



Synthesis of PEG-Fe₃O₄/ZnO Magnetic Nanocomposites by Ultrasound Assisted Co-Precipitation Process and their Antibacterial Activity

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Received: 15 September 2020;

Accepted: 5 November 2020;

Published online: 10 December 2020;

AJC-20201

In this study, PEG-Fe₃O₄/ZnO magnetic nanocomposites were synthesized by a ultrasound assisted co-precipitation method. The magnetic nanocomposites were characterized by XRD, FT-IR, FESEM and VSM techniques. The effect of Fe₃O₄:ZnO ratio on the crystallite size and the lattice parameter of the nanoparticles was also studied using XRD. Magnetic nanoparticles of PEG-Fe₃O₄/ZnO nanocomposites exhibit the particles size in the range of 20-30 nm. The VSM measurements at room temperature showed that PEG-Fe₃O₄/ZnO nanocomposites had superparamagnetic properties. These results indicated that ultrasonication time and ration (Fe₃O₄/ZnO) greatly affect the characteristics of the nanoparticles. The antibacterial efficiency of PEG-Fe₃O₄/ZnO nanoparticles was tested against *Sacharomyces cerevisiae*, *Bacillus subtilis* and *E. coli* bacteria using disc diffusion method.

Keywords: PEG-Fe₃O₄/ZnO, Ultrasonication, Crystallite, Lattice parameter, Antibacterial activity.

INTRODUCTION

Applications of nanocomposite materials in magnetic resonance imaging, cancer cell burning, drug transmission or bactericidal of nanocomposite materials are deeply concerned by scientists [1-3]. ZnO is a n-type semiconductor material with a wide band gap of 3.1-3.3 eV wide and a large excitation energy (60 meV) at room temperature [2]. Therefore, it is stimulated by the green near-UV emission band that creates holes [3]. The study of ZnO as an antibacterial agent was first introduced in 1950s [4,5]. Bacteria cells, when absorbed by fluorescent substance, are irradiated with a wavelength of light and the appropriate dosage produces an oxidation reaction (ROS) that kills pathogenic bacteria [3,6]. To adjust the optical band gap, emission properties and improve the applicability of ZnO nanoparticles, scientists added Fe₃O₄ in certain proportions and reduced their size to nanometers [7]. Fe₃O₄ material has properties such as magnetic properties, high magnetization, biodegradable [1,8]. Thanh *et al.* [9] successfully synthesized Fe₃O₄ nanomaterials coated by PEG using a microwave-assisted co-precipitation method with average size of 8-40 nm, global shape and high magnetization of 68.21 emu/g [9].

Sawai [10] investigated the resistance of some bacterial strains of ZnO powder and metal oxides. There are many methods for synthesizing ZnO, Fe₃O₄ nanocomposite materials with other oxides such as: co-precipitation method, hydrothermal, sol-gel [11], reverse micelle [12], *etc.* But the disadvantages of these classical methods use expensive equipment and often arise toxic chemicals negatively affecting to environment [10-14]. Currently, green synthesis using natural extracts such as bark of plants such as mangosteen, aloe vera [15-17], *etc.* is a new method that has been interested by scientists recently. Yuvakkumar *et al.* [18] successfully synthesized ZnO nano by green method using rambutan extract. In this research, PEG-Fe₃O₄/ZnO and ZnO nanocomposites were synthesized by the coprecipitation-ultrasonication method and characterized. The effects of Fe₃O₄:ZnO ratio, magnetic property and their antibacterial activity have also been investigated.

EXPERIMENTAL

The chemicals *viz.* ferric chloride hexahydrate (FeCl₃·6H₂O), ferrous chloride tetrahydrate (FeCl₂·4H₂O), zinc nitrate hexahydrate (Zn(NO₃)₂·6H₂O) and ethanol were purchased

form Merck. The PEG polymer (PEG-6000) and liquid NH_3 were obtained from Macklin, China. Rambutan (*Nephelium lappaceum* L.) peel was collected locally and deionized water was used for the preparation of all aqueous solutions.

Characterization: X-ray diffraction patterns were recorded with a P'Pret Pro-PANalytical X-ray diffractometer operating at 1.8 kW (40 mA/45KV) using $\text{CuK}\alpha$ ($\lambda = 1.5406 \text{ \AA}$) radiation. FT-IR spectrum was recorded by KBr pellet method using a Bruker FT-IR spectrometer. FESEM were carried out using a Hitachi S-4800. The minimal amount of solid sample was dispersed in ethanol and small drops were placed on an aluminum grid. The grid was dried for 1-2 h in a vacuum over at 40°C prior to the FESEM studies. Magnetic measurements of the solid samples were performed at room temperature (25°C) using a Magnet B-10 vibrating sample magnetometer (VSM).

Preparation of PEG-coated Fe_3O_4 nanoparticles: The PEG-coated Fe_3O_4 nanoparticles were prepared by mixing $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ with ratio of 2:1 into 100 mL of deionized water. The preparation method of PEG-coated Fe_3O_4 nanoparticles was prepared according to the reported method [9,19]. The reaction mixture was placed on a sonicator (Sonics & materials-VCX500; 500 W, 20 kHz) and 25-28% NH_3 solution was added dropwise using a syringe pump at flow rate of 50 mL/h. These procedures resulted in the formation of black precipitates of PEG-coated Fe_3O_4 nanoparticles. The solid was washed with distilled water several times until the distillate becomes neutral and finally washed two times with ethanol. The product was dried in oven at a 65°C .

Preparation of ZnO nanoparticles: The ZnO nanoparticles was prepared as per reported method [17,18]. Rambutan (*Nephelium lappaceum* L.) peels were washed with distilled water and subsequently incised into small pieces, and finally dried at 60°C . A dried rambutan peels (Using 3 g) was boiled with a mixture of ethanol and distilled water (1:2 ratio) for 30 min. Extract was filtered with Whatman No.1 and stored in refrigerator. Rambutan peel extract (50 mL) was added drop wise in a zinc nitrate hexahydrate (0.1 M) solution under ultrasonic waves (500 w, 20 kHz) with constant magnetic stirring at temperature room for 1 h to obtain a zinc-ellagat complex formation. The zinc-ellagat complex formed was collected by centrifugation at 7000 rpm for 20 min. Then, the solid was

washed with distilled water and dried in oven at 40°C for 8 h, and calcinated in a muffle furnace at 450°C to get ZnO nanoparticles.

Preparation of PEG- Fe_3O_4 /ZnO nanoparticles: The PEG-coated Fe_3O_4 and ZnO nanoparticles were mixed with 100 mL deionized water using a magnetic stirrer until a homogenous solution obtained. Then, the reaction mixture was placed on a sonicator (VCX500; 500 W, 20 kHz) for 30 min. The different mass ratio of PEG- Fe_3O_4 and ZnO nanoparticles as 1:0.5; 1:1; 1:2 and 1:3 were investigated. Fig. 1 showed the schematic illustration of the preparation process of PEG- Fe_3O_4 /ZnO magnetic nanocomposites.

Antibacterial activity: The antibacterial efficiency of the ZnO and PEG- Fe_3O_4 /ZnO nanoparticles was tested against *Sacharomyces cerevisiae* and *Bacillus subtilis* bacteria using Kirby-Bauer disk diffusion method [20]. The antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms.

RESULTS AND DISCUSSION

Morphological studies: During the preparation of PEG-coated Fe_3O_4 nanoparticles, ZnO nanoparticles and Fe_3O_4 /ZnO magnetic nanoparticles, the colour of the reaction mixtures changed several times due to the influence of the compound phase formed. First, ammonia solution, which was added into reaction solutions, produces OH^- anion to react with Fe^{2+} and Fe^{3+} cations to form Fe_3O_4 nanoparticles. The addition of PEG to reaction mixtures resulted in the coating of Fe_3O_4 with PEG, which was happened due to the interaction between -OH group of PEG compound and -OH group on the surface of Fe_3O_4 nanoparticles, the H atom would be released and the C-H chain interacted with the Fe_3O_4 surface [8].

In the preparation of ZnO nanoparticles, formation of ester oxygen atom and phenolic hydroxy groups of polyphenols form p-track conjugation effect, when hydroxyl groups binded with metal. The zinc-ellagat complex was obtained due to the chelating effect. The zinc-ellagat complex decomposed at 450°C and leads to the formation of ZnO nanoparticles [18]. Then, ZnO nanoparticles was functionalized to the PEG- Fe_3O_4 as shown in Fig. 1. The products were further characterized by XRD, FT-IR and VSM methods.

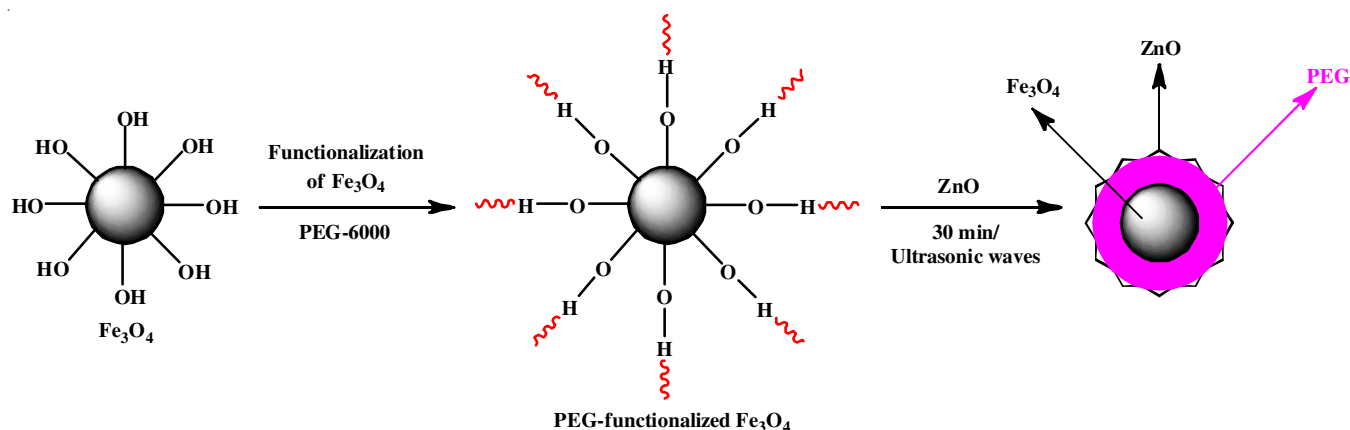


Fig. 1. Schematic illustration of preparation process of PEG- Fe_3O_4 /ZnO magnetic nanocomposites

XRD studies: The position and relative intensity of all diffraction peaks in all XRD patterns are fully matched (Fig. 2). The characteristic peaks of Fe₃O₄, namely the peaks at 2θ of 30.0°; 35.4°; 57.2° and 62.6°, indicated that Fe₃O₄ nanoparticles had a cubic spinel structure [10]. The peaks of ZnO

at 2θ of 31.76°; 34.28°; 36.12°, 47.30°, 56.37° and 68.77° indicated that ZnO nanoparticles had a hexagonal structure [17,18].

The characteristic peaks of PEG-Fe₃O₄/ZnO samples showed that the characteristic peaks of both Fe₃O₄ and ZnO crystals. Table-1 shows the influence of Fe₃O₄:ZnO ratio at 30 min ultrasonication towards the crystallite size and lattice parameters in PEG-Fe₃O₄/ZnO nanoparticle formation. It is found that the size crystallite of Fe₃O₄ nanoparticle and the lattice parameter is not different from the PEG-Fe₃O₄ sample, while the crystal size of ZnO increased in PEG-Fe₃O₄/ZnO compared to the ZnO sample. The reason is attributed due to the fact that Fe₃O₄ crystal was coated by PEG, so less affected than ZnO crystal in PEG-Fe₃O₄/ZnO nanocomposite synthesis.

FESEM images (Fig. 3) showed that PEG-Fe₃O₄ nanoparticles after surface modification with ZnO have a softer surface and consist of more uniform particle size distribution than the ZnO nanoparticles. The PEG-Fe₃O₄/ZnO samples exhibit the sizes of nano-Fe₃O₄ and ZnO particles in the range of 15-20 nm.

FTIR analysis: The FT-IR spectrum of PEG-Fe₃O₄/ZnO (Fig. 4) shows the fingerprint regions at 547 and 456 cm⁻¹ indicating the presence of Fe-O bond in bare Fe₃O₄ and Fe₃O₄-PEG samples [21] and can be attributed to the stretching vibration of Zn-O [4]. The Fe-O-C bond interaction was iden-

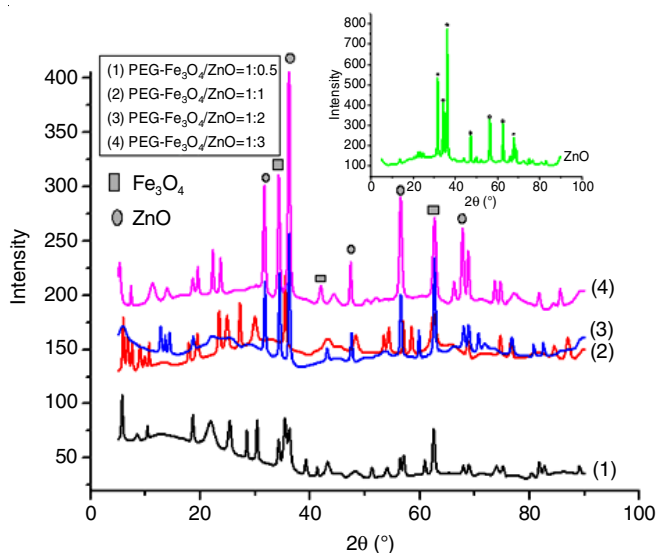
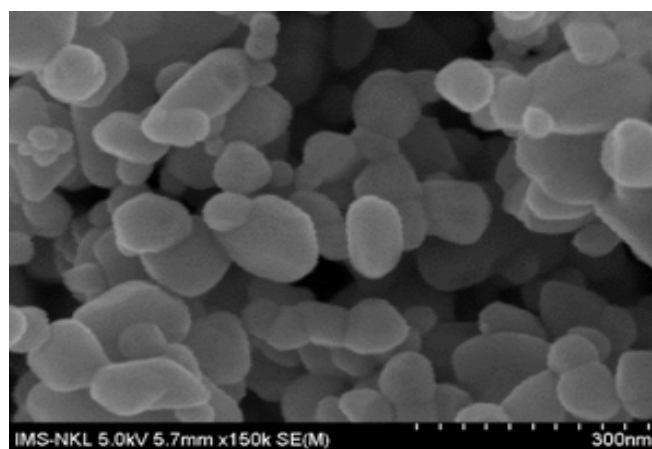
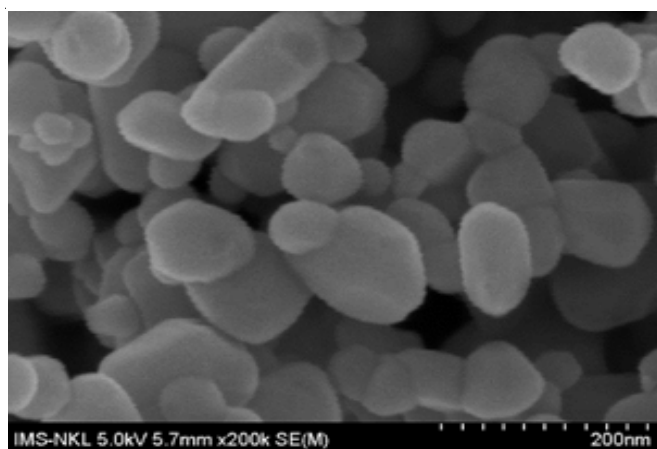
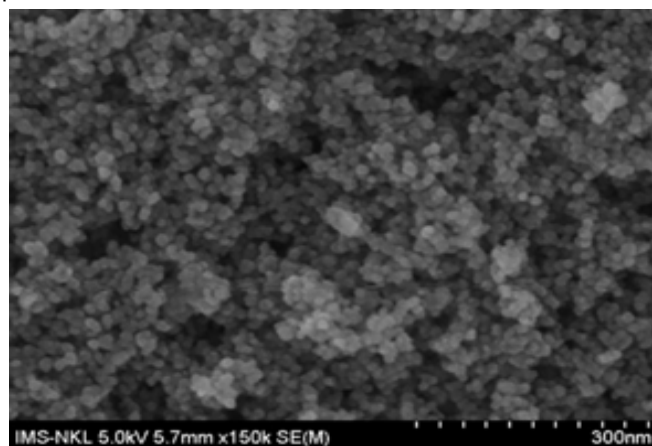
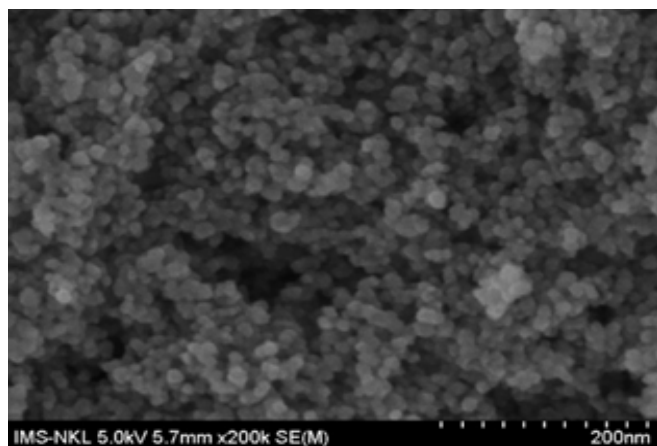


Fig. 2. XRD pattern of ZnO NPs and PEG-Fe₃O₄/ZnO (1:1) nanoparticles



ZnO nanoparticles



PEG-Fe₃O₄/ZnO

Fig. 3. FESEM images of ZnO nanoparticles and PEG-Fe₃O₄/ZnO (1:1) nanocomposites

TABLE-1
EFFECT OF THE RATIO ($\text{Fe}_3\text{O}_4:\text{ZnO}$) TOWARD CRYSTALLITE SIZE AND LATTICE PARAMETER OF PEG- $\text{Fe}_3\text{O}_4/\text{ZnO}$ MAGNETIC NANOCOMPOSITES

Samples	ZnO	PEG- Fe_3O_4	PEG- $\text{Fe}_3\text{O}_4/\text{ZnO}$				
			1:0.5	1:1	1:2	1:3	
Crystallite (nm)	ZnO Fe_3O_4	20.7465 – 20.9798	– 13.9673	20.8856 24,165 27.9376	27.8622 20.2293	20.8882 18.1205	
Lattice parameter (Å)	ZnO	a = b = 3.2427 c = 5.1948 $\alpha = \beta = 90^\circ$ $\gamma = 120^\circ$	–	a = b = 3.2357 c = 5.2357	a = b = 2.7325 c = 4.2147	a = b = 3,086 c = 5.2068	a = b = 3.5141 c = 5.2290
	Fe_3O_4	–	a = b = c = 8.3232 $\alpha = \beta = \gamma = 90^\circ$	a = b = c = 8.4105	a = b = c = 8.4016	a = b = c = 8.4599	a = b = c = 8.4464
Saturation magnetization (emu/g)		65.71	53.55	63.51	34.43	23.788	

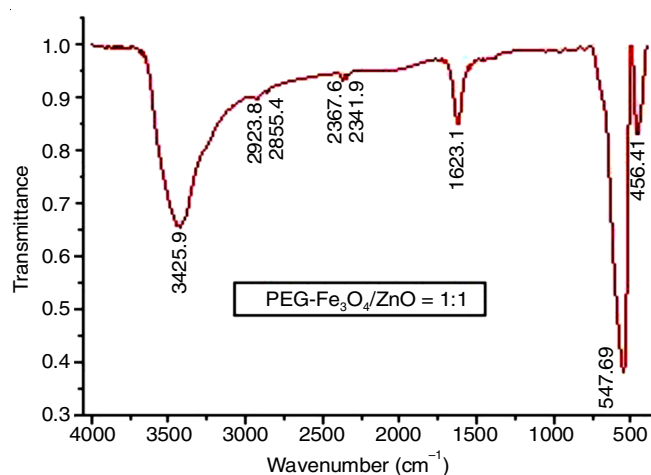


Fig. 4. FT-IR spectrum of PEG- $\text{Fe}_3\text{O}_4/\text{ZnO}$ (1:1) nanocomposites

tified by absorption around 1050 cm^{-1} indicates that Fe_3O_4 has been coated with PEG [21].

Magnetic measurements: The magnetization curves were obtained at room temperature and parameters such as coercive field (H_c) and initial susceptibility (χ_i) were obtained. The saturation magnetization (M_s) was obtained by extrapolation to infinite field of the experimental data obtained in the high field range where the magnetization varies linearly with the inverse of the applied field (Fig. 5). Saturation magnetization values were calculated by considering the ratio of $\text{Fe}_3\text{O}_4/\text{ZnO}$ from the samples.

The results showed that the saturation magnetization of bare Fe_3O_4 nanoparticles reached 67.77 emu/g . When using PEG as dispersant, the saturation magnetization of PEG- Fe_3O_4 were 65.71 emu/g [19]. When the PEG- Fe_3O_4 core modified

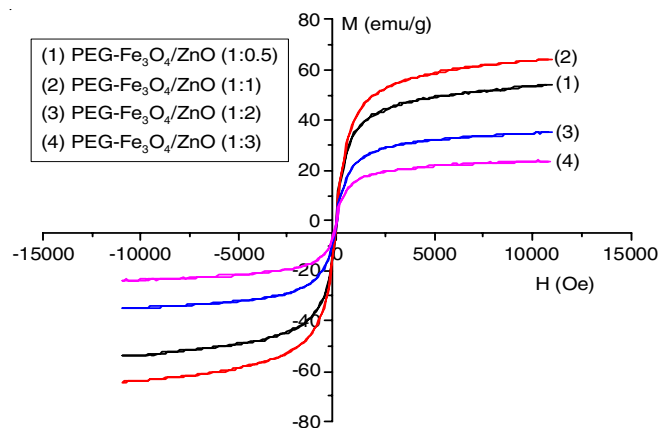


Fig. 5. M-H curves for PEG- $\text{Fe}_3\text{O}_4/\text{ZnO}$ nanocomposites

with ZnO, the saturation magnetization of PEG- $\text{Fe}_3\text{O}_4/\text{ZnO}$ nanocomposite decreased from 30% to 5% depending on the ratio of $\text{Fe}_3\text{O}_4\text{-ZnO}$ in the samples (Table-1).

Antibacterial activity: When a certain amount of ZnO and PEG- $\text{Fe}_3\text{O}_4/\text{ZnO}$ nanoparticles were sprinkled on the nutrient medium incubated under appropriate conditions. After 72 h, two of the petri dishes did not have any bacteria or mold growing on the surface of the nutrient (Figs. 6a and Fig. 7a). This indicated that bitg nanoparticles had an ability to kill bacteria and mold cells when adhered to the sample.

The results also showed that ZnO and PEG- $\text{Fe}_3\text{O}_4/\text{ZnO}$ has an ability to inhibit *Bacillus subtilis* bacteria. At the point of sprinkling of the test sample on a disk with *Bacillus subtilis* implant, the inner region of the sample (aseptic zone) is formed (Fig 6.b-c and Fig. 7b-c) and has the best ability to inhibit *E. coli* (Fig. 6i-f and Fig. 7i-f).

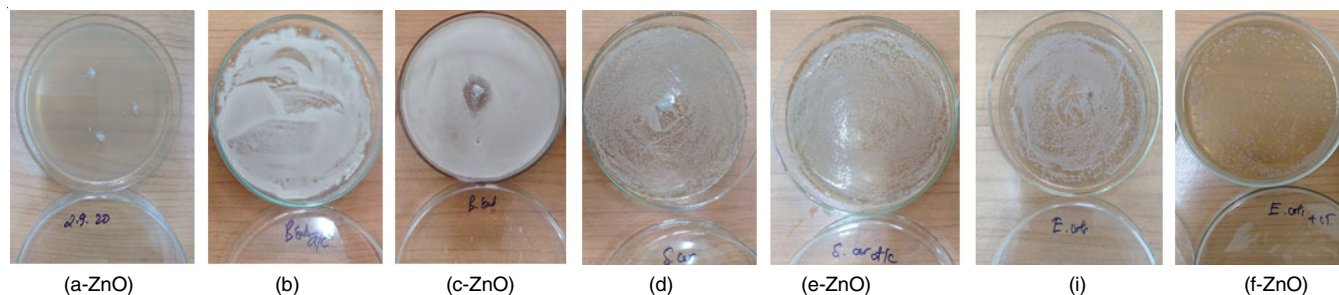


Fig. 6. Antibacterial and antifungal activity of ZnO

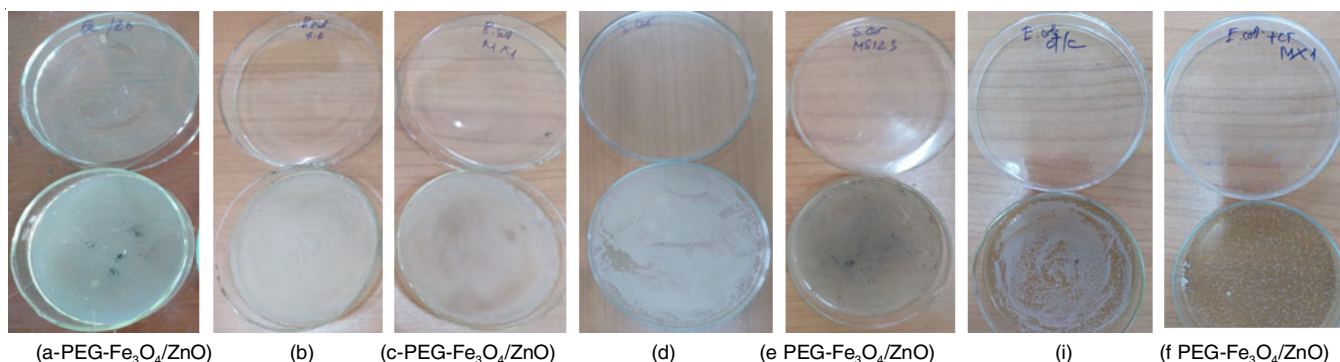


Fig. 7. Antibacterial and antifungal activity of PEG-Fe₃O₄/ZnO

For *S. cerevisiae* strain, when the suspension is in contact with ZnO and PEG-Fe₃O₄/ZnO, it makes cell density significantly reduced (Fig. 6d-e and Fig. 7d-e). Therefore, it is concluded that PEG-Fe₃O₄/ZnO has an ability to kill *S. cerevisiae* yeast cells. In summary, PEG-Fe₃O₄/ZnO has the best ability to kill yeast, while ZnO has an best ability to kill *E. coli*.

Conclusion

The PEG-Fe₃O₄/ZnO magnetic nanocomposites was successfully prepared by ultrasound assisted co-precipitation method. Based on the analysis results, the XRD data of the samples obtained with the variation of the ratio of Fe₃O₄:ZnO showed that lattice parameters of Fe₃O₄ nanoparticle had no difference from the PEG-Fe₃O₄ sample, while the crystal size of ZnO increased when compared to the bare ZnO nanoparticles. The modified surface of PEG-Fe₃O₄/ZnO nanoparticles has resulted in softening the magnetization. The antibacterial activity results showed that PEG-Fe₃O₄/ZnO nanoparticles had an ability to kill bacterial and mold cells when adhering to samples, especially the best inhibited with *Bacillus subtilis*.

ACKNOWLEDGEMENTS

This research is funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant no. 104.06-2018.320.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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