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Antibacterial Properties of Synthesized Ag and Ag@SiO₂ Core-Shell Nanoparticles: A Comparative Study

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Abstract: Spherical bare silver nanoparticles (AgNPs) and silica coated silver nanoparticles (Ag@SiO₂) have been prepared by one step chemical reduction method. The optical, structural and morphological studies were done by UV-Vis absorption spectroscopy, X-ray Diffraction (XRD) and Transmission Electron Microscopy (TEM). The antibacterial effects of AgNP and Ag@SiO₂ were further compared based on diameter of zone of inhibition and minimum inhibitory concentration (MIC) test against *B. subtilis* AST5-2, *S. aureus* ATCC 25923, *S. marcescens* AL2-16, and *K. pneumoniae* AWD5. Enhanced antibacterial activities were observed for Ag@SiO₂ core-shell NPs as compared to AgNPs against all tested bacteria. The results were attributed to the prolonged release of Ag (I) through porous silica shell that inhibits the growth of tested bacteria and also infers the possibility to be used in potential antibacterial applications.

Keywords: Antibacterial, enhanced, porous, silver, core-shell

1. Introduction

The infection by microorganisms such as bacteria, yeasts, viruses and molds are creating serious life and health problems to humans and other organisms [1]. Because of the regular emergence of drug-resistant bacteria, scientists and researchers are trying to develop cost-effective antimicrobial agents to make them affordable to all. In this regard, metals nanoparticles have attracted a lot of attention among the researchers due to their novel properties and

potential applications in biomedical fields [2]. Among them silver based nanoparticles are in extensive use due to their strong antimicrobial action towards a broad range of bacteria and low toxicity towards mammalian cells [2]. Advances in the synthesis techniques have also made it possible to synthesize other novel structures of nanomaterials such as core-shell, hollow core-shell etc. Core-shell or nano shell materials are broadly defined as nanostructure comprising a core (inner materials) and a shell (outer materials). Such materials are made with wide range of different combinations, inorganic/inorganic, inorganic/organic, organic/inorganic, organic/organic materials. The choice of different shell materials on the core particle depend on the type of applications. These are highly functional materials with the tailored properties, which make them unique among others. One of the special features that the core-shell has is the tunable core-shell ratio. By tuning the core-shell ratio, one can achieve various goals like enhancing luminescence properties [3], engineering band structure [3], using as drug carriers [4], and applications in light emitting device [5]. Out of various classes of core-shell materials, dielectric shell (silica) over metal core has become very popular because of their high thermal stability, low colloidal reactivity and, water solubility [6]. However antibacterial activity of silica coated metal nanoparticles has not been explored yet. Antibacterial properties of silver ions Ag (I) were well known since ancient times and are widely used as bactericide in various cases like in burn wound, dental work and catheters [7-8]. It is believed that Ag (I) rather than Ag (0) is the main active species responsible for the growth inhibition of bacteria, virus and fungi [9-10]. Ag (0) on the nanoparticles surface is oxidised to Ag (I) in presence of dissolved oxygen species which disturbs the biochemical processes of cellular enzymes, DNA and respiratory systems [9-10]. However indispensable oxidation process of these nanoparticles may cause low release of Ag(I) that results in low antibacterial efficiencies and also makes the nanoparticles unstable. So with the aim of increasing stability, we have synthesized silver and silica coated silver nanoparticles by simple chemical reduction method. The objective of this study was to compare the optical and antibacterial properties of synthesized silver and silver-silica core-shell nanoparticles. We believe that such comparative study would reveal a better understanding and would lead to better utilization of nanoparticles for specific applications.

2. Experimental section

2.1. Synthesis of Silver Nanoparticles (AgNPs)

0.425g of silver nitrate (AgNO_3) was dissolved in 50 ml deionized water to make 4mM solution. 25 ml of the prepared solution was then added to 100 ml deionized water. Another solution of tri-sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 3\text{H}_2\text{O}$) was prepared by dissolving 0.3g $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ in 30 ml water. Then 125 ml solution of AgNO_3 was kept under stirring and allowed to boil. The stirring was continued for 15 min and 5ml of the prepared $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 3\text{H}_2\text{O}$ solution was added as soon as boiling commence. The color of the solution changes to light yellow that confirms the formation of AgNPs.

2.2. Synthesis of Silica Coated Silver Core-Shell Nanoparticles ($\text{Ag}@\text{SiO}_2$ NPs)

5 ml of prepared AgNPs solution was added to the mixer of 10 ml deionized water and 20 ml ethanol. The resultant solution stirred at room temperature. After 10 min of stirring, 1 ml of DMA and 20 μl of TEOS solution were added drop wise. The color of the solution turns from light yellow to wine color in 20 min. The final solution was kept under continuous stirring for 2 hours so that active silica coating was done on silver core. The addition of DMA in the above synthesis has no significant role but acted as catalyst in the overall process [11].

2.3 Characterization of NPs

The morphology of the synthesized nanoparticles was studied by TEM (JEOL JEM 2100) operated at 200 kV. The samples for TEM analysis were prepared by placing a drop of homogeneous suspension on a copper grid film and allowing it to dry at room temperature. The crystalline phase and structure of the AgNPs and $\text{Ag}@\text{SiO}_2$ NPs were further analyzed by XRD spectrometer (Rigaku Corporation Japan/Model: Miniflex) by taking small amount of solution and drying it on a quartz plates. $\text{CuK}\alpha$ radiation ($\lambda=1.54056 \text{ \AA}$) was used as source x-ray. The optical properties of the NPs were characterized by UV-Vis (Perkin Elmer, Lambda 35) with a 1 cm quartz cell at room temperature. DLS measurements were performed using Delsa Nano. The measurement parameters are as follows: a laser (He-Ne) wavelength of 633 nm, a measurement temperature of 25.8°C , a medium viscosity of 0.8719 cP and a medium refractive index of 1.3372. Before the DLS measurement, the samples were equilibrated for 2 min and after that samples were loaded into quartz cuvette and measurements were performed. The concentrations of Ag (I) released in AgNPs and $\text{Ag}@\text{SiO}_2$ core-shell nanoparticles over period of time (0 to 120 h) were determined using Atomic absorption spectroscopy (AAS) techniques. To determine the concentrations of Ag (I), samples were firstly filtered with 0.22 μm filter membrane and centrifuged with ultra-filtration centrifuge (10000 r/min for 20 min) to remove nanoparticles from solution. Then the filtrate was acidified with HNO_3 and measured with AAS.

2.4. Microorganisms and growth conditions:

Luria Bertani (LB), and nutrient medium (NM) and constituents of growth media were purchased from HI Media. *B. subtilis* AST5-2, *S. aureus* ATCC 25923, *Serratia marcescens* (*S. marcescens*) AL2-16 and *K. pneumoniae* AWD5 are type strains, which were taken from departmental culture collection, while *B. subtilis* AST5-2 was isolated in an unrelated work, and identified by 16S rDNA sequence analysis (data not given). *S. marcescens* AL2-16, *B. subtilis* AST5-2 and *K. pneumoniae* AWD5 were isolated from soil. The bacterial strains were grown on LB or NM as per the requirements, and stored on nutrient agar (NA) slants at -20 °C. The cultures were revived on LB broth and sub-cultured after regular intervals.

The AgNPs and Ag@SiO₂ NPs were further purified by centrifugation at 10000 r/min for 20 min at 27 °C and a dried powder of the nanosized silver was obtained by freeze-drying as described by Abdelwahed *et al.* (2006) [12]. To remove the excess impurity, the silver pellet was washed three to four times with deionized water by centrifugation (10000 r/min for 20 min). The re-suspended aqueous homogenate of NPs were diluted for different trials. The original concentrations of stock solution, and dilution factors were considered for determination of concentrations of different type of AgNPs

3. Results and discussion

3.1. UV Visible Spectroscopy

Optical properties of the noble metals like Ag, Au, etc. depend on the morphology of the nanoparticles. When sizes of the nanoparticles become comparable with the wavelength of incoming electromagnetic wave, the electric field of the light induces a polarization with respect to the heavier ionic core of the nanoparticles which creates a dipole oscillation of the free electrons at resonant frequency of incoming light [13]. The phenomenon is known as surface plasmon resonance (SPR), which is the origin of different colors that appear. The absorption strongly depends upon the particles size, shapes and dielectric constant of surrounding medium [13]. As the size of the nanoparticles increases (>20 nm), the SPR gets red-shifted due to the retardation effects as well as contribution from multipolar terms (extrinsic size effect). For smaller sized nanoparticles (<20 nm) extrinsic size effect is negligible, while intrinsic size effect plays the significant role [13]. Here, the SPR peak of prepared AgNPs was found at 425 nm while for silica coated silver nanoparticles, the SPR peak shifts to 426 nm. The red shift observed is due to the coating of silica shell which increased the local refractive index of the surrounding medium.

$$\lambda = \lambda_p \left[\varepsilon^\infty + 2n_{EtOH}^2 + \frac{2g(n_{SiO_2}^2 - n_{EtOH}^2)}{3} \right]^{\frac{1}{2}} \quad (1)$$

$$\lambda_p = \left[\frac{4\pi^2 c^2 m_{eff} \varepsilon_0}{Ne^2} \right]^{\frac{1}{2}} \quad (2)$$

Theoretically, the peak positions of SPR can be obtained by using Equ (1) [14]. Where n , ε^∞ , g are the refractive index of the surrounding medium, high frequency of the core material, volume fraction of the shell material while λ is the calculated peak position of nanoparticles and λ_p is the bulk plasmon wavelength represented in equation (2).

Here m_{eff} and N are the effective mass and number density of core material (Ag). For Ag clusters having no medium, we expect λ_p to be at around 136.3 nm while AgNPs dispersed in water or ethanol medium λ is found at around ~420 nm. We performed similar calculations based on the above Equ (1) for silica coated silver nanoparticles dispersed in ethanol medium. By substituting the parameters $\lambda_p = 136.3\text{nm}$, $\varepsilon^\infty = 5.9$, $n_{EtOH} = 1.359$, $n_{SiO_2} = 1.4675$ (at $\lambda_{SiO_2} = 426\text{nm}$) [15], we get $\lambda = 427\text{ nm}$ for Ag@SiO₂ core-shell materials. The redshift in the plasmon absorption is observed due to increase in refractive index of the medium which is confirmed by theoretical analysis. Here the values of g for bare Ag and silica coated Ag nanoparticles are taken as 0 and 1 respectively [14].

Here in the synthesis process, the amount of TEOS determines the thickness of silica coating around silver core while DMA acts as a catalyst in overall process [11]. Usually, the homogenous coating of silica around chemically stable Ag core in presence of amine is a complicated task because the presence of amine group causes the aerial oxidation of Ag and forms complex ions $[\text{Ag}(\text{NH}_3)_2]^+$ that are soluble and color less. This complex compound adsorbs on the surface of Ag causing relative damping effect and makes particles unstable [11]. Our approach overcomes this difficulty of instability and also prevents the dissolution of silver core as excess use of DMA in presence of TEOS may results core free silica nanoparticles [11]. In our case of DMA addition, the damping and red shift were found very limited which gives an additional stability to Ag@SiO₂ core-shell materials for a long time in the solution. After synthesis, the absorbance of the prepared samples (AgNP and Ag@SiO₂) was taken after one hour and three months (Fig. 1). The SPR peak of AgNP was found to be red shifted (425 to 432 nm) while no change in SPR peak for Ag@SiO₂ was found. This signifies the stability of prepared core-shell materials.

3.2 XRD analysis

XRD patterns of Ag and Ag@SiO₂ core-shell NPs were shown in Fig. 2. The indices were found as (111), (200) and (220) with the corresponding $2\theta=38^\circ$, 45.02° and 62.65° for AgNPs while 38.17° , 45.22° and 62.84° for Ag@SiO₂ core-shell NPs respectively. The analysis suggests that the synthesized NPs are in face centred cubic (FCC) structure and the positions of peaks are in agreements with JCPDS data (File No. 03-0921 and 89-3722). Additionally, the intensity of (111) plane for both the Ag and Ag@SiO₂ core-shell NPs clearly indicates that the AgNPs are highly crystalline. After coating the silica shell, intensity of all the peaks decreases except (111), suggesting the cubic nature of AgNPs is preserved [9]. The matching of SAED (selected area diffraction pattern) and XRD results also confirmed the crystalline nature of silver (Fig. 2 and 3 c, f).

3.3 TEM and DLS analysis

Morphology of the prepared nanoparticles has been obtained by JEM-2100 Transmission electron microscope (TEM) operating at 200 kV. Samples for TEM analysis was prepared by placing a drop of the nanoparticles solution on carbon coated TEM grids. The obtained images confirm the presence of spherical shaped AgNPs and silica coated silver nanoparticles with nearly uniform size distributions. The averages size obtained by TEM and DLS analysis (Fig. 3 b, e) were found in good agreement. The average size of AgNP was found as ~14 nm (Fig. 3 a-b) whereas for core-shell nano particles, the average sizes was found as ~17 nm with shell thickness ~3 nm respectively (Fig. 3 d-e).

3.4 Antibacterial Study

Bacterial sensitivity to antibiotics is commonly tested using disk diffusion test, employing antibiotic impregnated disk [16]. A similar test with nanoparticles laden disks was used in this study. Layers of bacterial suspension (5 ml) were applied uniformly on the surface of nutrient agar plate. Immediately afterwards small disks of uniform size (5

mm diameter) containing nanoparticles of 706, 692, 673 and 642 $\mu\text{g/ml}$ designated as A1, A2, A3 and A4 were placed carefully on the plates and incubated at 37°C for 24 hours. Positions of A1, A2, A3 and A4 in all the plates are as shown in Fig. 4I. Afterword the zones of inhibition (ZOI) were measured by a standard ruler (table 1).

Table 1: Zone of inhibition (mm) against various microorganisms at different concentration ($\mu\text{g/ml}$) of AgNPs and Ag@SiO₂

Conc.($\mu\text{g/ml}$)	<i>B. subtilis</i>		<i>S. aureus</i>		<i>Serratia</i>		<i>K. pneumoniae</i>	
	AgNPs	Ag@SiO ₂	AgNPs	Ag@SiO ₂	AgNPs	Ag@SiO ₂	AgNPs	Ag@SiO ₂
A1=706	9.3	10.4	10.	14.7	6.6	8.8	8.3	14.4
A2=692	8.6	11.5	13	16.2	9	9	10	10.45
A3=673	11	11	13.	16	8.6	8.6	6	11.85
A4=642	7.5	12	12	16	7.2	9.7	6	12.5

For our study, two sets of plates S1 (Fig. 4 A, C, E and G) and S2 (B, D, F and H) were prepared in this way. S1 contained AgNPs of four different concentrations (A1, A2, A3 and A4) and set S2 contained Ag@SiO₂ NPs of same concentrations respectively. Each set is associated with different types of Gram-positive and Gram-negative bacteria.

Table 2: Percentage of different elements present in Ag@SiO₂ NPs obtained from EDX analysis.

Element	Weight %	Atomic %
O	8.72	30.09
Na	7.45	17.88
Al	0.65	1.33
Si	5.01	9.86

Cl	0.95	1.48
Ag	69.56	35.60
Cd	7.66	3.76

From EDX analysis, % wt of Ag and Si in Ag@SiO₂ nanocomposite was found as 69.56 and 5.01 %. Thus, the amount of Ag and Si was calculated and found as 491.09 and 35.37 µg/ml in 706 µg/ml Ag@SiO₂ nanocomposite. Similarly, for 692, 673 and 642 µg/ml of Ag@SiO₂ nanocomposite, the amount of Ag and Si were found as 481.35, 34.66 µg/ml, 468.13, 33.71 µg/ml and 446.57, 32.16 µg/ml respectively (table 2).

Here, the antibacterial activity of Ag and Ag@SiO₂ NPs was tested and compared against various microorganisms by measuring the diameter of zone inhibition by disk diffusion test. The strain susceptible to disinfectant exhibits the larger zone of the disk, while small zone reflects the resistant nature of microorganism. All plates in set S2 were found to be affected more than the corresponding plates in S1 against all tested bacteria. It was observed that for all four doses (A1 to A4), antibacterial activities of Ag@SiO₂ NPs were enhanced indicating the more antibacterial role than AgNPs. (MIC) test were employed to reveal the lowest concentration of prepared AgNP and Ag@SiO₂ NPs against various microorganisms. For MIC test, the experiment has been repeated eight times at various concentrations. And 197µg/ml for AgNP and 177µg/ml for Ag@SiO₂ was found the least concentration based on least absorbance values against tested bacteria. Though, the absorbance data differed, yet minimum inhibitory concentration remained 197µg/ml and 177µg/ml for AgNP and Ag@SiO₂ core-shell NPs respectively. MIC was further confirmed by sub-culturing from said tubes, with no apparent growth of respective bacteria. The MIC results suggest a non-specific antibacterial mechanism of both NPs, against different types of Gram-positive and Gram-negative bacteria. The lethal action of both nanoparticles (AgNP and Ag@SiO₂) is similar irrespective of types of bacteria.

Also, its relevant to mention that the antibacterial effects using nanoparticles were studied in CTAB free environment as it has been reported to provide positive charge to nano particles when used as stabilizing agent, which then interact with negatively charged cell wall of bacteria very strongly, and therefore may affect the data of bactericidal effect [17].

As observed from the AAS measurements, concentration of Ag (I) released from core-shell nanoparticles was more (99 $\mu\text{g/ml}$ for Ag@SiO_2) in comparison to AgNPs (92 $\mu\text{g/ml}$) after 100 h (Fig. 5). Generally, the MIC of Ag (I) is typically found in the range of 5–40 $\mu\text{g/ml}$ depending on the growth media [18]. Thus, herein the main contributor to the bacterial killing in the case of AgNPs and core-shell NPs is Ag (I), though the particles effect for bactericidal activity cannot be negligible.

Recently, the antibacterial mechanisms of Ag-based NPs have focused on the effects on Ag (I) more as compared to AgNPs. Xiu *et al.* 2012 demonstrated that released silver ions are the definite factor for antibacterial action rather than particle effect. Tan *et al.* 2016 demonstrated the enhanced antibacterial action of AgCl@SiO_2 NPs as compared to AgCl NPs. The enhanced antibacterial activities were attributed to the prolonged release of Ag (I) through porous silica shell. They argued that the solution first diffuses into the porous channel of the silica shell and contacts the surface of nanoparticles (AgNPs) to form electrophoretic mobility. As a result, silver ions released in the bacterial suspension through the pore channels and caused antibacterial action. Moreover, parts the free Ag (I) present in the solution attaches to the cell surface due to electrostatic attraction of silanol and concentrates on the surface of Ag@SiO_2 NPs, which enhances the antibacterial performances of Ag@SiO_2 [9]. Previously, the antibacterial action of Ag-based NPs based on dissolved Ag (I) had been reported [9, 10, 19]. In this work, our results showed that compared to AgNP, Ag (I)-supporting SiO_2 has a significant increase in antibacterial activity. Moreover, Ag@SiO_2 NPs exhibited a better long acting antibacterial performance than that of AgNPs.

4. Conclusion

In this work, spherical AgNP and Ag@SiO_2 core-shell NPs were successfully prepared and their structure was analyzed by UV-Vis, XRD and TEM techniques. The enhanced antibacterial effects of Ag@SiO_2 core-shell NPs were observed and attributed to the release of silver ions through the porous silica shell and the silver ions attached on the surface of NPs. In conclusion, we can say that our work integrates nanotechnology and bacteriology leading to possible new bactericides for long lasting period. However, a detailed study is needed to evaluate the exact mechanism of antibacterial action of silica coated metal NPs.

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Figures with captions

Fig. (1). UV-Vis spectra of (a) AgNP and (b) Ag@SiO₂ core-shell NPs taken after one hour and three months duration.

Fig. (2). XRD pattern of Ag and Ag@SiO₂ core-shell nanoparticles.

Fig. (3). TEM images, size distributions and SAED pattern of (a, b, c) AgNPs and (d, e, f) Ag@SiO₂ core-shell nanoparticles.

Fig. (4). Representative Images of disk diffusion test of Ag and Ag@SiO₂ nanoparticles at different concentrations (A1, A2, A3 and A4) against *Bacillus* (A, B), *Staphylococcus* (C, D), *Serratia* (E, F) and *Klebsiella* (G, H).

Fig. (5). Release kinetics of Ag (I) from AgNPs and Ag@SiO₂ core-shell NPs.

Fig. (1).

Fig. (2)

Fig. (3)

Fig. (4)

Fig. (5)