

Synthesis and Physicochemical Studies on Morphlinecarbamic Acid Capped Silver Nanoparticles

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ABSTRACT: A systematic and detailed study for antibacterial efficacy of silver nanoparticles (AgNPs) synthesized with a passivating monolayer of morphlinecarbamic acid using a chemical reduction approach is presented here. As synthesized silver nanoparticles were obtained with good yield, monodispersity, high stability and crystallinity. These AgNPs were characterized by elemental analysis and energy dispersive analysis of X-rays (EDAX). SEM images of powdered sample revealed spherical shape with particle size 100-200 nm. XRD patterns indicated arrangement of silver nanoparticles with a face centered cubic (fcc) structure. A surface plasmon resonance peak was observed at 430nm. As synthesized silver nanoparticles were tested for antimicrobial activity against different pathogenic microorganisms such as *Proteus vulgaris* (Gram negative), *Staphylococcus citrus* (Gram positive) and *Staphylococcus epidermidis* (Gram positive). Silver nanoparticles with ligand metal feed ratio (1×1) showed prominent inhibition in activity towards *S. Epidermidis* with maximum zone of inhibition value of 14 \pm 1.5 mm. Lipase activity of morphlinecarbamic acid capped silver nanoparticles showed reduction from 191.37 - 16.74 µmol/ml/min in activity with ligand metal feed ratio (1×1).

Keywords: Silver; nanoparticles; capping agent; Morphlinecarbamic Acid (MorphcbmH); FESEM; XRD; antimicrobial activity and lipase activity.

INTRODUCTION: Silver nanoparticles have attracted particularly considerable attention due to their innumerable diverse properties and uses, like magnetic and optical polarizability, electrical use due to the highest electrical and thermal conductivity among metals¹ catalysis, surface-enhanced Raman scattering (SERS), textile engineering, biotechnology and bioengineering,² water treatment,³ electronics,⁴ antibacterial/antifungal agents in a diverse range of consumer products: air sanitizer sprays, socks, pillows, slippers, respirators, wet wipes, detergents, soaps, shampoos, toothpastes, air filters, coatings of refrigerators, vacuum cleaners, washing machines, food storage containers, cellular phones⁵ etc. Due to the fluorescence and surface plasmon resonance characteristics of silver, it is continuously being used as sensor in DNA sequences, mass spectrometry of peptides, colorimetric determination of histidine and ammonia⁶ and most importantly, in the medicinal field as anti-microbial agents⁷ precisely exhibiting antibacterial proper-ties.^{8,9,10,11}

There are so many important applications of silver nanoparticles including spectrally selective coating for solar energy absorption and intercalation material for electrical batteries, as optical receptors, polarizing filters, catalysts in chemical reaction (selective oxidation of styrene)¹² and as antimicrobial agents. Nano form of silver is being incorporated into textiles and other materials to eliminate bacteria and odour from clothing, food packaging, disinfectants, household appliances and other items where antimicrobial properties are desirable. Bandages are being infused with silver nanoparticles to heal cuts faster. Silver's antibiotic properties have made the metal a popular treatment for wounds and burns. In recent years, there has been an extraordinary growth in nanoscience and technology due to development of new techniques to synthesize nanomaterials and the accessibility of tools for the classification and manipulation of nanoparticles. Fundamental understanding of nanoscale chemistry and physics is required for the production of nanoparticles as well as the knowledge to commercialize them is must.

The increasing impact of metallic nanoparticles in sciences has stimulated the development of new techniques and multiple improvements of the existing



methods of manufacturing nanoparticles with requisite properties. Nanoparticles can be synthesized through a variety of physical and chemical methods. The choice of preparation procedure will depend on the physical and chemical characteristics required for the final product, such as size, dispersion, chemical miscibility, optical properties, among others.

Chemical methods are usually used to synthesize silver nanoparticles (AgNPs) and among them chemical reduction is the most frequently applied method for the preparation of their stable, colloidal dispersions in water or organic solvent. Typical reducing agents include polyols, NaBH₄, N₂H₄, sodium citrate and N,N-dimethyformamide.^{13,14} In order to prevent aggregation AgNPs have been stabilized with capping agents such as sodium dodecyl sulphate (SDS), polypyrrolidone tri-sodium vinyl (pvp), citrate, alkanethiols, glutathione, thiolated polyethyleneglycol (PEG), p-mercaptophenol, aromaticalkylthiols, (phenylalkanethiol) and mercaptopropyl trimethoxysilane.

MATERIAL AND METHODS:

Chemicals: Ethyl alcohol (99.5%, Bengal chemicals), toluene, Morpholine, silver nitrate and dimethyl sulphoxide all from Merck, AR.

Preparation of Chemical Capping Agent/Ligand: Morpholine (1ml, 1000 μ l) was dissolved in hexane (3-5ml). To this continuously stirred solution dry carbon dioxide gas was passed for about five minutes. A white coloured solid separated immediately. It was filtered, washed with hexane and dried in air. Final drying was done by keeping the white colored solid overnight in a calcium chloride desiccator.

Preparation of Morpholinecarbamic Acid Complex of Ag (I): Morpholinecarbamic acid complex reported under present investigation has been prepared by addition of ethanolic solution of MorphcbmH to a continuously stirred ethanolic solution of AgNO₃ in 1:1 stoichiometric ratio. The black grey precipitates of the complex were washed two to three times with ethanol and dried in a CaCl₂ desiccator overnight.

Preparation of MorphcbmH capped silver nanoparticles: To a solution of silver nitrate (0.100g; 0.592 mmol) prepared in ethanol (2-3 ml) was added with stirring 10 ml of toluene. Ethanolic solution of freshly prepared carbamic acid MorphcbmH: 0.077g; 0.592 mmol, was added to it. Reaction mixture was stirred for 5 minutes and ethanolic solution of NaBH₄ (0.023g; 0.592 m mol) was added dropwise with continuous stirring when greyish black colored product was obtained which was stirred for another one hour for completion of reduction process. It was filtered, washed several times with ethanol and dried in CaCl₂ desiccator overnight.

Elemental analyses and physical measurements: Silver and sulphur were determined gravimetrically as silver oxide and barium sulphate respectively. Carbon and hydrogen analyses were performed on an automatic Coleman-33 analyser while nitrogen was analysed by Kjeldahl's method. Field Emission Scanning electron microscopy (FESEM) measurements of MorphcbmH capped silver nanoparticles were made on a Hitachi ultra – high resolution scanning electron microscope SU8010. Infrared spectra of ligand MorphcbmH and MorphcbmH capped silver nanoparticles were recorded as KBr pellets on Nicolet 5700 FT Infrared Spectrophotometer in the $4000 - 600 \text{ cm}^{-1}$ region. Powder XRD patterns for different samples were taken in θ range of 10°- 80° with a step size of $0.050 (2 \theta)$ with 1 sec counting time at each step using $Cu - K\alpha$ ($\lambda = 1.540$) radiation source and a diffractometer system [XPERT-PRO]. Electronic absorption spectra of AgNPs were recorded on Bio Chem UV spectrophotometer (range 900 - 200 nm). Cyclic voltammograms of methanolic solutions (10^{-3}) M) of AgNO₃, free ligand (MorphcbmH) and its silver(I) complex were recorded using Autolab Potentiostat 128N Electrochemical Analyzer with scanning potential range from -10 to +10 volts. Methanolic solutions of KCl/CTAB (10⁻¹M) were used as supporting electrolytes in the potential range of -1 to +1 V and -3 to +3 V at a scan rate of 100 mV s⁻¹.

Biological studies: Antimicrobial activity of MorphcbmH capped silver nanoparticles, i.e. [Ag n(MorphcbmH)]NO₃ was screened against three strains of microbes, viz. a) *Proteus vulgarius*, b) *Staphylococcus epidermidis* and c) *Staphylococcus aureus*.

The activity of lipase enzyme (μ mol/ml/min) in presence of compounds, i.e. MorphcbmH capped silver nanoparticles ((0.5×1), (1×1) and (1.5×1)) and [(Ag((MorphcbmH)](NO₃) was assayed by colorimetric method using chromogenic p-nitrophenyl palmitate (pNPP) as substrate and subsequent measurement of absorbance of p-nitrophenol released (A₄₁₀ nm). Different concentrations of test solution of compounds were used. Effect of incubation time for optimum concentration of compounds has also been studied. An



appropriate control (without addition of compound) was also used for each assay.

RESULTS AND DISCUSSION:

Morpholinecarbamate (MorphcbmH), a carbamate derivative has been used as a chemical capping agent for synthesis of silver nanoparticles. It has been obtained from a heterocyclic secondary amine,

morpholine (Morph). Great interest in chemistry of morpholine and its carbamate derivatives arises because of their potential pharmacological applications and biological activities. Morpholinecarbamic acid [MorphcbmH] ligand has been obtained from insertion of carbon dioxide into the N-H bond of the heterocyclic amine, i.e. morpholine in 1:1 stoichiometry (eq 1).



Preparation of Morpholinecarbamic Acid Complex of Ag (I): Synthesis of this complex follows the reaction (eq 2):

 $AgNO_3 + MorphcbmH \longrightarrow [Ag(MorphcbmH)]NO_3$ (eq 2)

Such type of silver(I) dimethylcarbamate has also been reported by 15 Alessio, et al.

Preparation of MorphcbmH capped silver nanoparticles:

AgNO₃ + nL + NaBH₄
$$\longrightarrow$$
 Ag(0).nL + NaNO₃ + $\frac{1}{2}$ B₂H₆ + $\frac{1}{2}$ H₂ (eq 3)

(L = MorphebmH; n = 0.5, 1 and 1)

By varying initial molar ratio of carbamic acid to AgNO₃ from 0.5:1, 1:1 and 1.5:1 three batches of carbamic acid capped AgNPs were prepared by reduction method using the procedure as detailed above and these were denoted as (0.5×1) , (1×1) and (1.5×1) respectively.

EDAX: EDAX analysis of silver nanoparticles provides evidence that sample contains silver (68.96%), oxygen (12.59%) and carbon (18.45%) all by weight percent and also confirms the presence of capping agent around silver nanoparticles. The data matches well to calculated percentage values of respective elements.



Figure 1: EDAX spectrum of MorphcbmH capped silver nanoparticles (1×1).







Figure 2: Particle size distribution (percentiles) of: a)[Ag(MorphcbmH)] (NO₃) complex and b) silver nanoparticles (1×0.5).

SEM: SEM micrographs of MorphcbmH capped silver nanoparticles $(1 \times 0.5; 1 \times 1; 1 \times 1.5)$ were recorded. Fig. 3 shows the SEM micrographs of AgNPs with 1×1 feed ratio and revealed that the nanoparticles are spherical in shape which are arranged in clusters. Micrographs exhibit uniform distribution of clusters. The range of particle size lies between 100-200 nm. From SEM micrograph it is observed that MorphcbmH is acting as a capping agent, resulting into formation of nanoparticles with definite shape.



Figure 3: SEM Micrographs of MorphcbmH capped silver nanoparticles (1×1) (a, b).

TEM: TEM images have revealed that particles are spherical in shape and are well monodispersed without any aggregation. It has been observed that core size of the prepared silver nanoparticles fall in the range 2.7 to 5.7 nm in diameter. Significant interactions between the nano-Ag and MorphcbmH molecules have been observed which indicates that MorphcbmH has been strongly chemisorbed onto silver surface as a capping agent. Stabilization of Ag¹⁶ and Au¹⁷ nanoparticles using sulphur bonding dithiocarbamate ligands as capping agent has earlier been reported by Shaowei Chen et al¹⁸. Average core diameter of [1×1] sample is 5.5nm.







Figure 4: TEM Micrographs of MorphcbmH capped silver nanoparticles (1×1).

IR Studies: Formation of zwitterionic form of MorphebmH ligand has been indicated by the observation of new FTIR absorptions due to v(O-H) at 3400 cm⁻¹ and v_a(CO₂) at 1655 cm⁻¹. The chemisorption of morpholinecarbamic acid on silver nanoparticles surface has been examined by FTIR spectroscopy. FTIR spectrum of MorphebmH capped silver nanoparticle shows strong v(O-H) absorption at 3400 cm⁻¹. Shift of this band to lower frequency as compared to free ligand (3427 cm⁻¹) indicates interaction of nitrate anion with proton of O-H in the Zwitterionic structure, thus weakening O-H bond. The v(C===N) has been observed at higher frequency, i.e. 1435 cm⁻¹ compared with that of free ligand.

XRD: XRD pattern of MorphcbmH capped silver nanoparticles exhibit four broad diffraction peaks confirming to the formation of silver nanoparticles. The samples denoted as $(.5\times1)$, (1×1) and (1.5×1) corresponding to MorphcbmH capped Ag nanoparticles showed four distinct XRD peaks at (111), (200), (220) and (311) corresponding to face centered cubic structure (fcc). The size of particles for different feed ratios of ligand to metal, i.e. (0.5×1) , (1×1) and (1.5×1) has been determined from Debye Scherrer's equation as 24.72 nm, 24.49 nm, 24.77 nm respectively. The XRD pattern of [Ag(MorphcbmH)](NO₃) complex exhibits several sharp diffraction peaks (Fig. 5(a-d)) which indicates its crystalline nature.





Figure 5: XRD Patterns of [Ag(MorphcbmH)] (NO₃) complex and MorphcbmH capped silver nanoparticles Ag nMorphcbmH where n=0.5,1,1.5 respectively (b, c & d).

SPR: Surface Plasmon Resonance of of (MorphcbmH) capped silver nanoparticles exhibit absorption peak at 426 nm (Fig. 6), which is characteristic of silver nanoparticles. The existence of peak at 426 nm shows good agreement with literature data reported for silver nanoparticles¹⁹. The localized surface plasmon absorption band depends heavily on size, shape, interparticle spacing, the dielectric con-

stant of the medium, and surface-adsorbed species^{20,21} (chemical surrounding).



Figure 6: Electronic absorption spectrum of MorphcbmH capped silver nanoparticles (1×1).

CV Studies: AgNO₃ exhibits a reversible cyclic voltammogram because of Ag(I)/Ag(0) couple with $E_{pc} = -1.264$ V, $E_{pa} = 1.547$ V, $\Delta E = 2.811$ V and $I_{pa}/I_{pc} = 1.5$ A. Voltammogram of free ligand MorphcbmH exhibits a reversible couple with $E_{pc} = 1.5$ V, $E_{pa} = 1.2$ V, $\Delta E = 0.30$ V and $I_{pa}/I_{pc} = 1.33$. The cyclic voltammogram of Ag n(MorphcbmH) exhibits $E_{pc} = -1.12$ V, $E_{pa} = -1.31$ V, $\Delta E = -0.19$ V and $I_{pa}/I_{pc} = 1.16$ A. While on the other hand, voltammogram of Ag(MorphcbmH)]NO₃ shows $E_{pc} = -0.60$ V, $E_{pa} = -0.68$ V, $\Delta E = -0.08$ V and $I_{pa}/I_{pc} = 2A$. The reduction potential of the couple Ag(I) / Ag(0) has been observed to be shifted towards higher value. This may be attributed to complexity of mechanistic electronic interactions during compound formation.

Biological studies:

Lipase Activity: Activity of lipase enzyme has been observed to be reduced by $(1 \times 1, 1 \times 1.5 \text{ and } 1 \times 0.5)$ AgNPs while negligible effect has been noticed for [Ag(MorphcbmH)]NO₃ compound. Colorimetric method used in present study gives the amount of hydrolyzed p-nitrophenol, which was proportional to the enzyme activity. The amount of hydrolyzed phenol in enzyme treatment with AgNPs was significantly lowered with increasing concentration of the test compound (MorphcbmH capped AgNPs). The nanoparticles with composition (1×1) gives the best results for inhibition of catalytic activity of lipase. The data for inhibition of activity lies between (191.37 -16.74, 159.86 - 53.28, 196.71 - 179.20 and 174.54 - 163.36 for 1×1 ; 1×1.5 ; 1×0.5 and [Ag(MorphcbmH)]NO₃ respectively for test samples. Effect of incubation time has also been studied for the sample with composition (1×1) . Activity of lipase enzyme has been observed to be lowered with increasing incubation time, viz. 5, 10, 20, 30 and 40 minutes.



Antimicrobial Activity: In our work we have investigated the application of silver nanoparticles as an antimicrobial agent and observed better antimicrobial activity against all three human pathogens Proteus vulgaris (P. vulgaris) (Gram negative), Staphylococcus citrus (S. citrus) (Gram positive) and Staphylococcus epidermidis (Gram positive). However, the antimicrobial effect depends upon the concentration of test chemicals. Silver nanoparticles showed good inhibition activity towards S. Epidermidis with maximum zone of inhibition value of 14.67 ± 1.5 mm at 40 µl. The mechanism of inhibitory action of AgNPs is not very well clear.^{22,23} However, several mechanisms have been proposed to explain the inhibitory effect of silver nanoparticles on microbes. The high affinity of silver towards sulfur and phosphorus was assumed to be the key element of the antimicrobial effect. Due to the abundance of sulphur containing proteins on the bacterial cell membrane, silver nanoparticles can bind with sulfur-containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. It was also suggested that silver ions (particularly Ag⁺) released from silver nanoparticles can interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication, or reacting with sulphur containing proteins, leading to the inhibition of enzyme functions which results in loss of cell viability and eventually resulting in cell death.^{24,25}

CONCLUSION: Stable silver nanoparticles (25-90 nm) have been synthesized using MorphcbmH as passivating agent. Powder XRD spectroscopy of feed ratios, i.e 0.5×1 , 1×1 and 1.5×1 revealed fcc arrangement of silver nanoparticles. Spherical shape of particles with size of 24-90 nm has been indicated on the basis of SEM study. As synthesized silver nanoparticles showed good antimicrobial activity against Proteus vulgaris (P. vulgaris) (Gram negative), Staphylococcus citrus (S. citrus) (Gram positive) and Staphylococcus epidermidis (Gram positive), however best results were obtained for Staphylococcus epidermidis (Gram positive) with maximum zone of inhibition of 14.67 ± 1.5 mm. Enzyme inhibition study of silver nanoparticles with different core diameters has exhibited reduction in lipase activity with decreasing ligand-metal feed ratio. Sample (1×1) showed maximum inhibition of lipase activity.

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