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Research Note

Synthesis and antimicrobial properties of ZnO/PVA, CuO/PVA, and TiO_2/PVA nanocomposites

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Drop test method.

Abstract. In this paper, zinc oxide (ZnO), copper oxide (CuO), and titanium oxide (TiO_2) nanoparticles were prepared using sol-gel method. The structural properties of these nanoparticles were characterized using X-Ray Diffraction (XRD) and Transmission Electron Microscopy (TEM). SEM images revealed that the average sizes of zinc oxide, copper oxide, and titanium oxide nanoparticles are about 37 nm, 86 nm, and 50 nm, respectively. Then, nanocomposites of metal oxide were produced by Polyvinyl Alcohol (PVA). The antibacterial activities of zinc oxide, copper oxide, and titanium oxide nanocomposites against human pathogenic bacteria, mainly Escherichia coli HB101 (gram-negative), have been studied using drop test during the time periods of 15 minutes, 30 minutes, 1 hour, 2 hours, 12 hours, and 24 hours of generated reviews. According to the results obtained with the nanocomposite, zinc oxide nanoparticles exhibit the highest antibacterial properties.

1. Introduction

In recent years, nanocomposite materials have received great interests for both industrial and academic applications [1]. Addition of small amount of nanomaterial could improve the performance of polymeric materials due to their small size, large specific surface area, quantum confinement effects, and strong interfacial interactions [2]. Among these polymers, Poly (Vinyl Alcohol) (PVA) is a polymer that has been studied intensively due to its good film forming and physical properties, high hydrophilicity, processability, biocompatibility, and good chemical resistance [3].

Many kinds of nanomaterials have been used to prepare organic/inorganic nanocomposites among these inorganic fillers, ZnO, CuO, and TiO_2. Nanoparticles have a special place due to their good stability, high refractive index, hydrophilicity, UV absorbance, nontoxicity, and excellent transparency for the visible light. These materials have increased in the field of basic and applied sciences due to their many applications in microelectronic and optoelectronic devices [4].

In order to achieve desirable properties, two or more oxides are combined. This makes it possible to change the composition ratio of two oxides and produce a new material with special preset characteristics [5].

An important application of zinc oxide, copper oxide, and titanium oxide nanoparticles is their ability to eliminate germs and bacteria in medicine and sterilize

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instruments to eradicate the infection. They are also used to prevent the growth of bacteria in the canning industry [6].

Zinc oxide has three states which are wurtzite, zincblende, and rock salt. Wurtzite structure is the most stable form (with lattice parameters $a = 0.3296$ nm and $c = 0.52065$ nm) [7]. Zinc oxide (ZnO) based polymeric nanocomposite materials are very interesting due to their good transparency, high electrical conductivity [8,9], and wide band gap (3.37) eV at room temperature [10]. This enables the applications of optoelectronics in the blue/UV region, including light-emitting diodes, laser diodes and photo detectors [11-13], short-wavelength light emitting diodes, dye-sensitized solar cells electrodes, piezoelectric materials, gas sensors, varistors (also called as variable resistors), and also in fully transparent thin film transistors [14].

Copper oxides have two most common forms: cupric oxides (CuO) and cuprous oxide (Cu_2O). The cupric oxide is a p-type semi-conductor with a monoclinic structure with energy band gap of 1.2 eV to 1.7 eV at room temperature with lattice parameters $c = 1/1288$ Å, $b = 3/4226$ Å, $a = 4/6837$ Å, and $\beta = 99/54$ Å, while the cuprous oxide is a p-type semiconductor with a cubic lattice structure with direct band energy gap of 2 eV [15,16].

Copper oxide (CuO) is a semiconducting material used in photoconductive and photothermal applications [17]. CuO has also been used in antimicrobial applications and in preparation of nanofluids [18,19].

Many kinds of nanomaterials have been used to prepare organic/inorganic nanocomposites among these inorganic fillers; TiO_2 nanoparticles have a special place due to their good stability, high refractive index, hydrophilicity, UV absorbance, nontoxicity, and excellent transparency for the visible light. TiO_2 is well known as a matter with strong redox ability used for photo electrochemical cell [20] and water or air purification [21] to degrade the organic pollutants killing the bacteria due to high photocatalytic activity and low cost [22]. High dielectric constant features of the titanium oxide have many applications for layers in insulation and protection of this material [23].

Titanium oxide can be found in three crystalline forms such as rutile, anatase, and Brookite [24].

Titanium oxide thin films have high transparency in range of 70-100% in the visible wavelength region (400-800); it can be used as an anti-scratch transparent coating because it has high hardness and high transparency [25].

In this paper, nanoparticles of zinc oxide, titanium oxide, and copper oxide were synthesized by sol-gel method, and then were prepared ZnO/PVA, CuO/PVA, and TiO_2/PVA nanocomposite films. The size, morphology, and crystallinity of the resulting ZnO, CuO, and TiO_2 nanoparticles were investigated. In addition, E. coli bacterial strain is chosen to evaluate the in-vitro antimicrobial activity of ZnO, CuO, and TiO_2 nanoparticles using drop test method.

2. Experimental

There are different methods for synthesis of nanoparticles of zinc oxide, copper oxide, and titanium oxide. For example, magnetron sputtering [26], CVD [27,28], pulsed laser [29], and sol-gel [30]. The sol-gel method has special and simpler advantages compared to other methods due to homogeneous particle distribution and higher reactivity of chemical components, reducing synthesis steps, controllable particle size (on a scale of nanometers), and higher density.

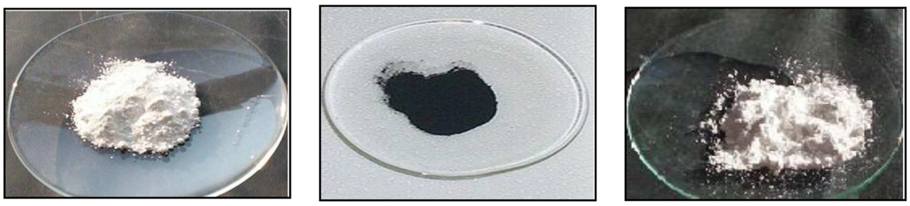
2.1. Synthesis of zinc oxide nanoparticles

ZnO nanoparticles were synthesized by sol-gel technique. The mixture of ethanol 99% (C_2H_5OH, MERK) and deionized water as a solvent was prepared at a ratio of 1:1. Then, the zinc nitrate [$Zn(NO_3)_2.6H_2O$] was used as the main material starter. Ethylene glycol and citric acid were added to the mixture as complex maker and polymer maker, respectively.

This solution was refluxed for 14 hours at 80°C. Zinc ions gels were dried for 2 hours at 160°C, and then nanoparticles of zinc oxide (after grinding) were annealed for an hour at 600°C (Figure 1(a)).

2.2. Synthesis of copper oxide nanoparticles

In this synthesis, a mixture of ethanol 99% (C_2H_5OH, MERK) and deionized water as the solvent was prepared at a ratio of 1:1. Then, 0.25 M of copper nitrate [$Cu\ (NO_3)_2.3H_2O$] was used as the main material starter. Ethylene glycol and citric acid, as a complex maker, are added to the mixture. The solution was



(a) (b) (c)

Figure 1. Nanoparticles of (a) zinc oxide, (b) copper oxide, and (c) titanium oxide synthesis by sol-gel method.

refluxed in temperatures' range of 90-110°C for 4 hours. Then, bloated gel was calcined at 120°C for 7 hours.

The final black powder, containing copper oxide nanoparticles after grinding, was calcined for one hour at 400°C (Figure 1(b)).

2.3. Synthesis of titanium oxide nanoparticles

In this synthesis, 40 cc ethanol 99% (C_2H_5OH, MERK) was added to 60 cc titanium isopropoxide and 40 cc acetylacetone. The resulting solution was stirred by a magnetic stirrer at 40°C. Then, the obtained solution was refluxed at 130°C for 4 hours.

Next, this solution was put in oil bath at 80°C for 36 hours. The color of resulting powder is gray. This powder was calcined for one hour at 500°C (Figure 1(c)).

2.3.1. Preparing PVA

270 cc deionized water was solved in 30 gr powder of polyvinyl alcohol (PVA) at 70°C by magnetic stirring, and was then put in an oil bath at the same temperature overnight until a clear sol was obtained. Then, it was decanted in three separate petri dishes, and 84 gr of zinc oxide, copper oxide, and titanium oxide nanoparticles were added, prepared from the previous stage to this sol by ultrasonic irradiation.

The nanocomposite polymer films were prepared by casting technique with the aid of Ultrasonic irradiation. Casting method is based on liquid particle dispersion, where the water was used as PVA polymer solvent, and then the polymer solution was used as nanoparticles dispersant. Once we get a homogeneous dispersed mixture, the solvent evaporation yields a homogeneous nanocomposite solid film. Since the nanoparticles tend to agglomerate, the ultrasonic radiation was employed to get a good dispersion of TiO_2 nanoparticles in the PVA solution. Ultrasonic radiation produces a harsh environment for some chemical interactions to take place between the polymer and nanoparticles which would result in better dispersion and less agglomeration.

This prepared material was put in the oven at 70°C for drying this sol and determining the antibacterial property. 20 cc of each sample was poured to the petri dish and was put in the oven at 80°C until this material was dried to a laminar form. The obtained polymer nano composites, containing of zinc oxide, copper oxide and titanium oxide, are observed in Figure 2.

3. Results and discussion

3.1. Structural properties

X-Ray Diffraction (XRD) was used to identify crystalline phases and estimate the crystalline sizes. XRD patterns of nanoparticles of zinc oxide, copper oxide, and titanium oxide are displayed in Figure 3(a) to (c), respectively.

As can be seen in Figure 3, samples are grown to a polycrystalline form, and the nanoparticles of zinc oxide and copper oxide have a preferred orientation of (101) and (111), respectively. Nanoparticles of titanium oxide have a preferred orientation of (101). Size of the crystallites was estimated by the Scherrer equation [31]:

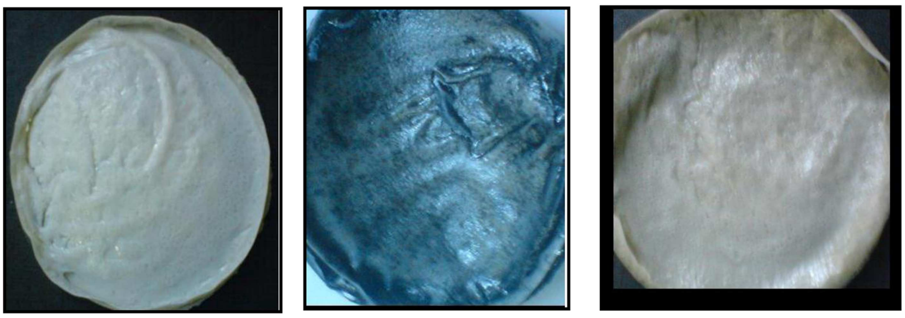
$$D = \frac{0.89\lambda}{B\cos\theta}. \tag{1}$$

where D is the mean size of the ordered (crystalline) domains, which may be smaller or equal to the grain size; λ is the X-ray wavelength; β is the line broadening at half the maximum intensity (FWHM) after subtracting the instrumental line broadening in radians. This quantity is also sometimes denoted as $\Delta(2\theta)$, and θ is the Bragg angle.

3.1.1. The surface morphology

The morphology and size distribution of the particles were further studied by TEM. Figure 4 is the TEM image of nanocrystals, showing that the particles have nearly spherical and ZnO nanoparticles with the lowest average grain size of 37 nm.

Nanoparticles of titanium oxide and copper oxide are located with average grain sizes of 86 nm and



(a) (b) (c)

Figure 2. Polymer nano composite containing (a) nanoparticles of zinc oxide, (b) nanoparticles of copper oxide, and (c) nanoparticles of titanium oxide.

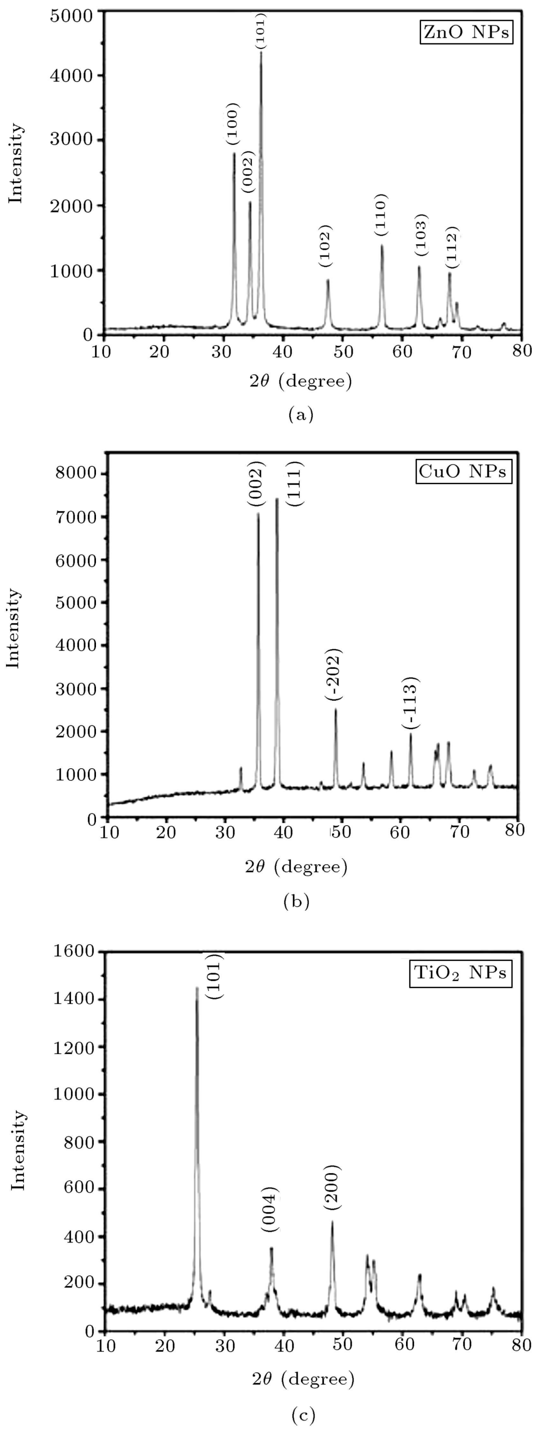


Figure 3. X-ray diffraction patterns of: (a) Nanoparticle zinc oxide, (b) nanoparticle copper oxide, and (c) nanoparticle titanium oxide.

50 nm, respectively. The titanium oxide nanoparticles have the most homogeneous distribution of nano-sized particles.

3.2. Antibacterial results

The antibacterial properties of ZnO, CuO, and TiO_2 nanoparticles were studied against E. coli HB101, and then were examined by drop method. In this method, the number of surviving colonies after exposing nanocomposite layer was compared with that of colonies in connection with the control layer (polymeric layer uncoated nanoparticles). This ratio can be used as an indicator to estimate antibacterial powder layers. For this purpose, first, liquid medium LB, as the combination of solution of sodium chloride, yeast extract, tryptone, and double-distilled water, will be produced. Afterwards, 100 μl liquid medium was put in polymer metal oxide layer with drop method. After certain times of 15 minutes and 30 minutes, the remaining bacteria on layers with 1 cc of PBS buffer solution were washed for 1 hour, 2 hours, 12 hours, and 24 hours and diluted to 10-8.

In the next stage, 10 μl of the remaining solution, containing bacteria on the surface layers, was transferred to solid medium; the number of bacteria was grown and divided by the number of alive bacteria on the surface of the control sample (time zero). This action was done for the control layer, without considering that these results can be seen in Table 1. Eventually, curve N/N_0 (quotient of the number of bacteria surviving on the surface of the control sample at time zero) was plotted in terms of the time periods of 2 hours and 24 hours, respectively (Figures 5 and 6). After 24 hours, it is observed that only a handful of surviving bacteria, indicating significant antimicrobial properties of nanocomposites, were obtained.

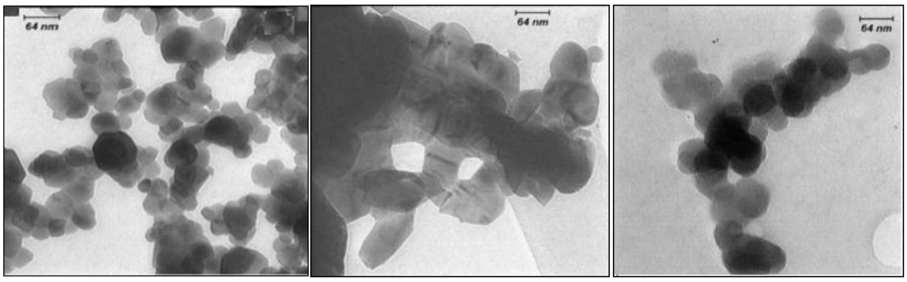
Tables 1 and 2 show the relative number of bacteria remaining on the prepared oxide nanocomposites: zinc oxide, copper oxide, and titanium oxide; these nanocomposites were compared with a control layer (made of pure polymer) multiple times.

4. Conclusion

In this respect, various cases of bacterial resistance to conventional antibiotics and antimicrobial agents have been researched for finding new types of effective antimicrobial agents.

In this paper, at first, zinc oxide, copper oxide, and titanium oxide nanoparticles were synthesized by sol-gel method. Then, the structural and morphology of these nanoparticles were measured by XRD and TEM. Afterwards, nanocomposites of zinc oxide, copper oxide, and titanium oxide were made using polyvinyl alcohol. To evaluate the anti-microbial properties of the nanocomposites, they were put into the exposed E. coli HB101.

The few number of surviving bacteria after 24 hours only showed significant anti-microbial activity in the nanocomposites. According to the obtained results, the nanocomposites of nanoparticles of zinc oxide exhibited the highest antibacterial properties; next in line are the nanocomposites of copper oxide and titanium



(a) (b) (c)

Figure 4. TEM images of (a) nanoparticles of zinc oxide, (b) nanoparticles of copper oxide, and (c) nanoparticles of titanium oxide.

Table 1. Number of the remaining colonies on the samples after certain time periods of 15 minutes, 30 minutes, 1 hour, 2 hours, and 24 hours.

Samples	Control layer	ZnO/PVA	CuO/PVA	TiO_2/PVA
Remaining colonies after 15 min	124950	46975	56675	10095
Remaining colonies after 30 min	104100	13925	83850	13420
Remaining colonies after 1 h	113775	74350	81775	99300
Remaining colonies after 2 h	113775	8440	12135	9440
Remaining colonies after 24 h	1115400	6.15	2483.2	69200

Table 2. Curve N/N_0 (quotient of the number of bacteria surviving on the surface of the control sample at time zero.)

Samples	Control layer	ZnO/PVA	TiO_2/PVA	CuO/PVA
Remaining colonies after 24 h	1115400	6.15	69200	2483.2
N/N_0	0.998925	5.5×10^{-5}	0.6197	2.224×10^{-3}

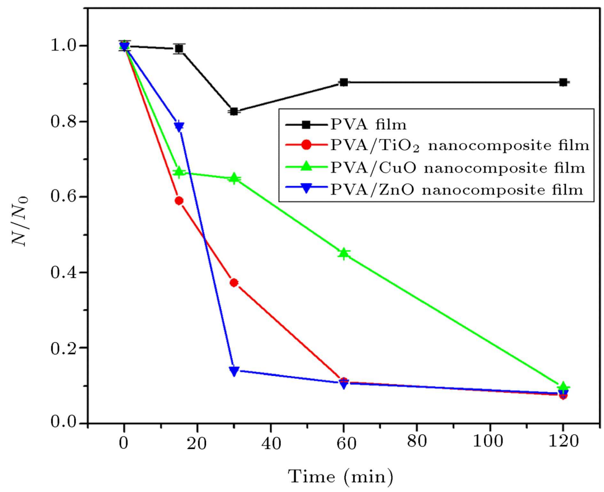


Figure 5. The relative number of alive bacteria on samples compared to those control samples after 2 hours.

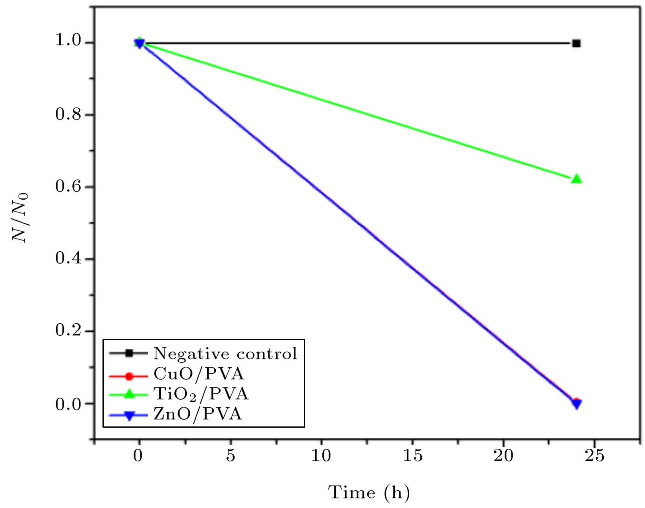


Figure 6. The relative number of alive bacteria on samples compared to those of control samples after 24 hours.

oxide with the highest antibacterial properties. The cause of severe nanocomposite antibacterial activity, including nanoparticles of zinc oxide, can be found in the small-sized particles, because the small size of particles increases the surface-to-volume ratio and leads to more activity levels in antibacterial processes.

Although the nanoparticles of titanium oxide have an average size smaller than those of copper oxide, but due to high-band gap, UV radiation is needed to activate the photocatalytic activity.

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