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Improvement Antibacterial Activity of ZnO through Modification with Zinc Chromite and Polypyrrole

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ABSTRACT

Recently, organic–inorganic composites have received great attention due to combining the advantages of all components. In order to improve the antibacterial activity of inorganic compounds, Zinc oxide (ZnO) nanoparticles modified with chromium containing metal oxide such as Zinc chromite ($ZnCr_2O_4$) and conducting Polymer of Polypyrrole (PPy) were synthesized. For this purpose, ZnO was synthesized. Then, ZnO/$ZnCr_2O_4$ composite using co-precipitation method was synthesized and finally, the synthesized ZnO/$ZnCr_2O_4$ composite was coated by PPy. The synthesized samples were characterized by X-ray Diffraction (XRD), Fourier Transform Infrared (FT-IR), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDXA). Furthermore, the antibacterial activity of ZnO, ZnO/$ZnCr_2O_4$ and PPy/ZnO/$ZnCr_2O_4$ samples were determined against Gram-negative bacterum Escherichia coli and Gram-positive bacterial species Staphylococcus aureus. Results showed that PPy/ZnO/$ZnCr_2O_4$ composite demonstrate enhanced antibacterial activity in comparison with ZnO and ZnO/$ZnCr_2O_4$ samples.

Keywords: Polypyrrole, ZnO, $ZnCr_2O_4$, Antibacterial, Composite

INTRODUCTION

Antibacterial materials are used in various industries such as medicine, textile and food [1,2]. They can be classified into two types, organic and inorganic ones. The disadvantages of using conventional organic substances for the elimination of microorganisms are toxicity and susceptibility to high temperature and pressure [3]. For this reason, the researchers gradually focus on inorganic antibacterial compounds which not only show better antibacterial activity, but have low toxicity, high stability and little environmental pollution. Recently, inorganic materials such as metal oxides [4] have received more attention due to superior durability, less toxicity, high selectivity and heat resistance [5,6]. Zinc oxide due to significant properties such as catalytic [7], electronic [8], chemical stability [9], has been attended. It also shows high potential in electrical and optical devices [10,11], solar cells [12], cosmetic materials [13] and so on. In addition, ZnO is nontoxic and environmentally friendly which is valuable for bio applications [14]. Furthermore, ZnO nanoparticles have been incorporated into a number of various polymers including low density polyethylene, polypropylene, polyvinyl acetate, polyamide and cotton woven fabric for more than antimicrobial purposes [15]. Polypyrrole (PPy) is a conjugated polymer that displays particular properties including conductivity, good biocompatibility, easy synthesis and flexibility. In biomedical applications, it is usually electrochemically generated with the incorporation of negatively charged biological macromolecules such as proteins to give composite materials [16]. Herein, we report preparation and characterization of PPy/ZnO/$ZnCr_2O_4$. In this study, ZnO nanoparticles modified with chromium containing zinc compound and PPy to improve the antibacterial performance of the synthesized PPy/ZnO/$ZnCr_2O_4$ composite. The present study showed the antibacterial of PPy/ZnO/$ZnCr_2O_4$ composite against two pathogenic bacteria, Gram-negative bacterial species *Escherichia coli* and Gram-positive bacterial species *Staphylococcus aureus*. In addition, for comparison the antimicrobial activity of ZnO and ZnO/$ZnCr_2O_4$ composite was examined. To our knowledge, this is the first report on the antibacterial effect of PPy/ZnO/$ZnCr_2O_4$ composite.

EXPERIMENTAL

Materials and characterization

All the analytical chemicals $Cr(NO_3)_3.9H_2O$, $Zn(NO_3)_2.6H_2O$, $Zn(CH_3COO)_2.2H_2O$, Sodium Dodecyl Benzenesulfonate (SDBS), Triton X-100, pyrrole, ammonium persulfate, sodium hydroxide and Congo red dye were purchased from Merck and used without further purification. *S. aureus* PTCC 1431 and *E. coli* PTCC 1399 were obtained from Persian type culture collection. All these strains were grown aerobically in nutrient broth at 37°C for 24 h before their use as target organisms. The cell density of the bacterial isolates was adjusted to an optimal density of 0.5 McFarland standards. The structural analysis of the samples was performed by powder X-ray Diffraction (Holland Philips Xpert, X-ray diffractometer with Cu-Kα radiation) and FT-IR analysis using a FTIR (JASCO FTIR-4200, Japan) in KBr pellet, in the range of 4000-400 cm^{-1}. The particle size, external morphology, and analysis of elements in the samples were characterized by SEM (JEOL JEM-3010 SEM). The UV-Vis absorption spectra were recorded using a Shimadzu UV-2550 spectrophotometer.

Synthesis of the ZnO sample

The ZnO sample was prepared via precipitation method. In this method, 5.27 g (0.024 mol) of $Zn(CH_3COO)_2$. $2H_2O$, 2.8 g (0.07 mol) of NaOH, 15 ml distilled water and 0.012 mol of Triton X-100 was used. Above chemicals vigorous stirring via magnetic stirrer for 120 min at room temperature. The product was washed with double distilled water several times and dried at room temperature. Then, the product was annealed at 500°C for 1 h.

Synthesis of the $ZnCr_2O_4$ sample

The procedure used for synthesis of the $ZnCr_2O_4$ reported previously [17]. In this synthesis, $Cr(NO_3)_3$. $9H_2O$ (6×10^{-3} mol), $Zn(NO_3)_2$. $6H_2O$ (3×10^{-3} mol), NaOH (0.040 mol), SDBS (5.74×10^{-4} mol) and 10 ml distilled water was used. Above chemicals stirring vigorously via magnetic stirrer for 30 min at 60°C, the final product was washed with double distilled water and dried at room temperature. Then, the product was annealed at 700°C for 3 h.

Synthesis of the PPy/ZnO/$ZnCr_2O_4$ composite

The procedure used for the synthesis of the ZnO/$ZnCr_2O_4$ followed by in-situ chemical polymerization of pyrrole that is similar to the previous reports [18]. In this procedure, 0.04 g (2×10^{-4} mol) $ZnCr_2O_4$ and 0.05 g (6×10^{-4} mol) ZnO was mixed with a 30 ml aqueous solution containing of 1.4×10^{-5} mol SDBS followed by sonication for 30 min and stirrer mechanically for 3 h. After adding of 26.1 ml pyrrole monomer, the solution was continuously stirred for another 1 h. Then, 11.5 ml of the 0.1 M ammonium persulfate solution was added drop wise into the above solution. The polymerization process was conducted while stirring for 2 h at room temperature. The product was magnetically separated and washed with deionized water, ethanol and then dried in an oven at 80°C.

Antibacterial studies

Disk diffusion method

The inhibitory effect on the bacterial growth was determined using the disk diffusion method [19]. First, the bacterial suspensions were added on the surface of Muller Hinton agar (MHA) at 4°C. Then, molten MHA was incubated with the concentration range of 10^6 CFU/ml of the respective bacterial strains and poured over washed and sterilized Petri dishes. The synthesized samples were dissolved in DMSO with concentration of 10 mg/cm^{-1}, and sterile Whatman filter paper No. 1 (6 mm) discs were separately impregnated with each sample to be tested at 5 mg/cm^{-1} and placed on the inoculated agar. The plates were incubated at 37°C for 24 h, and the zones of inhibition were measured at the end of the incubation period. Antibacterial activity was expressed as the zone of inhibition (mm) produced by the synthesized samples compared with the penicillin (positive control) and tetracycline (negative control). The experiments were repeated three times, and the mean values with standard deviation were obtained.

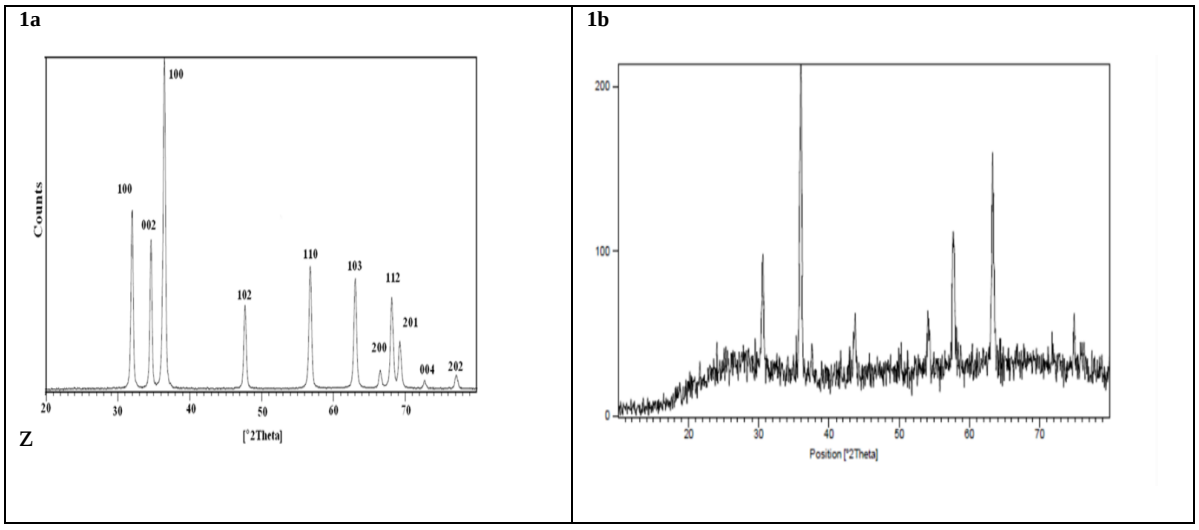
Minimum Inhibitory Concentration (MIC)

The MIC is the lowest concentration of an agent that inhibits the growth of an organism, that determined by the micro-well dilution method [20]. The inoculum of each bacterium was prepared, and the suspension was adjusted to 10^6 CFU/ml. All synthesized samples were dissolved in DMSO and dilutions series were then prepared. Each plate included 30 µl of nutrient broth, 40 µl of 24 hold bacterial inoculum and 10 µl of the diluted samples. The plates were incubated at 37°C for 24 h. As an indicator of bacterial growth, 30 ml of 0.2 mg/cm^{-1} of p-iodonitrotetrazolium solution was added to each plate and incubated at 37°C for 30 min. The colorless tetrazolium salt was reduced to a red colored compound by the biochemical activity of the organisms, thereby making the inhibition of bacterial growth visible as a clear well. Tetracycline was used as control. Each treatment was repeated three times.

RESULTS AND DISCUSSION

Characterization of the samples

Figure 1a-1c shows the XRD patterns of ZnO, $ZnCr_2O_4$, and PPy/ZnO/$ZnCr_2O_4$ samples after annealed at 700°C for 3 h respectively. The crystal phases corresponding to peaks at 31.8°C, 34.4°C, 36.3°C, 47.5°C, 68.0°C and 69.1°C are corresponded to ZnO (JCPDS No. 36-1451) (Figure 1a). The XRD results shows that the $ZnCr_2O_4$ with cubic phase was obtained (JCPDS No. 22-1107), and the distinctive peaks at 18.45°C, 30.35°C, 35.75°C, 37.35°C, 43.45°C, 53.93°C, 57.45°C and 63.21°C matched well with cubic phase of $ZnFeCr_2O_4$ (Figure 1b) [17]. In the XRD spectrum of PPy/ZnO/$ZnCr_2O_4$, the peaks corresponding to the polypyrrole are not detected, indicating that polypyrrole was amorphous in PPy/ZnO/$ZnCr_2O_4$ composite (Figure 1c).



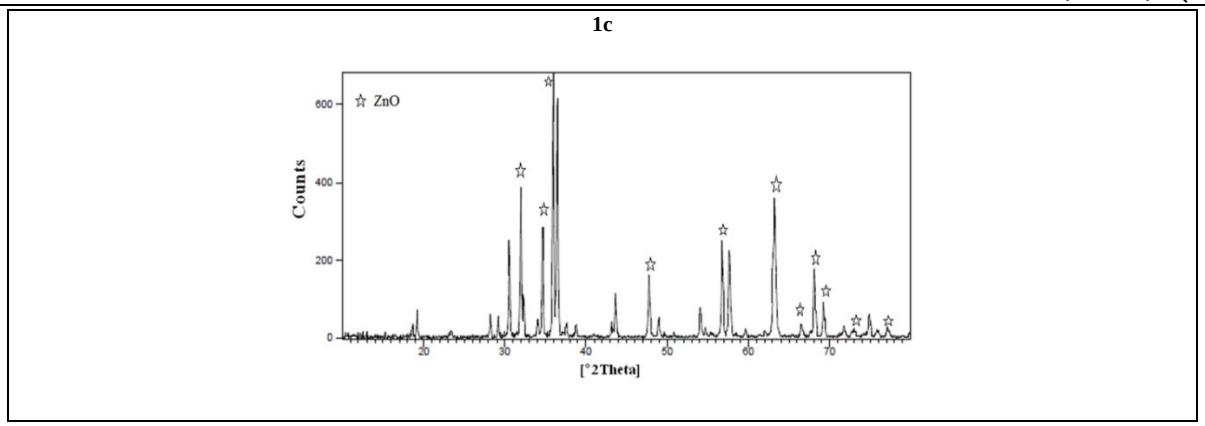


Figure 1: The XRD patterns of (a) ZnO, (b) $ZnCr_2O_4$ and (c) /PPy/ZnO/$ZnCr_2O_4$

The chemical structure of the PPy/ZnO/$ZnCr_2O_4$ composite was confirmed by FT-IR analysis. In the FT-IR spectrum of ZnO, the band at 436 cm^{-1} was ascribed to Zn-O stretching vibrations [21]. In the FT-IR spectrum of $ZnCr_2O_4$, two characteristic peaks observed at around 513 and 625 cm^{-1} corresponds to octahedral-metal stretching and to intrinsic stretching vibrations of the metal at the tetrahedral site, respectively [17]. These bands indicate the formation of spinel $ZnCr_2O_4$. The FT-IR spectrum of PPy/ZnO/$ZnCr_2O_4$ composite, the band at 441 cm^{-1} confirmed the presence of ZnO in prepared composite [22]. Also, some shift as compared to PPy confirmed that the PPy had incorporated with ZnO and $ZnCr_2O_4$ [23,24]. From the results it can be inferred that the polymerization was successfully carried out. The morphology of the ZnO, ZnO/$ZnCr_2O_4$ and PPy/ZnO/$ZnCr_2O_4$ is presented in Figure 2a-2c.

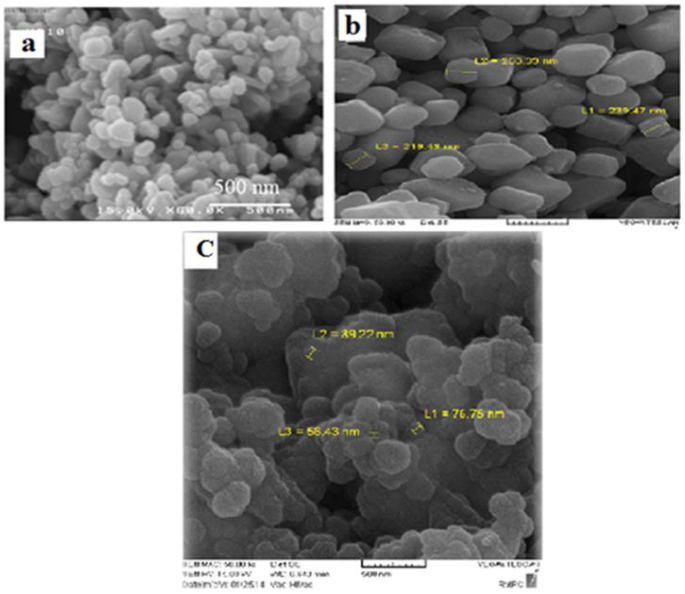


Figure 2: The SEM images of (a) ZnO, (b) ZnO/$ZnCr_2O_4$ and (c) PPy/ZnO/$ZnCr_2O_4$

Figure 2a shows that the morphology of the ZnO is nanoparticle and particle size is of about (30-100 nm). Figure 2b for the ZnO/$ZnCr_2O_4$ sample shows that the particles are quite polydispersed with a mean diameter of 213 nm. Figure 2c shows a PPy layer coated on mixed ZnO/$ZnCr_2O_4$ particles. The EDAX analysis of the ZnO/$ZnCr_2O_4$ and PPy/ZnO/$ZnCr_2O_4$ samples sintered at 700°C was performed along with its elemental analysis in order to check the purity of sample. The EDAX analysis revealed that the entire samples synthesized without any impurities (Figure 3a and 3b).

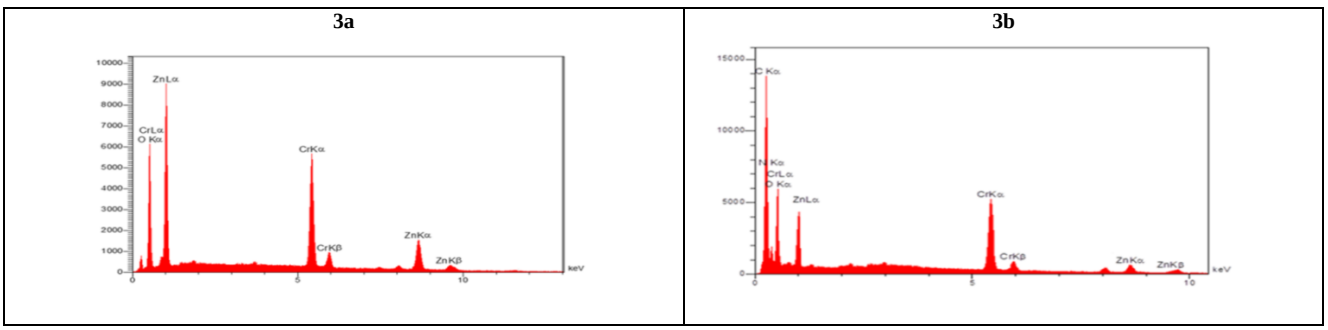


Figure 3: The EDAX spectrum of ZnO/$ZnCr_2O_4$, (b) The EDAX spectrum of PPy/ZnO/$ZnCr_2O_4$

Based on the experimental results and analysis of the XRD, FTIR, SEM and EDAX it was found that the PPy/ZnO/$ZnCr_2O_4$ composite was obtained.

Antibacterial activity

The antibacterial activity of ZnO, ZnO/$ZnCr_2O_4$ and PPy/ZnO/$ZnCr_2O_4$ for *E. coli* and *S. aureus* was compared using the Kirby Bauer method and Muller-Hinton agar. The Diameter of Inhibition Zone (DIZ) represents the susceptibility of the bacterial strains. The inhibition zones of synthesized samples against both *E. coli* and *S. aureus* are summarized in Table 1.

Table 1: Disc diffusion test and Agar dilution test of samples for *Escherichia coli* and *Staphylococcus aureus*

	Sample	[a]Agar dilution Test (DIZ)	[a]Disk diffusion Test (DIZ)
Escherichia coli	ZnO	18 ± 0.25 mm	24 ± 0.1 mm
	ZnO/$ZnCr_2O_4$	22 ± 0 mm	28 ± 0.02 mm
	PPy/ ZnO/$ZnCr_2O_4$	31 ± 0.1 mm	30 ± 0.5 mm
	Tetracycline	40 ± 0.0 mm	33 ± 0.3 mm
Staphylococcus mucus	ZnO	24 ± 0.1 mm	18 ± 0.1 mm
	ZnO/$ZnCr_2O_4$	27 ± 0.3 mm	19 ± 0.3 mm
	PPy/ ZnO/$ZnCr_2O_4$	34 ± 0 mm	28 ± 0.2 mm
	Penicillin	44 ± 0.1 mm	40 ± 0 mm

[a]Calculated from three values

In the agar dilution method, ZnO and ZnO/$ZnCr_2O_4$ samples produced 18 mm and 22 mm of ZOI, while PPy/ZnO/$ZnCr_2O_4$ produced 31 mm of ZOI against *E. coli*, respectively. While induced 24, 27 mm and 34 mm of ZOI against *S. aureus*, respectively. In the ZnO/$ZnCr_2O_4$ composite the inhibition zone indicates that the mechanism of the biocidal action of ZnO nanoparticles involves disruption of the membrane with generation of surface oxygen species and finally death of pathogens. Results showed that $ZnCr_2O_4$ composited with ZnO nanoparticles had higher antibacterial activity compared to ZnO individually, suggesting a synergistic effect [25,26]. Interestingly, the incorporation of PPy into ZnO/$ZnCr_2O_4$ composite led to a considerable increase in the bacterial activity. This can be explained by the continuous release of Zn^{2+} ions which are responsible for antibacterial activity [27]. It is believed that the positively charged biomacromolecular components and nucleic acids cause structural changes and deformation in bacteria cell walls and membranes, which lead to disruption of metabolic processes followed by cell death. The antimicrobial mechanism of ZnO nanoparticles has also been suggested to be related to microorganisms damage due to free radicals derived from the surface of the ZnO nanoparticles that can penetrate the cell membrane and kill the bacteria [28]. We also analyzed the MIC of synthesized samples against both the bacterial strains (Table 2).

Table 2: Minimum inhibitory concentration (MIC) (µg/ml) of samples against *Escherichia coli* and *Staphylococcus aureus bacteria*

Nanoparticles	*Escherichia coli*	*Staphylococcus aureus*
ZnO	22	19
ZnO/$ZnCr_2O_4$	28	21
PPy/ZnO/ $ZnCr_2O_4$	37	48
Tetracycline	30	28

The results showed that the bactericidal pattern of our synthesized samples against both *E. coli* and *S. aureus* strains was again PPy/ZnO/$ZnCr_2O_4$ >ZnO/$ZnCr_2O_4$ >ZnO.

CONCLUSION

Recently, because of increasing microbial resistance to common disinfectants and antibiotics, numerous studies have been performed to improve antimicrobial strategies. Several studies have been documented in the field of antibacterial compounds in the recent years. Application of inorganic metal oxides could be considered as a suitable alternative for some antimicrobial methods. In this work, novel mixed metal oxide of ZnO/$ZnCr_2O_4$ and PPy/ZnO/$ZnCr_2O_4$ have been successfully synthesized by simple and efficient method. The structural characterizations have confirmed the formation of synthesized samples. The antibacterial activity of synthesized samples against *E. coli* and *S. aureus* bacteria was assessed by the agar dilution and disk diffusion test. PPy/ZnO/$ZnCr_2O_4$ composite exhibited maximum antibacterial activity against *E. coli* and *S. aureus*.

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