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Antibacterial activity of viologen pendant indole stabilized silver nanoparticles

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ABSTRACT

Synthesis and stabilization of water dispersed silver nanoparticles was achieved by a simple one step procedure using N-methyl-N'-(5-indol-yl-pentyl)-4,4'-bipyridinium dibromide as reducing and stabilizing agent. The viologen pendant stabilized silver nanoparticles were characterized using UV-vis spectroscopy, Transmission Electron Microscope (TEM) and Atomic Force Microscopy (AFM). Further, viologen pendant stabilized silver nanoparticles were studied for antibacterial activity against *Staphylococcus aureus, Escherichia coli, Bacillus cereus* and *Klebsiella nemoniae.*

INTRODUCTION

Outbreaks of enterohemorrhagic *Escherichia coli* infections associated with lettuce and other leaf crops have occurred with increasing frequency in recent years (Islam *et al*., 2004). *Bacillus cereus* is associated with rice seed infection in the tropical environment (Cottyn *et al*., 2001). Food (Portnoy *et al.,* 1976), seed and vegetable (Harmon *et al*., 1987) contamination were caused by *B. cereus.* However, it has been used to manage early (Kokalis-Burelle *et al*., 1992) and late (Zhang *et al*., 2001) leaf spot disease of peanut using *Cercosporidium personatum*. *Klebsiella pneumoniae* is associated with the maize root and also with stem (Chelius and Triplett, 2000). Ingested *Klebsiella pneumoniae* within adult Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), in a mass rearing facility. This examination revealed the establishment of both bacterial strains as biofilms within the adult intestines, on the apical end of developing and developed eggs and throughout all subsequent life stages (Lauzon *et al.,* 2009). *Manduca sexta* (Vicki Fleming *et al.,* 2004), *Bombyx mori* (Kaito *et al.,* 2002), the fruit fly *Drosophila melanogaster* (Needham *et al.,* 2004) have been used to develop virulence for human pathogens including *S. aureus.*

In nanoscience and technology the fast developing area is the synthesis and study of nanocomposite materials. Metal nanoparticles are of importance due to their potential applications in catalysis, photonics, biomedicine, antimicrobial activity and optics (Wang *et al.,* 2004; Biswas *et al.,* 2004; Shipway and Willner, 2001; Nie and Emory, 1997; Govindaraju *et al.,* 2008; Govindaraju *et al.,* 2009). Size, shape and surface morphology play pivotal roles in controlling the physical, chemical and electronic properties of the nanoscopic materials. The interest arises due to the fascinating aspects of nanoparticles, such as size related electronic magnetic and optical properties. To be more precise polymer metal nanocomposites are promising candidates based on the fact that the very small sized particles enhance the optical properties while the polymer matrix offers flexible functionalities to control host-guest interactions to ensure the growth and distribution of metal nanoparticles (Tamilselvan *et al*., 1998; Gioco *et al.,* 2004; Pothukuchi *et al*., 2004).

Preparation of metal nanoparticles involves the reduction of metal ions in solutions or in high temperature gaseous environments. Several methods have been reported earlier in relation to the formation of metal nanoparticles on the surface of matrix polymers in hope that the resulting nanocomposites can exhibit synergistic or combinational properties (Chen *et al.,* 2002; Forster and Antonietti, 1998; Chen *et al.,* 1999). Different ways of achieving metal deposition on polymer surfaces and preparing metal clusters or nanoparticles have been reported (Ookoutchaev *et al.,*1999). Viologen group of compounds finds application in herbicides, anticoccidiostats and other industrial uses due to its physiochemical properties and its non toxic nature. Viologens has been reported for residue analysis, biochemical research and mechanistic studies (Ross and Krieger, 1980). Viologens are also used as sensors in many studies and used for surface grafting in antibacterial studies (Zhilong Shi *et al*., 2004). In the present work we describe a simple method to prepare highly stabilized silver nanoparticles functionalized viologen pendant indole moieties and their antibacterial activity against *B. cereus, E. coli, K. pneumoniae* and *S. aureus.*

MATERIAL AND METHODS

UV-vis absorption spectra were recorded using a Perkin-Elmer Lamda spectrophotometer. The solutions were analyzed in quartz cells, and solid films of about 100μm in thickness were analyzed as cast onto non-absorbing glass slides. The double beam spectrophotometer was standardized using double distilled water. Transmission electron microscopy (TEM) was performed using a JEOL transmission electron microscope operating at 300 keV. The samples for TEM were prepared by dropping the dispersion on copper grid supported amorphous carbon films. Atomic Force Microscope equipped with a Nanoscope IV controller was used for AFM measurements.

Preparation

The indole pendant viologen was synthesized as reported (Kelaidopoulou *et al*., 1998). Briefly, N-Bromopentyl indole was synthesized by taking 1.2mL of 1,5-dibromopentane and 0.6 g of indole was taken 2gms of KOH pellets were taken in a solution of 10mL dry DMF. The solution was allowed to stir over night and later to the solution nearly 50mL of water was added and the solution was extracted with 30 x 4 mL of diethyl ether. The resulting solution was dried over $MgSO_4$. Further the solution was allowed to pass over the column containing CH_2Cl_2 /Petroleum ether and the compound bromopentyl indole was obtained as a colorless liquid Yield 67% 0.4g of 4,4'-bipyridine and 0.7 mL of bromopentyl indole was taken in 6mL of acetonitrile and the solution was heated and stirred in for 16 hours the yellow precipitate was collected. Later methylation of the compound was carried out using adding equimolar mixture of methyl iodide in acetone. In the preparation of silver nanoparticles, 20 mM of Viologen pendant indole was taken and dissolved in 100mL of water. This solution was stirred and an aqueous solution of 1x 10^{-3} M $AgNO_3$solution was added and the solution was subjected to stirring. The reaction was complete in two hours.

Antibacterial activity

Viologen pendant synthesized silver nanoparticles were tested for their antibacterial activity by the agar well diffusion method. The bacterial pathogens *Staphylococcus aureus, Escherichia coli, Bacillus cereus* and *Kellebsella nemoneae* were used for antibacterial analysis. Bacteria were cultured in agar plates by the streake plate technique. Wells were made using a cork borer and filled with various concentrations (10µg, 15µg and 20µg) of viologen pendant stabilized silver nanoparticles. After incubation at 37º C for 24 hrs clear zone were measured. The assays were performed in triplicate.

RESULTS AND DISCUSSIONS

Formation of silver nanoparticles by reduction of aqueous metal ions by viologen pendant indole was monitored continuously during the course of reaction. There were no absorption bands in the range of 400-800 initially, however after 20 minutes the one obvious band appeared at 432 nm. The spectra was recorded for nearly two hours the peak was stable after two hours and there was no marked difference in the course of the reaction, after two hours. The results of the UV-visible studies are presented in Fig.1. Transmission Electron Microscope images show that particles are well separated and there is no sign of aggregation. The polymeric silver core of Ag @ MV^{2+} is observed in the image recorded and the particle size is almost monodisperse with a size of 30 nm (Fig 2). Automic Force Microscopic (AFM) image of (Fig. 3) colloidal silver nanoparticles are evenly distributed in polyindole viologen matrix.

The viologen pendant stabilized silver nanoparticles of the present finding exhibited excellent antibacterial activity against the bacterial pathogens *Staphylococcus aureus, Escharichia coli, Bacillus cereus* and *Kellebsella nemoneae*. Table.1 shows the inhibition of bacterial growth in various concentrations of viologen pendant stabilized silver nanoparticles. Stoimenov *et al*., (2002) demonstrated that highly reactive metal oxide nanoparticles exhibit excellent biocidal activity against Gram-positive and Gram-negative bacteria. Hybrids of silver nanoparticles with amphiphilic hyper-branched macromolecules exhibit effective antimicrobial surface coatings (Aymonier *et al*., 2002). It is believed that DNA loses its replication ability and cellular proteins become inactivated on Ag+ treatment (Feng *et al*., 2000). It was also shown that Ag^+ binds to functional groups of proteins, resulting in protein denaturation (Spadaro *et al.*, 1974). Studies have reported that the positive charge of the Ag ions is crucial for its antimicrobial activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged

Table1. Zone of inhibition (mm) of viologen pendant stabilized silver nanoparticles against bacterial pathogens

Bacterial species	Nanoparticles (µg)		
	10µg	15µg	20µg
S. aureus	17.0	19.0	21.5
B. cerus	18.0	18.5	21.0
E. coli	17.5	18.0	20.0
K. nemoneae	18.5	19.0	20.5

Figure 1. UV-visible spectra recorded as a function of time of reaction of aqueous solution of silver nitrate with indole pendant viologen.

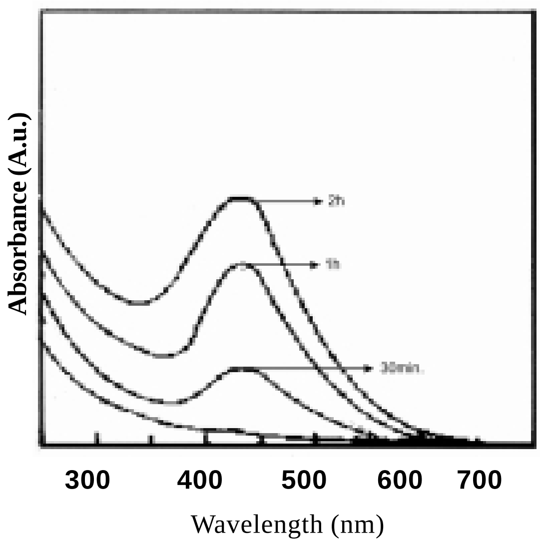
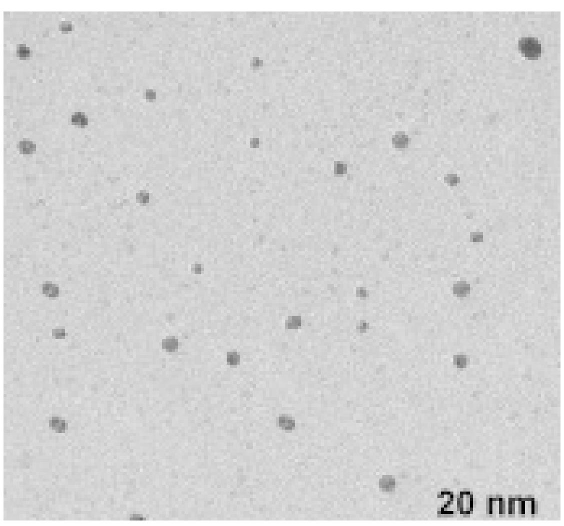
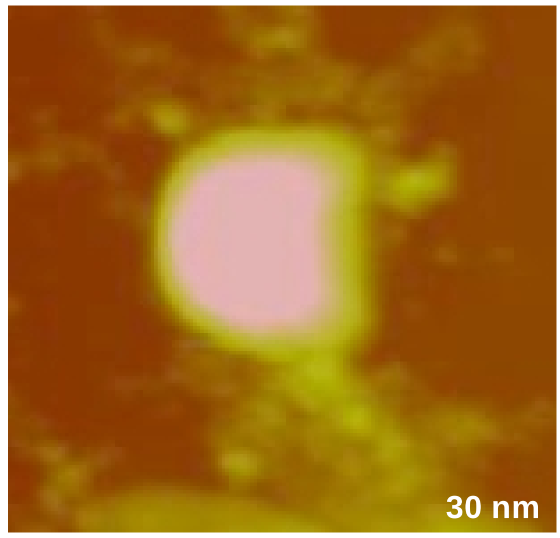


Figure 2. Transmission electron microscopic image of indole pendant viologen mediated silver nanoparticles.



nanoparticles (Stoimenov *et al*., 2002; Sondi *et al.*, 2004). We have demonstrated this aspect by establishing enhanced antibacterial activity of viologen pendant stabilized silver nanoparticles. Viologen pendant stabilized silver nanoparticles divulges that by the preparations, characterization, surface modification and functiona lization of nanosized inorganic particles, it is highly possible of a new generation of bactericidal materials.
Viologens are interesting materials with applications in industry and medicine (Bird and Kuhn, 1981). They are parent compounds of one of the most exciting types of

Figure 3. Atomic Force Microscopic image of silver nanoparticles formed by reduction of Ag ions using indole pendant viologen



herbicide discovered for many years. Viologen moieties in the course of the reaction not only act as reducing agents for formation of silver nanoparticles but also facilitate the stabilization of silver nanoparticles, by capitalizing the ability of viologen pendant indole to reduce metal ions, nanoparticles >30 nm were synthesized. The metallized polymers of heterocyclic compounds involved in the synthesis paves way to prepare high surface area substrates can be used as antimicrobial agent.

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