, as well as their constituents. **Figure 4** demonstrates the existence of additional elements O, Fe, Co, Bi, and I in the nanocomposite surface, which are precisely the same elements found in the EDAX test, and no peak was seen for another element, according to the corresponding elemental mappings results.

6.3 Cytotoxicity of CFO/BiOI nanocomposites

The cell survival MTT assay utilizing ELISA READER method was used to evaluate the cytotoxicity of the synthesized nanocomposite and in order to do this, they incubated 0.06 mg / ml and 0.48 mg / ml for 24 hours. As mentioned in the previous article, the same goes for this synthesis, the analysis indicated a survival rate of more than 50% for the maximum concentration of 0.06 mg/ml and the obtained data show that the nanocomposite synthesized by solvothermal method has the ability to kill bacteria and of course, this means that the ability is concentration dependent and we see more cell death with increasing concentration. In addition, based on the results, the IC50 value was determined to be 0.06 mg / ml. Energy Dispersive X-ray (EDAX) and corresponding elemental mappings test results were exactly equal and showed that the surface elements present on the surface of CFO/BiOI nanocomposite include O, Fe, Co, Bi, I.

Table 1. Bacterial growth inhibition zone (mm) based on the nanoparticle concentration

Bacteria	Inhibition Zone (mm)						
	BiOI (0.48 mg)	CFO/BiOI (0.48)	SXT	GM	AMX	CRO	CF
<i>Bacillus cereus</i>	18	18	25	25	R	22	R
<i>Pseudomonas aeruginosa</i>	15	15	R	R	R	16	R
<i>Escherichia coli</i>	17	17	32	R	R	32	R
<i>Staphylococcus aureus</i>	27	27	37	37	R	R	R

Abbreviations: **R**, resistant

Table 2. MIC and MBC CFO/BiOI, BiOI nanoparticles

Bacteria	MBC(mg/ml)	MIC(mg/ml)
<i>Escherichia coli</i>	0. 48	0.24
<i>Staphylococcus aureus</i>	0.48	0.24
<i>Pseudomonas aeruginosa</i>	0.48	0.24
<i>Bacillus cereus</i>	0.12	0.06

7. Conclusion

In the present study, CFO/BiOI nanocomposites were synthesized using the solvothermal method. The properties of CFO/BiOI nanocomposites were investigated by using various XRD, SEM, High Resolution Mapping and EDAX. Furthermore, the MIC, MBC and biological activity of CFO/BiOI nanocomposite against Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative (*Pseudomonas*

aeruginosa and *Escherichia coli*) bacteria were tested under visible light with different weight nits of CFO/BiOI. The result of this study is a decrease in the susceptibility of *Staphylococcus aureus* from nanocomposites compared to similar studies, which indicates an increase in the antibacterial ability of BiOI nanocomposites. Based on the results, the IC50 value of CFO/BiOI nanocomposite was determined to be 0.06 mg / ml and by confirming other antibacterial tests, it showed a suitable concentration to inhibit 50% of bacterial growth. According to the results of the antibacterial test, MBC of the nanocomposites, 50 µl from all the tubes which showed no visible bacterial growth were distributed on BHI agar plates and incubated for 24 h at 37 °C. The lowest concentration that killed 98% of the bacterial population is termed as MBC endpoint. So, the nanoparticles showed good potency against all of the microorganisms which were used in this study.

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Figures

Figure 1

X-ray diffraction patterns. (a) BiOI. (b) CFO/BiOI.

Figure 2

(a) FE-SEM image of BiOI on a scale of 200 nm. (b) FE-SEM image of CFO/BiOI on a scale of 200 nm.

Figure 3

EDAX spectrum. (a) BiOI. (b) CFO/BiOI with percentage of elements.

Figure 4

The elemental mapping images and EDAX spectrum of CFO/BiOI nanocomposites.