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SILVER OXIDE NANOPARTICLES: AN EFFICIENT ANTIBACTERIAL AGENT SYNTHESIZED BY ELECTROCHEMICAL REDUCTION METHOD

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ABSTRACT

Silver oxide nanoparticles were prepared by electrochemical reduction method which is environmental bening. The tetra butyl ammonium bromide (TBAB) used as stabilizing agent in an organic medium by optimizing current density. The synthesized silver oxide nanoparticles were characterized by using UV-Visible, FT-IR, XRD, EDS and TEM analysis techniques. The nanoparticles were tested for antibacterial activity against human pathogens like gram negative *Escherichia coli (E.coli),Salmonella typhi*and gram positive *Staphylococcus aureus, Bacillus subtilis*strains and which was proved to be excellent.

Keywords: Electrochemical cell, Tetra butyl ammonium bromide, Silver oxide nanoparticles, Human pathogens, Antibacterial activity.

INTRODUCTION

In recent years, researchers in the field of nanotechnology takes a lot of effort in order to provide new and improved biomaterials with specific applications in medicine [1,2]. The continuing appearance of antibiotic resistance in microorganisms challenges the compounds and drug targets with high biocompatibility and antibacterial properties [3,5]. Scientists are looking towards developing new bioactive compounds with silver at a nanometric scale because silver is a nontoxic, safe inorganic antibacterial agent used for centuries and is capable of killing about 650 types of diseases causing microorganisms [6]. Silver has been described as being oligodynamic because of its ability to exert a bactericidal effect at minute concentrations [7]. It has a significant potential for a wide range of biological applications such as antifungal agent, antibacterial agent for antibiotic resistant bacteria, preventing infections, healing wounds and anti-inflammatory [8]. The effect of nanoparticles was found to be significantly pronounced on both gram positive as well as gram negative strains. Nowadays, there is a worldwide concern due to social problem caused by harmful microorganisms such as legionella infections, hospital infections due to medicine resistant bacteria such as MRSA (contd.) [9]. We attribute this enhanced antibacterial effect of the nanoparticles to their stability in the medium as a colloid which modulates the phosphotyrosine profile of the bacterial proteins and arrests bacterial growth.

In the present work, silver nanoparticles have been synthesized by the electrochemical method. Interesting specifications of this technique are simplicity, control on particle size and purity of yield. The big challenge in the preparation of nanoparticles is their agglomeration, to prevent this problem various types of stabilizers have been used such as long chain fatty acids (stearic, palmitic and lauric acids) [10], polyvinyl pyrrolidone [11], soluble starch [12], gelatin [13] etc. for current study we used TBAB as stabilizer which also serve as supporting electrolyte. Antibacterial studies were carried out on both antibiotic resistant and non-resistant strains of gram negative and non-resistant strain of gram-positive bacteria. Efforts have been made to understand the underlying molecular mechanism of such antibacterial actions. The effect of the nanoparticles was found to be significantly pronounced on both gram positive as well as gram negative strains.

Experimental methods

Materials

All chemicals (up to 98.99% purity) are purchased from Aldrich and Rankem chemical suppliers and used as received. *Bacillus subtilis* (ATCC No. 6633), *Staphylococcus aureus* (ATCC No. 25923) and *Escherichia coli* (ATCC No. 25922), *Salmonella typhi* (ATCC No. 23564) provided by Govt. Institute of Science, Aurangabad 431004. They were clinical isolates and are used as international reference standards for disc susceptibility assessment of many antibiotics. Antibiotics Ampicillin were subjected to analysis. Nutrient agar was used for growth and maintainers of bacterial strains.

Synthesis of silver oxide nanoparticles

The synthesis of silver oxide nanoparticles by electrochemical reduction method originally reported by Reetz et al [14], for narrow size distributed metal nanoparticles. Cluster size was found to decrease with increase in current density [15]. The process makes use of an inexpensive two electrode setup for 30 ml electrolyte solutions in which sacrificial anode consists of the bulk metal to be transformed into metal clusters. The supporting electrolyte consists of TBAB which also serve as stabilizers for the metal clusters. Thus in the overall process the bulk metal is oxidized at the anode, the metal cations migrate to the cathode and reduction takes place with formation of metal or metal oxide in the zero oxidation state. Agglomeration with formation of undesired metal powders is prevented by the presence of the ammonium stabilizers.

In the initial experiment we have used a silver sheet (1x1 cm) were used as anode and a platinum sheet (1x1cm) as the cathode. The two electrodes were kept 1 cm apart. Tetra butyl ammonium bromide (0.01 M) in acetonitrile / tetrahydrofuran (4:1) served as the supporting electrolyte. Upon applying current density of 10 mA/cm^2 we obtained > 95% of silver oxide clusters stabilized by TBAB. To remove dissolved oxygen from the electrolytic bath N_2 was bubbled for several hrs. before synthesis. Electrolysis was carried out in nitrogen atmosphere. The residual oxygen in the bath oxidizes silver to silver oxide. The reaction proceeds in the following way. On application of electric current, the anode slowly dissolves leading to formation and subsequently get passivated by active TBAB species.

The clusters size was found to decrease with increase in current density. Since the material is insoluble in the solvent mixture used, the work up turned out to be simple decantation and drying in vacuum and were stored as closed glass vials under ambient conditions for future experiments.

Characterization of silver oxide nanoparticles

The prepared silver oxide nanoparticles were characterized by UV-Visible spectrophotometer, FT-IR spectrophotometer, XRD, TEM and EDS techniques. The UV-Visible spectrophotometer [Jasco 503] using a quartz cuvette with acetonitrile/tetrahydrofuran as reference. The IR spectra were recorded on FT-IR spectrophotometer [Jasco, FT-IR/4100] Japan. Using dry KBr as standard reference in the range of 400-4000cm^{-1}. The X-ray powder diffraction patterns of the silver oxide nanoparticles were recorded on Bruker 8D advance X-ray diffractometer using CuKα radiation of wavelength = 1.54056 Å. The presence and elemental composition in AgO nanoparticles were examined using energy dispersive spectrophotometer (EDS) were carried out with JEOL; JSM- 6330 LA operated at 20.0kV and 1.0000 nA. HRTEM analysis was carried out to study the surface morphology and size of Ago Nanoparticles with Tecnai $20G^2$ operated at 200kV.

Antibacterial activity experiment

The antibacterial activity of silver nanoparticles was screened *in vitro* against gram positive and gram negative human pathogens using ampicillin as standard.

Types of Bacteria

- Gram (+ve) bacteria–*Bacillus subtilis* (ATCC No. 6633), *Staphylococcus aureus* (ATCC No. 25923).
- Gram (-ve) bacteria–*Escherichia coli* (ATCC No. 25922), *Salmonella typhi* (ATCC No. 23564).

Materials

- Nutrient agar (12-15 mL)
- Sterile petri dishes
- Old grown culture (24 hours) in test tube
- Sterile pipettes
- Test tube containing solution of the compound to be tested with known concentration in ACN:THF

Preparation of Sub–Culture

Antibacterial activities were studied by the paper disc plate method [16]. A uniform suspension of test organism of 24 hours old culture was prepared in test tube containing sterile saline solution. A sterile nutrient agar was then added in each of the petri dishes. The dishes were related to ensure the uniform mixing of the microorganism in the agar medium which was then allowed to solidify. The agar cups were prepared with sterile cork borer bearing suitable dimension. The solution of silver oxide nanoparticles to be tested for aseptically into each cup. The ACN: THF was used a control of the solvent incubated at 37°C for 24 hours. The concentration of the silver oxide nanoparticles in ACN: THF was 25 and 50µg ml^{-1}. Ampicillin was used as a standard compound for comparison. After incubation the inhibitory zones around the agar cups were observed. The diameter of inhibition zones were measured in terms of mm.

Zones of Inhibition

- Strong growth inhibitor (zone size 15- 20mm)
- Moderate growth inhibitor (Zone size 9-14mm)
- Less growth inhibitor (Zones size 6-8 mm)
- No growth inhibitor

Keeping above mentioned points in view, the screening of the metal nanoparticles for antimicrobial activity has been performed.

RESULT AND DISCUSSION

The electrochemical synthesis of Ag nanoparticles capped with TBAB at 10 mA/cm^2 current density has been carried out in the present study. As the electrochemical reduction proceeds the change in colour of the reaction mixture with time is monitored. As current density increases its colors change from yellow to orange. It is seen that between 15 to 20 minutes a change in colour golden brown usually took place, which is indicative of the formation of silver nanoparticles [17]. Fig.1 shows the formation of yellow to orange colored silver nanoparticles with 2, 4, 6, 8, 10 and 12mA/cm^2 respectively.

Fig. 1: Yellow to orange colored silver colloidal nanoparticles

The optical properties of AgNPs were calculated by UV-Visible spectrophotometer, an important and most commonly used technique, to ascertain the formation stability of metal nanoparticles. Due to surface plasmon resonance (SPR), a strong absorption of electromagnetic waves is exhibited by metal nanoparticles in the visible range. The UV-Visible extinction spectra, such as the shifting, intensity and full width at half maxima (FWHM) of absorption peaks, have proven to be quite sensitive to the shape, size and size distribution of silver nanoparticles [18,19].

In the spectrum of silver nanoparticles Fig. 2 a single Surface Plasmon Resonance band (SPR) is observed. According to Mie's theory [20] only a single SPR band is expected in the absorption spectra of spherical metal nanoparticles, whereas anisotropic particles could give rise to two or more SPR bands depending on the shape of the particles [21]. It is also reported that the absorption spectrum of spherical silver nanoparticles present a maximum between 420 to 450nm with a blue shift when particle size diminishes or increases, respectively [22-25]. In our case, a single SPR band is observed which predicts that synthesized Ag nanoparticles particles are spherical in shape. Thus in the present study the colloidal silver nanoparticles prepared at a current density of 10 mA/cm^2 show the surface plasmon band on 413 nm. These results have been well correlated to the reports and can thus be said to be confirmed.

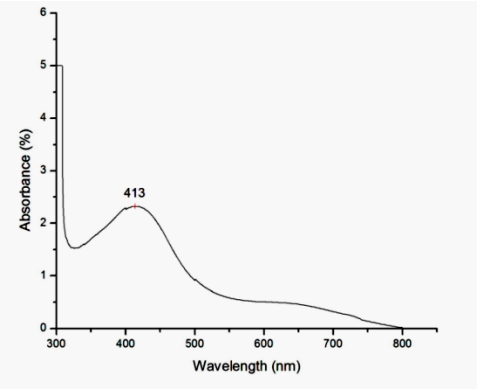

Fig. 2: Optical absorption spectrum for silver oxide nanoparticles.

Figure 3 shows the FTIR analysis results of the silver nanoparticles capped with TBAB at a current density of 10 mA/cm^2. For the sample the spectra show frequencies at 2978cm^{-1} and 2878cm^{-1} correspond to the C-H stretching vibrations. The peak at 1766cm^{-1} corresponds to the symmetrical ammonium ion (NR_4). The frequency corresponding to 1475cm^{-1} indicates the C-H bending, while the C-N linkage in the N^+R_4 ion gives a band at 1402cm^{-1}. The peak at 1172cm^{-}

[1] is due to the C-N stretching vibrations. The appearance of these frequencies in the IR spectra of silver nanoparticles confirms the stabilization is achieved by the TBAB. The spectrum also contains distinct peaks at 917cm^{-1}, 843cm^{-1}, 832cm^{-1} and 742cm^{-1} which correspond to the mixed phase that contains silver and silver oxide.

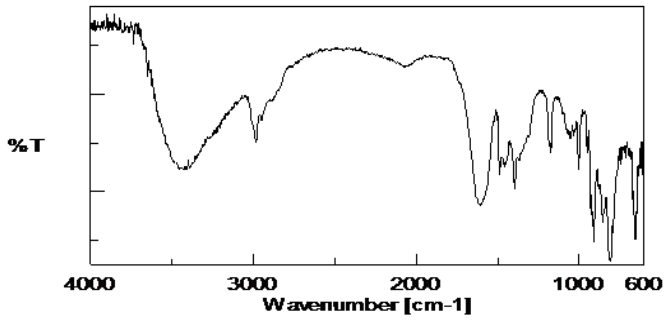

Fig. 3: FTIR spectrum of Ag NPs.

The XRD pattern of dried silver nanoparticles is shown in Fig. 4. The observed 2θ values and the inter planar spacing for the samples were compared with the ASTM data. The comparison indicates the presence of a mixed phase of silver and silver oxide with cubic structure. The presence of silver oxide in the sample may have arisen due to the possible oxidation of silver during drying, storing and analysis.

A formation of an ultrathin layer of silver oxide on the surface of the silver nanoparticle has also been reported [26]. These results indicate the presence of a mixed phase containing both silver and silver oxide in the samples, irrespective of the capping agent and the current densities used for synthesis. These are confirmed by comparison with the reports [27-30].

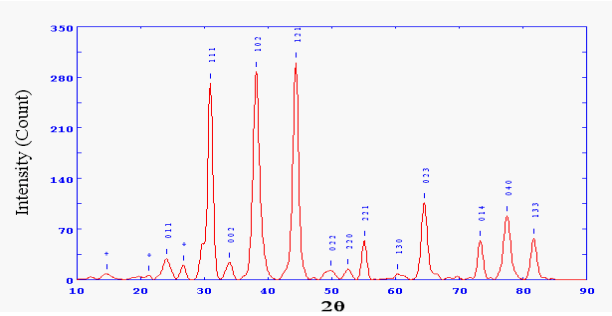

Fig. 4: XRD pattern for silver nanoparticles

Table 4.1: XRD analysis data for silver oxide nanoparticles

h	k	l	2θ(°) (Exp)	2θ(°) (Cal)	d(A°) (Exp)	d(A°) (Cal)	Intensity (Exp)
1	1	0	30.924	30.925	5.3627	5.3727	42.89
1	1	1	38.125	38.116	4.6636	4.6636	138.96
2	0	0	44.324	44.301	4.0626	4.0568	523.78
2	2	0	64.497	64.447	3.4345	3.4367	57.34
2	2	1	69.701	68.883	3.0243	3.0249	243.30
3	1	0	73.239	73.190	2.7738	2.7748	61.67
3	1	1	77.496	77.402	2.3529	2.3531	23.43
2	2	2	81.523	81.545	2.0472	2.0475	37.17

The lattice parameter observed a=4.086, b=4.086, c=4.086 at alpha=90º, beta=90º and gama=90º. Sharp peaks were obtained at corresponding to the planes (110), (111), (200) and (220) indicates the cubic structure of silver oxide nanoparticles and which was found to be highly crystalline in nature. The data obtained was matched with the database of Joint Committee on Powder Diffraction Standards (JCPDS) ASTM card no. 04-0783. Bragg reflections are weak and broadened relative to the intense (111) reflection. This feature indicates that the nanocrystals are (111) oriented [31]. The average particle size of silver nanoparticles was calculated by the use of full width at half maximum (FWHM) of cubic (111) using the Debye-Scherrer equation, Kλ/βcosθ, where K is the Scherrer constant with value from 0.9 to 1, λ is the wavelength of the X-ray, β is the full width at half maximum and θ is the Bragg angle in radians. From the Scherrer equation the average particle size of silver nanoparticles was

7.5 nm to 12.4 nm.

Energy dispersive spectra of silver nanoparticles in Fig. 5shows that silver, oxygen and bromine are present in the sample and its composition is given in Table 5.1. According to the atomic % of elements, it clearly shows the formation of silver oxide nanoparticles. Bromine is present in very less amount because of the addition of TBAB.

The HR-Transmission electron microgram and the particle size distribution for the sample of silver nanoparticles capped with TBAB at current density of 10 mA/cm^2 was obtained for various resolutions. Fig. 6 which shows spherical, well separated monodispersed silver nanoparticles. The average particle size is 5 to 20 nm in range. The inset fig. shows SAD pattern for silver nanoparticles which shows the cubic structure of silver oxide nanoparticles. These results are in accordance with the shape of the SPR band and with the particle size calculated from XRD analysis.

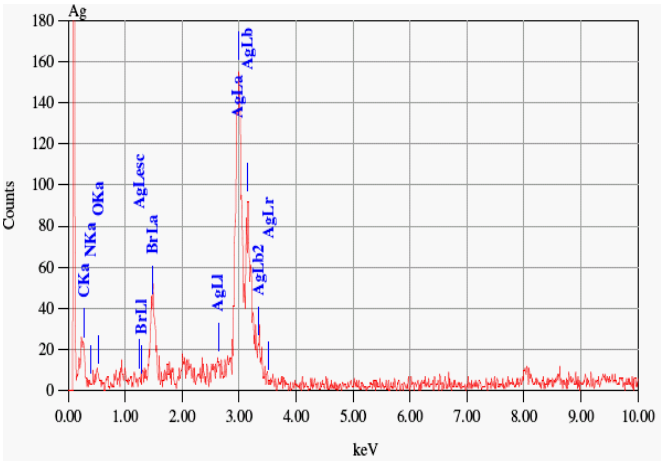

Fig. 5EDS spectrum of Ag NPs

Table 5.1: EDS analysis of Ag nanoparticles

Constituents	Mass (%)	Atom (%)
Ag	72.38	49.88
O	14.94	32.38
Br	1.05	0.46

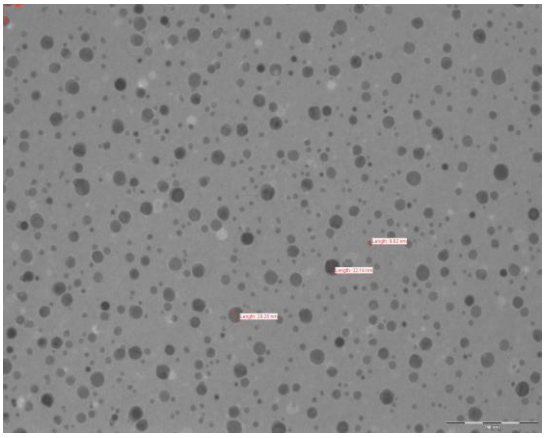

Fig. 6: HR-TEM micrograph of silver oxide NPs.

The size dependent bactericidal activity of silver nanoparticles against gram negative bacteria has already been reported [32-33] literature reports reveal the bactericidal activity of silver nanoparticles of either a simple or composite nature [34-35]. Metal atoms in nano size provide a significantly large surface area in contact with the bacterial effluent. Bacterial cell size usually ranges in micron range. These cells have cellular membranes which contains pores in nanometer range. The nanoparticles which were synthesized have a size less than that of pore size in the bacteria and thus they have a unique property of crossing the cell membrane without any hindrance. Such a large contact surface is expected to enhance the extent of bacterial elimination. Table 1. shows antibacterial activity of silver nanoparticles against human pathogens. The results were revealed that silver oxide nanoparticles acted as excellent antibacterial agents against both gram positive as well as gram negative bacteria.

Table 1: *In vitro* antibacterial screening of synthesized silver oxide NPs.

Human Pathogenic Bacteria	Silver oxide nanoparticles		AN + THF (4:1)		Ampicillin	
	50 µl	100 µl	50 µl	100 µl	50µl	100 µl
Zone of Inhibition (mm)						
B. subtilis	17	21	00	00	11	15
Staphylococcus aureus	17	19	00	00	14	16
E. coli	21	24	00	00	19	23
S. typhi	19	21	00	00	14	17

Fig. 7 shows the presence of antibacterial effect of the nanoparticles on the corresponding organisms.

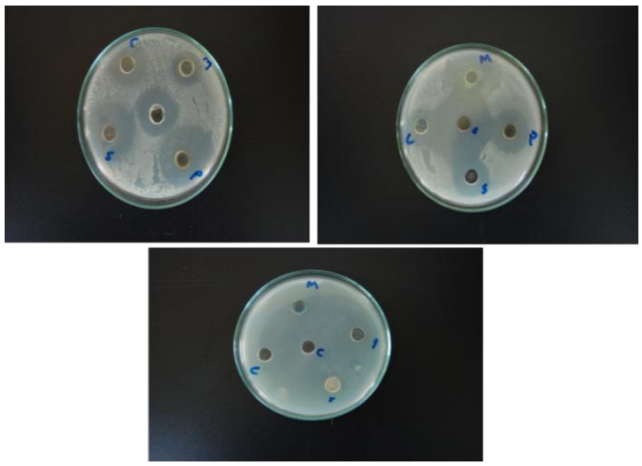

Fig. 7: Shows the zone of inhibition covered by silver oxide nanoparticles against human pathogens

Fig. 7 indicates the zone of inhibition of which became prominent after treatment with nanoparticles suspension in case of both gram positive as well as gram negative bacteria. The extent of inhibition of bacterial growth reported in this study was dependent on the concentration of nanoparticles in the medium. Interaction between nanoparticles and the cell wall of bacteria which was congerial to the fact that growth of gram negative bacteria was more profoundly affected by silver oxide nanoparticles than that of the gram +ve bacteria. Interactions with nanoparticles resulted in perforation in the cell wall, contributing to the *anti*-bacterial effects to the nanoparticles. *S. aureus* accounts for 30-50% of skin and soft tissue infections, some of the most common infections caused by *S. aureus* involve the skin, including furuncles, cellulitis, impetigo and postoperative wound infections of various sites. Some of the most serious infections produced by *S. aureus* are bacteremia, pneumonia, osteomyelitis, endocarditis [36-37] empyema, scalded skin syndrome etc. silver and silver based compounds have been in use for centuries in the treatment of burns and chronic wounds [38]. Since *S. aureus* are a major skin infection causing agent, it raised the possibility that a part of the antibacterial activity of silver nanoparticles could be due to its effects on the growth of *S. aureus*.

CONCLUSIONS

We have demonstrated the efficiency of electrochemical reduction method for the synthesis of silver oxide nanoparticles. The TBAB salts have played a significant role on controlling the particle size. The procedure offers several advantages including control the particles size (1-10nm), excellent yields, operational simplicity and minimum environmental effects. The synthesized silver nanoparticles exhibited gram positive and gram negative bacteria inhibitory activities and can be used as promising antibacterial agents in wide applications. Such a green and efficient process provides new opportunities for the rapid screening of a wide range of synthesis of metal nanoparticles, either for the development of new drugs for the material scientist.

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