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Research Article

Facile Synthesis of Zinc Carbonate and Zinc Oxide Nanoparticles and Their Antimicrobial Properties

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Abstract

$ZnCO_3$ nanoparticles (NPs) were prepared by the direct precipitation method using zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) and ammonium carbonate ($(NH_4)_2CO_3$) as precursor materials and polyvinyl alcohol (PVA) as capping agent (R1) whereas ZnO NPs were prepared by thermal decarbonation of the prepared $ZnCO_3$ NPs in the absence (R2) and the presence (R3) of polyvinyl alcohol (PVA) as capping agent. Thus, prepared NPs were subjected to characterization by Fourier transform infrared (FTIR) spectroscopy and phase identification by X-ray diffraction (XRD) study. The effects of the prepared NPs towards different microbial species were also considered. The FTIR spectra of the synthesized $ZnCO_3$ nanoparticles showed characteristic peaks at about 480 cm^{-1} and 1403 cm^{-1} correlated to the zinc oxide bond (Zn-O) and carbonate group respectively. Similarly, the FTIR spectra showing a peak at 400 cm^{-1} suggested the formation of ZnO NPs. Likewise, the XRD pattern of the synthesized NPs confirmed the formation of single-phase crystalline ZnO NPs with FCC lattice structure. Using Scherrer's equation, the crystallite diameter of the prepared nanoparticles was found to be 9, 21 and 17 nm for R1, R2 and R3 samples respectively. The antimicrobial actions against the different species of bacteria and fungi were also observed and it was found that ZnO NPs prepared by using a capping agent showed a higher zone of inhibition, than that without using the capping agent. Thus, in the present study, $ZnCO_3$ and ZnO NPs were prepared by simple, cost-effective precipitation followed by a decarbonation method and these NPs could be a potential antimicrobial agent.

Keywords: Antimicrobial, Capping agent, FTIR, Nanoparticles, XRD

Introduction

Nanomaterials are substances having a very small scale, usually in the range of 1–100 nm (Singh et al., 2011; Haque et al., 2020). The size of nanoparticles (NPs) largely affects the size properties correlations *viz.* surface volume ratio, optical properties, antimicrobial properties (Kumar, 2014). Nanomaterials have advanced applications as well as implications in a variety of fields like optics, electronics, material science, biomedical sciences (Singh et al., 2011). Metal oxide NPs have been synthesized widely due to their unique physical, chemical and biological properties (Oprea et al., 2014; Janjal et al., 2013; Rosei, 2004; Kumar et al., 2015). The most recent and successful application of NPs has emerged in medical applications as drug delivery systems, in the form of nanomedicine, biomedical sensors and curing different diseases (Govinda et al., 2015; Sundar & Prajapati, 2012). Among several NPs, ZnO and $ZnCO_3$ NPs can be regarded as magic nanomaterials as they have multifunctional properties (Salahuddin et al., 2015). These NPs have been commonly used in cosmetics, medicines, agriculture, energy harvesting, etc. (Pourmortazavi et al., 2015; Parthasarathi & Thilagavathi, 2011). ZnO NPs have been used as ingredients in sunscreens, nanosensors, water purification, solar cells, etc. (Janjal et al., 2013; Kotesh et al., 2021). Similarly, $ZnCO_3$ NPs have been extensively used in respirators as they help in removing toxic gases like SO_2 and HCN (Alhawi et al., 2015; Pan & Wang, 2015). Another important application of these NPs is that $ZnCO_3$ NPs act as precursors for the synthesis of ultrafine ZnO NPs, which have several applications in various fields (Matei et al., 2014).

Among various properties of $ZnCO_3$ and ZnO NPs, antimicrobial activities have been extensively studied (Govinda et al., 2015; Kermani et al., 2015). It has been reported that the NPs prepared using a capping agent being smaller in size show greater inhibition against bacteria and fungi, than the NPs without using a capping agent (Kermani et al., 2015). $ZnCO_3$ and ZnO NPs inhibit the growth of bacteria and fungi showing significant applications as antibacterial and antifungal agents (Tankhiwale and Bajpai, 2012). The antibacterial activity is due to the release of Zn^{2+} ions by increasing H_2O_2 generation causing damage to the cell membrane and eventual death of the bacterial cells (Oprea et al., 2014). On the other hand, the antifungal activities of the NPs are attributed to the production of excess nucleic acids and carbohydrates by the stress mechanism for self-protection against the NPs leading to cell death (Saeed et al., 2015). Several studies have been carried out in the synthesis of ZnO NPs. Direct precipitation method, aqueous chemical method, simple chemical method, sol-gel method and solvothermal method in the synthesis of ZnO NPs (Saeed et al., 2015; Rao et al., 2007; Kundu et al., 2011; Kolekar et al; 2013; Zak et al., 2011). Similarly, the electro-deposition method, hydrothermal method, direct precipitation method and solid-state reactions have been applied for the synthesis of $ZnCO_3$ NPs (Pourmortazavi et al., 2015; Pan & Wang, 2015; Shamsipur et al., 2012; Wu & Jiang, 2006).

The agglomeration of NPs increases the size of the particles and hinders the -good dispersion of NPs leading to altering the properties of NPs (Govinda et al., 2015). Similarly, excessive use of chemicals and time-consuming factors have been observed during the synthesis. Various capping agents like polyvinyl alcohol (PVA), polyaniline (PANi), anthocyanin, triethanolamine (TEA), oleic acid and thioglycerol have been applied for the synthesis of different kinds of NPs (Kermani et al., 2015; Septiani et al., 2017; Singh et al., 2009). To overcome the problems during the synthesis like excessive use of chemicals and time consumption, PVA is extensively used as a capping agent in the preparation of NPs because of its excellent physical and chemical properties. During the synthesis, alcohol (OH) groups are produced from PVA molecules to form metal–ion/polymer complexes by ligand reaction (Kermani et al., 2015).

Owing to different important applications of $ZnCO_3$ and ZnO NPs, the main aim of the research work was to prepare the NPs by a simple, cost-effective precipitation method in the presence and without using a capping agent and study their size-properties correlation against the different species of bacteria and fungi for the antimicrobial actions.

Materials and Methods

Materials

Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) and ammonium carbonate ($(NH_4)_2CO_3$) were purchased from Fisher Scientific. PVA, dimethyl sulfoxide (DMSO) and ethanol (C_2H_5OH) (99.5%) were purchased from the local market. The used reagents were of analytical grade and employed without further purification. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27853) were used as bacterial isolates while *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma* and *Rhizopus* were used as fungal isolates. These bacterial and fungal strains were collected from the biotechnology department of the National School of Sciences (NIST). Muller Hinton Broth (MHB) and Potato Dextrose Broth (PDB) were used as media for antibacterial and antifungal tests respectively.

Preparation of the NPs

$ZnCO_3$ NPs were prepared through direct precipitation using $Zn(NO_3)_2.6H_2O$ and $(NH_4)_2CO_3$ solution as precursor materials and PVA as capping agent whereas ZnO NPs were synthesized by decarbonation of the prepared $ZnCO_3$ NPs (Saeed et al., 2015). In short, 2g PVA was dissolved in 50 mL of 1.5M $Zn(NO_3)_2.6H_2O$ solution and it was added dropwise in 100 mL of 2.25M $(NH_4)_2CO_3$ solutions with constant stirring at 40ºC and continuous stirring was done till 60 minutes after adding all zinc nitrate solution. The white precipitate obtained from the experiment was filtered by using Whatman Number 1 filter paper. The precipitate was rinsed thoroughly with distilled water, then ethanol, and again with distilled water three times each respectively. The washed precipitate was dried in a hot-air oven at 80 ˚C for an hour. The white precipitate formed is $ZnCO_3$ NPs (R1), and a fraction of it was kept for characterization. The remaining dried precipitate was calcined in a muffle furnace at 550 °C for 2 h to obtain white nano-sized ZnO NPs. The ZnO NPs formed in absence of a capping agent is named R2 and in presence of the PVA as a capping agent are named R3.

Characterization techniques

Prepared ZnO NPs were subjected to Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and antimicrobial studies. FTIR characterization technique was carried out by using IR-Tracer-100 (Shimadzu, Japan) at the Central Department of Chemistry, Nepal. The phase identification of the synthesized NPs was investigated using X-ray diffraction (XRD).by using D-2 Phaser Advanced Diffractometer (Bruker, Germany) at the NAST, Kathmandu. It was done by Rigaku Multiflex diffractometer with monochromated CuKα radiation of wavelength 1.5406 Å. XRD scanning was performed under ambient conditions over the 2θ region of 10° to 80° at the rate of 2˚/min (40 kV, 20 mA). The crystalline domain size of synthesized ZnO and $ZnCO_3$ NPs was calculated using Scherrer's formula, $D = \frac{0.9\lambda}{\beta\cos\theta}$. where, D is the diameter of the nanoparticle, λ is the wavelength, β is the full width at half maximum value (FWHM) which is measured with the help of origin software and θ is half of the angle of diffraction.

Antimicrobial study

25 mg /mL suspension solution for both NPs was prepared in DMSO, and this suspension was used for an antimicrobial study. The bacteria were cultured in MHB, and fungi were cultured in PDB, and an analysis of the antimicrobial tests was carried out. Screening and evaluation of the antimicrobial activity were performed by the agar-well diffusion method (Balouiri et al., 2016).

Results and Discussion

Structural characterization

The FTIR spectra of the synthesized ZnONPs of both R2 and R3 along with the FTIR spectrum of $ZnCO_3$ NPs (R1) in presence of PVA as a capping agent are shown in Figure 1. As can be seen from the FTIR spectra, the synthesized $ZnCO_3$ NPs (R1) had a characteristic peak at about 480 cm^{-1} which correlated to the zinc oxide bond (Zn-O). Further, the peak at 1403 cm^{-1} corresponds to carbonate (CO_3^{-2}) and the absorption peak in the range of 500 to 1000 cm^{-1} range is due to the lattice vibration of CO_3^{-2}. In the literature, CO_3^{-2} peaks were observed at 1370 and 1504 cm^{-1}. Similarly, the lattice vibration of CO_3^{-2} was observed at 703 to 1029 cm^{-1} (Balouiri et al., 2016). This inferred the formation of $ZnCO_3$ NPs before the formation of ZnO NPs.

The FTIR spectra of synthesized ZnO NPs (R2 and R3) showed a characteristic peak at about 400 cm^{-1} which correlates to the zinc oxide bond (Zn-O). From the different literature, it can be found that the ZnO peak is observed between the range of 400 to 700 cm^{-1}. The peaks of ZnO from the different literature works are observed at 500, 521 and 375 cm^{-1} (Salahuddin et al., 2015; Matei et al., 2014; Zak et al., 2011; Shamsipur et al. 2012; Karthivelu et al., 2009; Raut et al., 2013) and hence it can easily infer that the prepared NPs are ZnO NPs. Similarly, the peaks in the range of 1400 to 1600 cm^{-1} correspond to C=O bonds, probably due to CO_2 absorption from the atmosphere (Santha et al., 1990). In addition, a broad peak at about 3400 cm^{-1} shows the characteristic peak for the -OH functional group indicating O-H bending vibrations, which may be due to the absorption of moisture from the atmosphere (Santha et al., 1990).

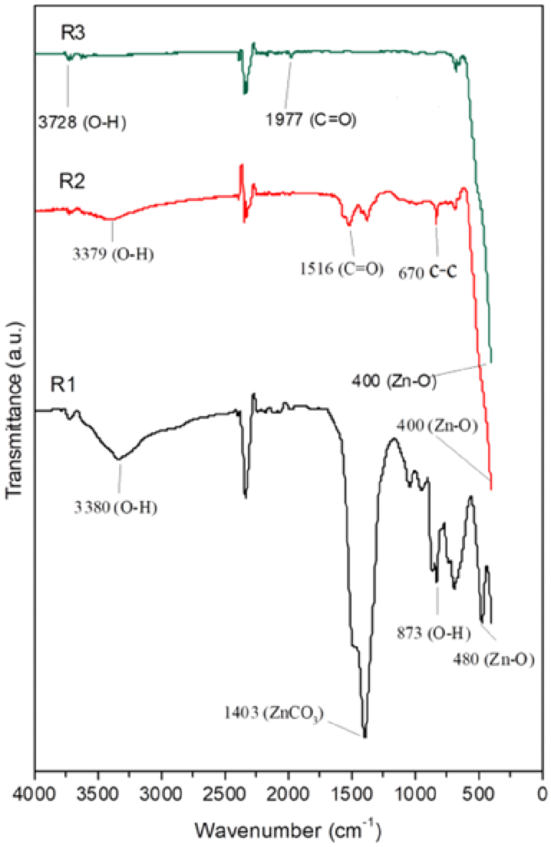

Figure 1: FTIR spectra of $ZnCO_3$ (R1) and ZnO NPs (R2 and R3).

Similarly, the X-ray diffraction (XRD) patterns of the synthesized $ZnCO_3$ NPs (R1) and ZnO NPs (R2 and R3) are shown in Figure 2. The sharp peaks show the synthesized particles are crystalline. The diffraction angles from the $ZnCO_3$ XRD pattern were observed at 2θ values of 25°, 32°, 33°, 36°, 39°, 43°, 54° and 67°. The result was matched with the Joint Committee on Powder Diffraction Standard (JCPDS) data for the correctness of the data obtained. The diffraction peaks were obtained at 2θ of 32°, 34°, 36°, 47°, 56°, 63°, 68° and 69° which can be indexed to (100), (002), (101), (102), (110), (103), (112) and (201) lattice planes respectively of ZnO crystals which are nearly matched with the JCPDS card No 36-1451. Further, there are no other abnormal peaks observed in the spectrum indicating the absence of other phases in the prepared samples. Moreover, the obtained XRD pattern further showed that the synthesized ZnO NPs possessed the FCC lattice structure (Janjal et al., 2013). Substituting the corresponding values on Scherrer’s equation on each of the peaks, the average crystallite diameter (D) of prepared $ZnCO_3$ NPs was calculated to be 9 nm. Similarly, the average crystallite size of the ZnO NPs without using and by using PVA as a capping agent was calculated to be 21 nm and 17 nm respectively. The smaller size of crystallite size of the prepared ZnO NPs with a capping agent (R3) compared to those without a capping agent (R2) may be due to the prevention of the agglomeration of the prepared NPs by the capping agent. Moreover, the obtained sizes of the NPs are comparable with the sizes mentioned in the literature (Singh et al., 2011; Janjal et al., 2013; Sundar & Prajapati, 2012; Saeed et al., 2015; Karthivelu et al., 2009).

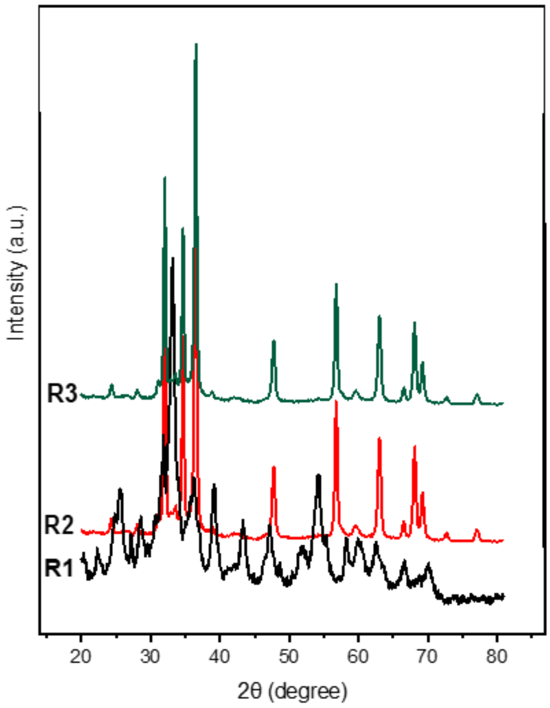

Figure 2: X-ray diffraction (XRD) pattern of $ZnCO_3$ (R1) and ZnO (R2 and R3) NPs.

Antimicrobial activities

Antibacterial properties: The antibacterial activity of $ZnCO_3$ and ZnO NPs was studied by agar well diffusion method against four bacterial species namely *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhii*, *Pseudomonas aeruginosa*. The zone of inhibition of the prepared compounds compared to the positive and negative controls is presented in Figure 3. The zone of inhibition by the NPs is observed to be lesser as compared to the positive control. Among all the pathogens, only *Staphylococcus aureus* was found to be inhibited by all the compounds while the rest of the bacteria were resistant. Further, the NPs prepared using a capping agent showed more inhibition on the bacteria, than the NPs synthesized without a capping agent this could be due to the smaller size of the prepared ZnO NPs. The observed results are summarized in Table 1.

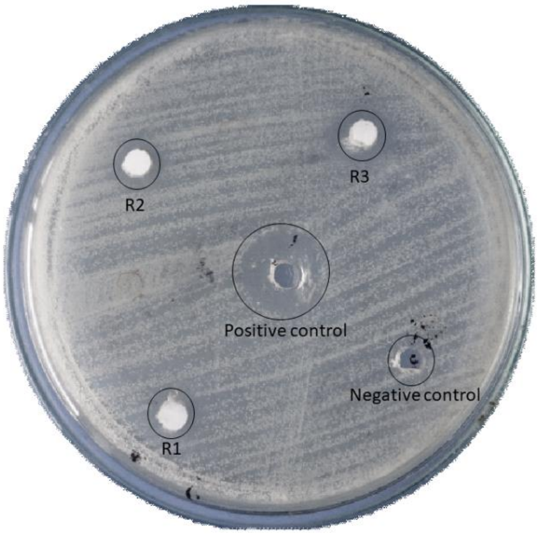

Figure 3: Antibacterial activity of the $ZnCO_3$ (R1) and ZnO (R2 and R3) NPs.

The antibacterial activities of the NPs have been attributed to different mechanisms such as (i) mechanical damage of the cell membrane by ZnO NPs and then leakage of the cell components which leads to the death of the microbes (ii) the generation of toxic reactive oxygen species (ROS) like OH^-, O_2^- and H_2O_2 from the ZnO NPs, with the particles penetrating the bacterial wall causing injuries and eventually the death of bacteria (iii) the release of Zn^{2+} ions, by the increase in H_2O_2 generation leading to cell membrane damage and then the death of the bacterial cells (Oprea et al., 2014; Weldegebrieal 2020; Yusof et al., 2019).

Table 1: Zone of inhibition of different NPs ($ZnCO_3$ and ZnO) against *Staphylococcus aureus*.

NPs	**Zone of Inhibition (mm)**		
	NPs	**Positive control**	**Negative control**
$ZnCO_3$ (R1)	10	15	0
ZnO (R2)	9	15	0
ZnO (R3)	11	15	0

Antifungal Properties: The antifungal activity of $ZnCO_3$ (R1) and ZnO (R3) NPs was studied by agar well diffusion method against 4 fungal strains (Figure 4). Among all the pathogens, only *Aspergillus flavus* was found to be inhibited by the compounds while the rest of the fungi were resistant, and the observed zone of inhibition results are summarized in Table 2.

Figure 4: Antifungal activity of the $ZnCO_3$ (R1) and ZnO (R3) NPs.

It is interesting to note that the inhibition by the NPs synthesized using a capping agent was observed against the fungal strains while the NPs prepared without a capping agent showed no antifungal behavior. The mechanism of antifungal activity by the NPs is rather different to the antibacterial mechanism. The fungal strains produce excess of nucleic acids and carbohydrates through the stress response, for self-protection reasons against the NPs, leading to cell death. The proteins, lipids and carbohydrates are released through the cell wall causing the death of the fungal species (Oprea et al., 2014).

Table 2: Zone of inhibition of different NPs ($ZnCO_3$ and ZnO) against *Aspergillus flavus*.

NPs	**Zone of Inhibition (mm)**		
	NPs	**Positive control**	**Negative control**
$ZnCO_3$ (R1)	12	28	0
ZnO (R2)	-	-	-
ZnO (R3)	10	28	0

Conclusion

$ZnCO_3$ NPs were synthesized successfully by a simple, cost-effective direct precipitation method whereas ZnO NPs were prepared from thermal decarbonation of the prepared $ZnCO_3$ NPs at 550°C in the absence and in presence of PVA as a capping agent. FTIR spectra peak at 480 and 1403 cm^{-1} is correlated to the formation of $ZnCO_3$ NPs. Similarly, the characteristic peak observed at about 400 cm^{-1} correlated to the zinc oxide bond (Zn-O). The XRD analysis give the formation of monophasic $ZnCO_3$ and ZnO NPs where the crystallite size of $ZnCO_3$ NPs was found to be 9 nm and for ZnO NPs 21 nm without capping agent and 17 nm by using the PVA as a capping agent. The synthesized NPs were found to be efficient towards the antimicrobial actions against bacteria and fungi. The antibacterial study with smaller-size ZnO NPs prepared using a capping agent is more effective against the bacteria than the NPs without using a capping agent.

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