RESEARCH PAPER

Chemistry

Volume : 4 | Issue : 6 | June 2014 | ISSN - 2249-555X

Evaluation of The Biological Activities of Ag Doped Bismuth Oxide Nanoparticles

KEYWORDS: Cleistanthus Collinus, Ag doped Bi_2O_3 nanoparticles, microbial activity, Scavenging activity.

Vennila Raj
Department of Chemistry, SRM University, Kattankulathur - 603203

*** P. Kamaraj**
Professor, Department of Chemistry, SRM University, Kattankulathur -603203. * Corresponding Author

M. Arthanareeswari
Professor, Department of Chemistry, SRM University, Kattankulathur - 603203

J. Deepika
School of BioEngineering , SRM University, Kattankulathur - 603203

ABSTRACT *The Ag doped Bi_2O_3 nanoparticles are prepared by using Cleistanthus Collinus plant methanolic extract. The Average grain size of Ag doped Bi_2O_3 is 23.98 nm. The Antimicrobial activity of Ag doped Bi_2O_3 is studied by using Gram-negative bacteria E.coli and fungi Aspergillus niger. Furthermore the antioxidant properties of Ag doped Bi_2O_3 have been studied by DPPH scavenging method.*

INTRODUCTION

A wide variety of nano materials possess some unique chemical, physical and electronic properties caused by nanoscale effect because of their small size compared to that of bulk materials. Ag nanoparticles are used in various fields like surface enhanced spectroscopy, chemical and biological sensing (Burda etal. 2005 And Haes 2004), disease diagnosis (Mieszawska etal), catalysis (Roucoux etal. 2002), microelectronics (Anderson etal. 2005, Maier etal. 2005 and Fichtner etal. 2005) and photochemical therapy of cancer (Thiruppathi etal 2010). These promising applications are due to the unique spectral response of Ag nanoparticles in the visible and infrared wave band. Bismuth oxide nanoparticles have been widely used in the fields of catalysis (Jinyi Deng etal. 2011), functional ceramics (Arda Aytimur etal. 2013), special glass and optical materials (Zhang etal. 2008), energy materials (Martirosyan etal.), medicine synthesis (Noori jassim etal. 2013), and semi conductor materials (Hoffman etal. 1995).The silver and bismuth oxides are prepared by using chemical methods like sol gel method(Anilkumar etal. 2005,), hydrothermal (Stotaw Talbachew Hayle etal. 2014 and Wildberger etal. 1998), Flame spray pyrolysis (Hiromichi Hayashi etal. 2010) and Co precipitation (Jha etal 2005) methods. These chemical synthesis methods are associated with the presence of some toxic chemicals adsorbed on the surface that may have adverse effect in the medical applications.

In recent years, plant mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and ecofriendliness. The use of environmentally benign living organisms such as bacteria (Fu etal 1999), fungi (Bhainsa etal. 2006), enzymes (Bankar etal. 2010), yeast and plant biomass like plant extract (Gnanadesigan etal. 2011, Mukunthan etal 2011 and Li etal 2007) are used for the biosynthesis of various metals and metal oxide nano particles.

In this study, the Ag and Bi_2O_3 nanoparticles are prepared by using Cleistanthus Collinus plant methanolic extract (vennila etal. 2013). This Cleistanthus Collinus plant contains lignan lactone glucosides like Cleistanthin A and Cleistanthin B. Genin (Diphyllin) is the major metabolite of these lignan lactone glycosides. The other lignin glycosidein in this plant is Collinusin. These are toxic aryl naphthalene lignin lactones. Cleistanthin A is used in Chinese medicine as sheng bai xin (Donald G. Barceloux etal. 1998). The Ag doped Bi_2O_3 nanoparticles were prepared in different composition of Ag and Bi_2O_3 by chemical method (Susha etal. 2012).The present work describes the study of Antioxidant and Antimicrobial activity of these nano particles.

MATERIALS AND METHODS

Ag doped Bi_2O_3 nanoparticles synthesized using Cleistanthus Collinus plant methanolic extract at room temperature have been subjected to characterization.

XRD

X-ray diffraction (XRD) patterns were obtained by using a Philips X'pert Diffractometer with Cu-Kα radiation source (λ=1.54Å). The diffractometer was operated at 30KV and 25mA and a scanning step of 0.05o in two theta and a dwell time of 1 second was used. Data was collected on a Bruker D8 Discover X - ray diffraction system. The diffraction patterns were collected in the 2 θ range 20°–70°. The width of the peaks, however, is relatively broad compared with the XRD pattern of Ag, Bi_2O_3 nanoparticles and Ag doped Bi_2O_3 nanoparticles, indicating a small crystal size. The average size $\langle D \rangle$ of the nanoparticles were computed using Scherer's formula (1),

$$D = 0.94\, \lambda / \beta \cos \theta \qquad (1)$$

where, β is the X ray wavelength (0.18 nm) and the full width half maximum of the diffraction peak (FWHM), respectively.

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDAX)

The morphology of the Ag doped Bi_2O_3 nanoparticles was observed by using a Hitachi S-4000 Field Emission SEM and a Hitachi S-900 SEM. Powder samples were glued onto a copper stub using carbon conductive tape. They were subsequently sputtered with gold to improve conductivity of the samples. The presence of the elemental Ag, Bismuth oxide and formation of Ag doped Bismuth oxide has been ensured by the EDAX analysis.

ANTIOXIDANT ACTIVITY

DPPH assay was carried out in two different ways using two different methods of mixing. The other parameters were same as described by Serpen et al (Serpen etal. 2007). In a typical process, 1.70 mL (100 μM) of DPPH was taken in a small glass bottle, and 10 mg of eight different Ag doped Bismuth oxide samples were dispersed in to it. DPPH was used as the radical source and Ag doped Bismuth oxide was used as radical scavenger. DPPH radical has a deep violet

Volume : 4 | Issue : 6 | June 2014 | ISSN - 2249-555X

color in solution, and gradually it becomes colorless or pale yellow in the presence of Ag doped Bismuth oxide nanoparticles. This property allows visual monitoring of the reaction, and the concentration of radicals is monitored from the change in percentage of absorption at 517 nm. The rate of the reaction was enhanced by vortexing the reaction mixture for 2 minutes at room temperature. The supernatant containing DPPH was collected for different time intervals. The time dependent DPPH scavenging was studied at an interval of 0, 15, 30 min. DPPH scavenging activity is calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = 1 - \frac{As}{Ac} \times 100$$

Where Ac and As are the intensity of peak at 517 nm for control (DPPH) and supernatant DPPH solvent respectively.

ANTIBACTERIAL ACTIVITY

Antimicrobial activities of the synthesized Ag doped Bismuth oxide nanoparticles were performed against both Gram-negative (E. coli). The antibacterial activity was done by modified Kirby-Bauer disk diffusion method. In brief, the pure cultures of organisms were subcultured in Muller-Hinton broth at 35°C ± 2°C on a rotary shaker at 160 rpm. For bacterial growth, a lawn of culture was prepared by spreading the 100 µL fresh culture having 106 colony-forming units (CFU)/mL of each test organism on nutrient agar plates with the help of a sterile glass-rod spreader. Plates were left standing for 10 minutes to let the culture get absorbed. Using a micropipette, 100 µL (25 and 50 µg) of the sample of nano particle suspension was absorbed in Whatmann filter paper discs placed on the nutrient agar plates for testing the nanomaterial antimicrobial activity. After overnight incubation at 35°C ± 2°C, the different levels of zone of inhibition were measured. Solvent blank was used as negative control. Antibiotic Streptomycin (100ug) was used as a positive control.

ANTI FUNGAL ACTIVITY

Antifungal activity of Silver doped bismuth oxide nanoparticles was studied using antifungal susceptibility test. PDB Broth and inoculate the culture. Then it is kept in a shaker for a day. The potato dextrose agar was weighed at 3.9gms and dissolved in 100ml distilled water and 1gm of agar was added. Then, the medium was kept for sterilization. After sterilization, the media was poured into sterile petriplates. This was allowed to solidify for twenty minutes. After solidification, the pure fungal culture was inoculated in the centre of the PDA plate in the form of discs and 25 and 50 µl of [sample concentration: 1000µg] each sample was placed in the disc. The plates were kept at room temperature. Then the antifungal activity was determined by measuring the diameter of zone of inhibition.

RESULTS AND DISCUSSION

Figure 1 shows XRD of Ag, Bi_2O_3 and Ag doped Bi_2O_3. These peaks are attributed to crystal planes (110), (200), (220), (311) for Ag, (110), (220), (321), (311), (102) for Bi_2O_3 nanoparticles and planes (311), (402), (501), (600), (503), (223), (811), (315), (723), (1021) for Ag doped Bi_2O_3. It shows the formation of Ag doped Bi_2O_3. These results confirm the formation of orthorhombic system and its primitive lattice (pdf Number-87-0866). Also the results show the uniform distribution of Ag and Bi_2O_3 nanoparticles. The average grain size of Ag nano particles was 158.11 nm and that of Bi_2O_3 was 45.29 nm. After doping, the 2θ values of planes in Ag doped bismuth oxide increased as the crystal size got decreased (Figure.1). FWHM of the peaks increased with increasing Bi_2O_3 concentration. The reason is that the crystalinity increased with increasing Bi_2O_3concentration from 33% to 80% in the Ag doped Tinoxide nanoparticles. Due to this, the micro strain (εL) and dislocation density (ρ) are increase with decrease in the particle size.

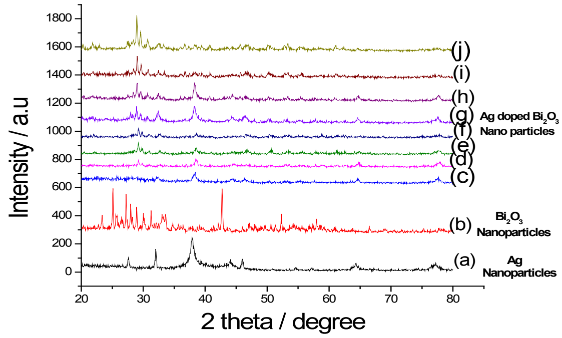


Figure 1: The XRD patterns of Ag Nanoparticles (b) Bi_2O_3 Nanoparticles, (c – j) Ag doped Bi_2O_3 nano particles -33.3% (c), 50% (d), 60% (e), 66.6%, 71.4% (g), 75% (h), 77.8% (i) and 80% (j).

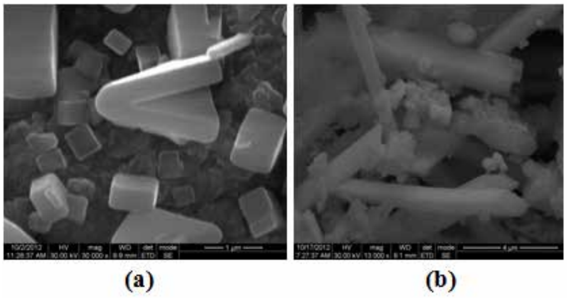
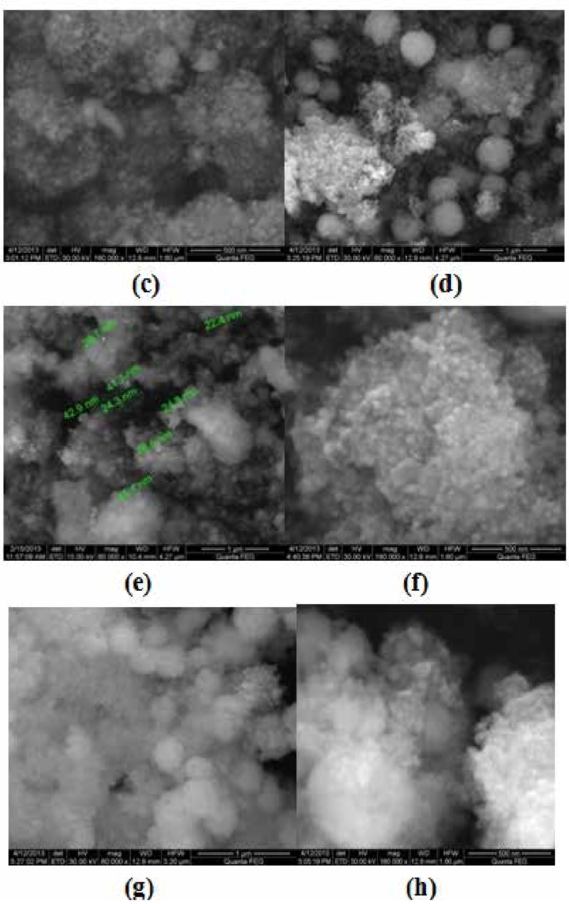
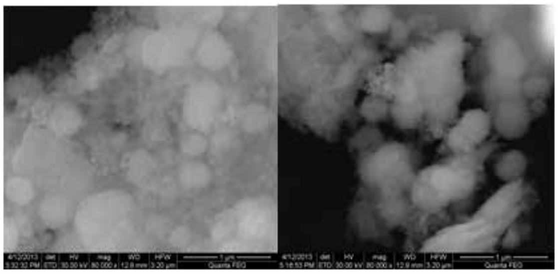


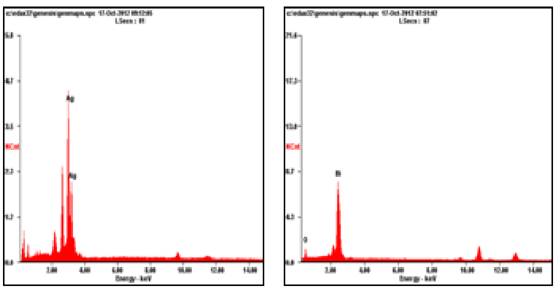
Figure 2: (a) The SEM for Ag Nanoparticles (b) The SEM for Bi_2O_3 Nanoparticles.



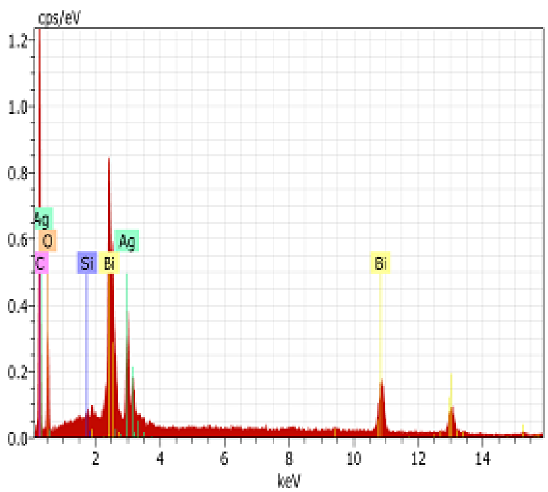


(i) (j)

(c – j) SEM images of Ag doped Bi2O3 nano composites -33.3% (c), 50% (d), 60% (e), 66.6%, 71.4% (g), 75% (h), 77.8% (i) and 80% (j).



(a) (b)



(c)

Figure 2: The EDAX for Ag (b). EDAX for Bi_2O_3 nanopartic (c). EDAX for Ag doped Bi_2O_3 nanoparticles

In EDAX results, the silver nanoparticles are represented by an optical absorption band peak at 3 keV and Bi_2O_3 nanoparticles at an optical absorption band peak at 2.5 keV. This represents the typical absorption of Ag and Bi_2O_3 nanoparticles .The SEM and EDAX results confirm the presence of noble Ag, Bi_2O_3 nanoparticles and Ag doped Bi_2O_3 nanoparticles (Figures.2-3). The average particle size of Ag doped Bi_2O_3 is 35.49.

DPPH SCAVENGING ACTIVITY

TABLE – 1

ANTIOXIDANT ACTIVITY OF Ag DOPED BISMUTH OXIDE NANOPARTICLES

Composition of Ag doped Bi2O3 (Ag: Bi2O3)	Anti oxidant activity		
	Initial	5 minutes	10 minutes
1:0.5 (33%)	30.4	34.7	38.3
1:1(50%)	30.9	40.0	42.9
1:1.5 (60%)	43.8	47.0	48.0
1:2 (66.6%)	48.9	52.2	54.3
1:2.5 (71%)	55.7	61.9	58.7
1:3 (75%)	70.4	72.4	71.3
1:3.5 (77.8%)	72.3	74.1	73.4
1:4 (80%)	74.6	78.1	77.2

DPPH scavenging activity of Ag doped Bismuth oxide nanoparticles increases with composition. The results of vortexed samples show an increase in the antioxidant potency of Ag doped Bismuth oxide nanoparticles. The vortexed reactions are in linear tendency which might be attributed to the increased chance of mixing of DPPH in methanol with nanoparticles. No DPPH scavenging was observed in the vortexed control DPPH solution. Compositions At 77.8% and 80% initially give better antioxidant activity and after vortex, the activity slowly decreases. This result clearly indicates the optimum level of antioxidant activity of Ag doped Bismuth oxide could be observed at 71.4% Composition.

TABLE – 2

ANTIMICROBIAL ACTIVITIES OF Ag DOPED BISMUTH OXIDE NANOPARTICLES

Composition of Ag doped Bi2O3 (Ag: Bi2O3)	Name of bacteria		Name of fungus	
	Escherichia coli (Zone of Inhibition) diameter in mm		Aspergillus niger (Zone of Inhibition) diameter in mm	
	25 µg	50 µg	25 µg	50 µg
1:0.5 (33%)	10	10.1	0.5	1
1:1(50%)	10	10.3	1.3	1.7
1:1.5 (60%)	10.5	11	2.8	2.9
1:2 (66.6%)	13	13.2	3.0	3.7
1:2.5 (71%)	13.4	13.6	3.5	3.8
1:3 (75%)	13.6	13.8	3.8	4.3
1:3.5 (77.8%)	14	14.4	3.9	4.6
1:4 (80%)	14	14.5	4.4	5.1

Increasing the concentration of bismuth oxide from 33.3% to 80% in Ag doped bismuth oxide, the antibacterial and anti fungal activity also increases. But the silver doped bismuth oxide showed significant antibacterial activity as well as anti fungal activity. Increasing the concentration of metal oxide from 33.3 % to 80 % the active oxide species concentration and antibacterial activity increases simultaneously. But these nanoparticles do not easily enter the fungal cell due to the presence of mitochondria in eukaryotic cell. The antimicrobial activity of Ag doped bismuth oxide is tabulated (Table 2).

CONCLUSION

The Ag and Bi2O3 nanoparticles successfully were green synthesized by using Cleistanthus Collinus plant methanolic extract. The antibacterial, antifungal and antioxidant activity of Ag doped Bi2O3 nanoparticles were evaluated. The formation of Ag doped Bi2O3 nanoparticles has been confirmed by XRD, SEM and EDAX methods. Ag doped Bi2O3 show an increasing DPPH free radical scavenging activity up to 71.4% and after that activity slowly decreases. The results confirm that the optimum concentration of Antioxidant activity could be 71.4% Ag doped Bi2O3. For Antimicrobial studies, increase in the concentration of Bi2O3 from 33.3% to 80% of Ag doped Bi2O3 nanoparticles, there is a significant decrease in bacterial and fungal growth. This implies the proficient antimicrobial activity of Ag doped Bi2O3 against Escherichia coli and Aspergillus niger.

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