Colloids and Surfaces B: Biointerfaces 113 (2014) 429–434

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Silver-doped manganese dioxide and trioxide nanoparticles inhibit both Gram positive and Gram negative pathogenic bacteria

R.K. Kunkalekar[a,*], M.S. Prabhu[b], M.M. Naik[c], A.V. Salker[a]

[a] Department of Chemistry, Goa University, Goa 403206, India
[b] Department of Biological Sciences, Birla Institute of Technology and Science Pilani, K. K. Birla Goa Campus, Zuarinagar, Goa 403726, India
[c] Department of Microbiology, Goa University, Goa 403206, India

ARTICLE INFO

Article history:
Received 1 July 2013
Received in revised form 2 September 2013
Accepted 18 September 2013
Available online 27 September 2013

Keywords:
Silver
Doped MnO_2
Doped Mn_2O_3
Antibacterial activity
Nanoparticles

ABSTRACT

Palladium, ruthenium and silver-doped MnO_2 and silver doped Mn_2O_3 nanoparticles were synthesized by simple co-precipitation technique. SEM–TEM analysis revealed the nano-size of these synthesized samples. XPS data illustrates that Mn is present in 4+ and 3+ oxidation states in MnO_2 and Mn_2O_3 respectively. Thermal analysis gave significant evidence for the phase changes with increasing temperature. Antibacterial activity of these synthesized nanoparticles on three Gram positive bacterial cultures (*Staphylococcus aureus* ATCC 6538, *Streptococcus epidermis* ATCC 12228, *Bacillus subtilis* ATCC 6633) and three Gram negative cultures (*Escherichia coli* ATCC 8739, *Salmonella abony* NCTC 6017 and *Klebsiella pneumoniae* ATCC 1003) was investigated using a disc diffusion method and live/dead assay. Only Ag-doped MnO_2 and Ag-doped Mn_2O_3 nanoparticles showed antibacterial property against all six-test bacteria but Ag-doped MnO_2 was found to be more effective than Ag-doped Mn_2O_3.

1. Introduction

The "golden age" of antibiotics is over, since misuse of antibiotics results in emergence of bacterial strains for which we have only one effective drug or in some cases none at all [1,2]. *Pseudomonas aeruginosa*, Methicillin resistant *Staphylococcus aureus*, *Mycobacterium tuberculosis*, vancomycin resistant *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Enterobacter* sp., *Acinetobacter* and *Escherichia coli* are often resistant to multiple antibiotics and consequently has joined the ranks of 'superbugs' due to their enormous capacity to engender antibiotic resistance [3–6]. Mobile genetic elements such as plasmid, integron and transposons often carry antibiotic resistance genes, thus their transfer results in immediate multidrug resistance in recipient strain [5,7,8]. The acquisition of mobile genetic elements has allowed certain pathogenic bacteria to thrive in hostile, antibiotic ridden environments.

Resistance due to antibiotic efflux, mediated by drug transport pump is an increasing problem worldwide. Efflux thus plays an important part in natural and acquired resistance of microorganisms to multiple antibiotics [9]. Efflux decreases the intracellular concentration of an antibiotic, thereby allowing bacterial survival [1]. Infectious bacteria now exist that are resistant to virtually all clinically used antibiotics, severely complicating treatment strategies. Vancomycin was considered as 'drug of last resort' in case of MRSA but emergence of vancomycin resistant *S. aureus* (VMSA) made this bacteria as 'superbug' and option to treat patients infected with VMSA are of very limited, leaving fever alternatives such as linezolid and quinupristin-dafopristin [6,10,11]. As resistance is observed to all classes of antibiotics, new arsenals for antibacterial therapy are desperately needed.

Silver is known to be a powerful, natural antimicrobial agent preventing serious infections. Antibacterial activity along with mechanism of action of silver nanoparticles on both Gram negative and Gram positive bacteria, including *E. coli*, *S. aureus*, *Bacillus subtilis*, *Streptococcus mutans*, and *Staphylococcus epidermidis* has already been investigated [12,13]. Recently due to their high antibacterial and catalytic activities, silver nanoparticles have attracted attention of researchers or medical microbiologists worldwide [14–16]. Nano-size and high surface-to-volume ratio of silver nanoparticles allows them to interact with microbial membranes. Ag nanoparticles (AgNP) have been shown to exhibit interesting catalytic, antibacterial and biosensing properties [14,16–19]. The antibacterial property of AgNP is currently exploited in many applications in a range of sectors, including medicine and dentistry [20–23]. Nanoparticles are capable of penetrating bacterial cells and act as a catalyst, to inactivate enzymes that microorganisms need for their metabolism by interacting with thiol groups of proteins, disrupt bacterial membranes and also affect DNA replication [16,24–26]. Silver in its free ionic form is highly toxic to human cells. It has been demonstrated that doping

* Corresponding author. Tel.: +91 9767649784; fax: +91 832 2452889.
E-mail address: kunkalekar.rohan@rediffmail.com (R.K. Kunkalekar).

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.09.036

of silver in metal oxide looses the toxicity of free silver to human cells and therefore it is advantageous to use silver doped metal oxide nanoparticles in the antibacterial study [12]. The doping of metal ions into the manganese oxide lattice can be useful, as manganese oxide can be easily prepare, it is environmental friendly, easy to handle and very economical. Also, these doped manganese oxides materials found to shows good catalytic activity [14,27].

In the present research work we report preparation of Pd, Ru and Ag-doped MnO_2 and Ag doped Mn_2O_3 nanoparticles. The prepared materials were characterized by using various instrumental techniques such as X-ray diffraction, scanning electron microscopy, transmission electron microscopy, BET surface area, X-ray photoelectron spectroscopy and thermal analysis. Antibacterial activity of these nanoparticles against both Gram positive and Gram negative bacterial strains has been investigated.

2. Experimental

2.1. Preparation of nanomaterials

Pd- and Ru-doped MnO_2 nanomaterials of composition $Mn_{1-X}A_XO_2$ (A = Pd and Ru, X = 0, 0.10) were prepared by dextrose aided co-precipitation technique [27]. All the chemicals used were of analytical grade. Appropriate amount of manganese acetate was dissolved in distilled water at room temperature, calculated amount of palladium or ruthenium chloride was taken in separate beaker to this little distilled water was added. In case of palladium chloride few millilitre of concentrated nitric acid was added and heated with stirring to dissolve completely. Both these solutions were mixed together at room temperature to get a clear homogeneous solution. This solution was added to 2% dextrose solution at 100 °C with stirring. The precipitation was carried out by adding drop by drop solution of sodium hydroxide (10%) to the above solution under vigorous stirring. Subsequently the suspension of precipitated metal hydroxide mixture (at pH = 9) was subjected to oxidation by drop wise addition of 30% H_2O_2 solution with constant stirring. The precipitate was then digested on a water bath for 3 h. The resulting precipitate was filtered, washed with distilled water and ethanol, and dried in air at 150 °C for 6 h. Finally the dried precipitate was homogenized well in mortar and calcined in air at 400 °C for 10 h.

Nano-sized Ag doped MnO_2 of composition $Mn_{1-X}Ag_XO_2$ (X = 0.10) were prepared by co-precipitation and drying method [14]. Stoichiometric amount of manganese acetate and silver nitrate were dissolved in distilled water at room temperature in separate beaker. Both these solutions were added to 200 ml distilled water at 100 °C with stirring. Liquid ammonia solution was added till pH = 9. Subsequently, the suspension was subjected to oxidation by dropwise addition of 30% H_2O_2 solution with constant stirring in order to adjust the appropriate oxidation state of metal ions. The suspension was then stirred continuously at 100 °C till dryness. The resulting product was dried at 150 °C for 6 h and homogenized well in mortar before calcination at 400 °C for 10 h in air.

Nano-sized Ag doped Mn_2O_3 of composition $Mn_{2-X}AgO_3$ (X = 0, 0.20) were prepared by starch assisted co-precipitation method. Calculated amount of manganese acetate and silver nitrate were dissolved in distilled water. Both these solutions were added to 2% starch solution at 100 °C with stirring. Liquid ammonia was added dropwise with stirring till pH = 9. Subsequently, the suspension was subjected to oxidation by dropwise addition of 30% H_2O_2 solution with constant stirring in order to adjust the oxidation state of metal ions. The suspension was then stirred continuously at 100 °C till dryness. The resulting product was dried at 150 °C for 6 h. Finally the dried precipitate was homogenized well in pestle mortar and calcined in air at 700 °C for 5 h.

2.2. Characterization of nanomaterials

The phase composition of the calcined samples was analyzed by X-ray diffraction (XRD) using RIGAKU Ultima IV diffractometer using Cu $K\alpha$ radiation (λ = 1.5418 Å) with 2θ scanning range 10–80°. Phase identification was made using the standard XRD reference database. The surface morphology was determined with JSM-5800LV scanning electron microscope (SEM) instrument operating at 20 kV. Transmission electron microscope (TEM) images were recorded on a PHILIPS CM 200 electron microscope operating with an accelerating voltage of 200 kV and providing a resolution of 2.4 Å. The BET surfaces area was measured by nitrogen adsorption at liquid Nitrogen temperature using a SMART SORB-91 surface area analyzer. The samples were regenerated at 200 °C for 2 h prior to the adsorption experiments. XPS (X-ray photoelectron spectroscopy) was performed with a VG Microtech Multilab ESCA 3000 spectrometer using a non-monochromatized MgKa X-ray source ($h\gamma$ = 1253.6 eV) at room temperature. Thermal analysis (TG/DTA) was carried out using a NETZCH STA 409 PC instrument in air at a heating rate of 10 K min^{-1} and heated from ambient to 1100 °C.

2.3. Antibacterial activity of the nanoparticles

Antibacterial activity of synthesized nanoparticles was performed by paper-disc diffusion method using Muller–Hinton agar on three Gram positive cultures (*S. aureus* ATCC 6538, *Streptococcus epidermis* ATCC 12228, *B. subtilis* ATCC 6633) and three Gram negative cultures (*E. coli* ATCC 8739, *Salmonella abony* NCTC 6017 and *K. pneumoniae* ATCC 1003). Test bacterial cultures were maintained as 20% glycerol stocks at −80 °C and were grown in Muller–Hinton broth (Himedia) at 37 °C to attain the OD value of ∼0.5 at 600 nm.

2.4. Preparation of antimicrobial paper-disc and antibacterial activity

5 mm diameter paper discs made of Whatman filter paper no. 41, were autoclaved at 121 °C for 15 min. On this paper disc each of the autoclaved nanoparticles were slowly applied in sterile conditions to get final concentration 100 μg/disc. These discs were then used to check the antibacterial activity of nanoparticles. Paper discs containing 100 μg/disc of each of the above synthesized nanoparticles were placed on surface of Muller–Hinton agar plate spread plated with 100 μl of above individual culture. The plates were incubated at 37 °C for 24 h and antimicrobial activity was checked as Inhibition Zone (IZ) around the disc. Compound was considered antimicrobial if the IZ was greater than 5 mm. The compounds showing the IZ were further checked for MIC (minimum inhibition concentration) on susceptible cultures. MIC assessed was described as the lowest concentration of the compound that visibly inhibited the colony growth. The MIC of nanoparticles was tested at concentration between 25 and 125 μg/disc and test was repeated thrice and results recorded as mean of the triplicate experiments.

2.5. Live/dead assay for Gram positive and Gram negative bacteria

Both Gram positive and Gram negative bacterial strains were grown overnight (to logarithmic phase) in Nutrient broth and then cells were harvested by centrifugation at 10,000 rpm at room temperature. Cell suspensions of all six test bacterial strains were prepared separately in 0.85% sterile physiological saline. Optical density (OD) of bacterial suspension was measured at 600 nm and dilution factor needed was then calculated and the dilution was carried out to obtain a bacterial count of 5×10^5 cfu/mL. Two millilitre of each bacterial suspension (5×10^5 cfu/mL) was then taken separately in sterile 10 ml Erlenmeyer flask and amended with 75 μg/ml