RESEARCH PAPER
Physics
Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

Synthesis of Silver Nanoparticles Using Bovine Serum Albumin, Characterization and Their Bioevaluation

KEYWORDS Nanoparticles, Silver nitrate, Bovine Serum Albumin and Antibacterial activity

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ABSTRACT *A simple and convenient method is synthesis of silver nanoparticles (AgNPs) in a foam matrix using the protein bovine serum albumin (BSA) is reported. BSA is an excellent foaming agent and by virtue of its zwitterionic character at the protein isoelectric point, may be used to bind to cationic silver (Ag+) ions in the foam. The metal ions in the foam are thereafter reduced in situ to yield silver nanoparticles. The BSA molecules coat and stabilize the nanoparticles thus eliminating the necessity of employing an additional stabilizing agent in the experimental procedure. The synthesized silver nanoparticles were characterized by FTIR, UV-Vis spectroscopic and powdered x-ray diffraction studies with Silver nanoparticles of size 26nm were obtained at pH 0.5.Antibacterial studies reveal that silver nano particles shows significant inhibition on Gram negative bacteria (E. coli and Brevibacterium) than Gram positive bacteria (Streptococci & Bacillus subtilis) tested.*

Introduction
Nanotechnology is rapidly growing by producing nanoproducts and nanoparticles (NPs) that can have novel and size-related physico-chemical properties differing significantly from larger matter [1]. These materials have many potential benefits to society with their development and deployment in science, engineering and technology. The novel properties of NPs have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies,

environmental remediation and biomedical devices [2-3]. Among them, silver nanoparticles (Ag-NPs or nanosilver) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts [4]. Reducing the particle size of materials is an efficient and reliable tool for improving their biocompatibility. Furthermore, nano materials can be modified for better efficiency to facilitate their applications in different field such as bioscience and medicine. Owing to the small size of the building blocks and high surface to-volume ratio, these materials are expected to demonstrate unique properties.

Synthesis, chemical and physical properties of metal nanoparticles are currently of considerable interest because of their potential application in commercial industries, material and medical science. The biological method of synthesis of nanoparticles have proved to be a better method than the chemical methods due to the large amount of capital involved in production and it involves an energy intensive process. The use of hazardous chemicals and generation of huge amount by products eliminates the method from being an eco-friendly one. These de-merits prompted us to explore the "greener synthesis [5] of nanoparticles".

Further the microorganisms such as bacteria, yeast and fungi play an important role in remediation of toxic metals through reduction of the metal ions recently this was also known as nanofactories. It has been well known that living cells are the best examples of machines that operate at the nano level and perform a number of jobs ranging from generation of energy to extraction of targeted materials at very high efficiency. A simple and convenient method for the synthesis of gold, silver and their alloy nanoparticles in a foam matrix using the protein bovine serum albumin (BSA) is also reported [6-7].

Antibiotics have revolutionized the medical care in the 20th century. However, the emergence and increase of microbial organisms (super bugs) resistant to multiple antibiotics and the continuing emphasis on health-care costs are the great challenges to the field of medicines. Many researchers have tried and been trying to develop new, effective antimicrobial [8-10] agents free of resistance and cost. Such problems and needs have led to the resurgence in the use of Ag-based antiseptics that may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics. Based on above findings, we have synthesized BSA-capped silver nanoparticles [11]at the room temperature by a chemical method at different pH .In our experiments BSA is used as a template to synthesize the nanoparticles. These Ag-NPs were characterized by FTIR,UV-V and powdered x-ray diffraction studies and further screened for antibacterial studies.

Materials and Methods
All the chemicals silver nitrate, BSA(Bovine Serum Albumin), ammonium hydroxide, nutrient agar, yeast were purchased from Coastal Enterprises and used as it is. All the solvents were of analytical grade and were distilled before use. FTIR in KBr, UV-Vis spectra and powdered X-ray diffraction studies were recorded on Perkin Elmer FTIR, Hitachi spectrophotometer and PAN Analytical XRD instrument with CuKα radiation of 1.54056Å at host university.

a) Biosynthesis of nanoparticles: Silver Nano particles were prepared by wet chemical synthesis. An albumin solution was prepared dissolving 0.1gms of BSA in 30ml of deionized water. The AgNO3 solution was prepared dissolving 0.05 gms of AgNO3 in 10 ml of deionized water. In the BSA solution ,AgNO3 solution was added while stirring on a magnetic stirrer and after 5 minutes, ammonium hydroxide was added drop wise to maintain the pH . It was stirred further for ̃15 minutes, and to let the solution settle down for 24 hours. The silver nano particles thus formed were isolated by filtration and washed with a minimum of amount of ethanol.

b) Preparation of culture media: Nutrient Agar was prepared by mixing Peptone (10gm),Yeast extract (5gm), NaCl (5gm)Agar (1.5gm) in 100ml distilled water. The ingredients were mixed and dissolved by heating. The pH was adjusted at 7.5 to 7.6 .The broth is solidified by adding agar and sterilized by autoclaving at 1210C for 15mins and poured into

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sterile petri dishes. After setting the media is preserved in refrigerator for use.

c)Antimicrobial activity assay was done by agar well diffusion method. The bacteria were grown in Muller-Hinton media (Himedia Pvt Ltd., Mumbai, India) at 370C and maintained on nutrient agar slants at 40C and stored at -200C. Inoculum of test organisms was prepared by growing pure isolate in nutrient broth at 370C for overnight. The overnight broth cultures were sub cultured in fresh nutrient broth and grown for 3hrs to obtain log phase culture. The agar plates were prepared by pouring 20ml M H medium in plate. The sterile M H agar medium is cooled to 450C and mixed thoroughly with 1ml of growth culture of concerned test organism (1 x 108 cells) and then poured into the sterile petridishes and allowed to solidify. Wells of 6mm size were made with sterile cork borer and AgNPs were added at different concentrations 4µl, 8µl, 12µl, 16µl, 20µl respectively. The agar plates were incubated at 370C for 24hrs. The diameter of zones of inhibition was measured in mm using Himedia Zone reader or normal centimeter scale.

Result and Discussion

Bovine Serum Albumin (BSA) is the most abundant plasma protein and a model globular protein that is widely used in bio-nanotechnology applications. It possesses a zwitterionic character at the isoelectric point (pH 4.7) with exposed ionic groups (C and N terminus) at the side chains of the globular protein, which are present in solution and are promising sites for binding cationic and anionic groups[12]. The general process for synthesis of nanomaterials in foam matrix involves the electrostatic complexation of metal ions with oppositely charged surfactant molecules, followed by the foam generation and subsequent in situ chemical reaction. BSA imparts greater flexibility to this method by allowing the complexation of metal ions with opposite charges simultaneously. Keeping these facts in consideration we prepared AgNPs, by mixing BSA and silver nitrate solution maintaining the pH. After one day standing we got nice silver nano particles that are characterized with spectral and diffraction studies .We monitored the formation of silver nano particles over the range of the pH 0.5–6.5 and silver nano particles with a bimodal primary particle size distribution with the most intense peak at 5.0 ± 7.4 nm were formed at a pH 0.5 depicted in Table 1.It is observed that silver nanoparticle dissolution showed size dependent behavior as larger, micron- sized silver particles show no dissolution at this pH further an increase in pH causes agglomeration of the nanoparticles which leads to the increase in size of the particles.

Table 1. Size of AgNP at various pH

Sl.No	pH	Size of particle
1	11	5000µm
2	04	158.8nm
3	02	73.5nm
4	0.5	26nm

The complexity of the protein structures which possess a number of amino acids and amide groups with significant changes in the IR spectra indicating wave number and %T (Absorbance) between the free Bovine serum albumin protein and silver nano particles obtained from this green synthesis are listed table 2.

Table 2 IR spectra of individual and complexes

BSA		Silver Nitrate		Composite	
Wave Number	%T(A)	Wave Number	%T(A)	Wave Number	%T(A)
3844	8.43(1.076)	3843	6.28(1.208)	3722	3.83(1.420)
3786	8.42(1.075)	3783	6.32(1.201)	3469	0.73(2.155)
3467	2.2(1.658)	3467	1.62(1.180)	1638	11.3(0.941)
1638	17.65(0.754)	2426	12.82(0.892)	1384	24.12(0.618)
1384	29.65(0.528)	1763	18.15(0.742)	603	30.55(0.515)
583	35.63(0.446)	1636	12.34(0.910)		
		1384	1.23(1.921)		
		825	23.62(0.627)		
		602	27.55(0.560)		

In the IR spectra of the free BSA the OH stretching frequency stemming from the free carboxylic acid groups can be seen as a broad peak centred at 3844-3786 cm^{-1}. The NH asymmetric and symmetric stretching frequencies of NH2 are observed at 3467 cm^{-1} regions [13-15] but are a little weaker than the OH stretching frequencies and may be overlapped with amino group.The difference between the IR spectrum of pure BSA and that of BSA-Ag^+ is obvious such as the intensity of NH groups peak decreasing from 2.2 to 0.7, suggesting coordination interaction between Ag^+ and NH groups of BSA that are crucial in the formation of Ag nano particles. In addition, this was further supported by the decrease in intensity of amide peak ($CONH_2$) at 1638 cm^{-1} . The strong peak at 1384 cm^{-1} in the BSA-Ag^+ spectra is attributed to the absorption of NO_3^{-1}, which was introduced by the addition of $AgNO_3$. Comparing the IR spectra of BSA-Ag with those of pure BSA, the characteristic peak of OH groups shifts to a lower wave number of about 14 cm^{-1} and the characteristic peak of NH groups hardly disappears. These results indicate that there might be conjugation between the Ag nanoparticles and OH groups and NH groups of BSA.

UV Visible Spectroscopy

UV visible spectral studies table 3 was studied for individual fragments BSA, Silver nitrate and the composite. The absorbance from spectra reveals uniform decreasing trend in the free and complexes with large variation in the complexes in comparison with BSA with increased wavelength. The relevance of study is to correlate absorbance from FTIR and UV-vis spectra responsible for agglomeration of nano particles. Studies of both infrared spectra and UV-Vis of BSA reveal the absorbance is reduced except the absorbance at 3467 cm^{-1} of NH_2 in IR at wavelength of 232 °A in UV–Vis with similar reduced phenomena in silver nitrate with both techniques. However in the composite the absorbance is high at low wavelength in contrast with individuals signifies that electronic, vibrational and rotational levels play crucial role in formation of composite that lead to agglomeration.

Table 3 UV Vis spectral data

BSA		Silver Nitrate		Composite	
Wavelength (oA)	Absorbance	Wavelength (oA)	Absorbance	Wavelength (oA)	Absorbance
232	2.078	232	2.100	217	1.98
276	1.618	302	0.505	259	0.238
348	1.741	342	0.750	342	0.746
379	0.723	378	0.730	370	0.73
600	0.252	600	0.250	600	0.3

X-ray Diffraction studies

XRD diffraction studies of the molecular complexes reveal that there is decrease in intensity of complexes than free molecules. The thickness of the particle 26nm is determined using Scherrar's formula [16-17].The data obtained for intensity and angle of diffraction for the complex are approximated with Gaussian fit as illustrated in figure 1 .At particular value of diffraction angle of 27.6 with corresponding intensity is 198 arbitrary units. Studies reveal that decreased intensity results in scattering leading to agglomeration of particles.

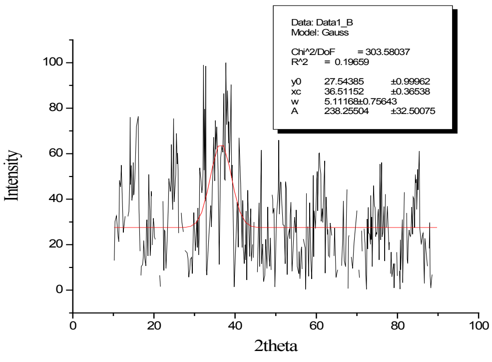

Fig 1: X-ray diffraction pattern of nanoparticle

Antimicrobial activity: The way in which bugs have become resistant to antibiotics within two generations of their discovery is one of the great tragedies of human history. However, the emergence of super bugs i.e. bacteria that resists the effects of the most powerful antibiotics are posing a great challenge to the field of medicines. In this continuation we also wanted to test the antibacterial effect of our newly synthesized silver nano material (AgNPs).We have chosen two bacterial strains one gram positive and other gram negative bacteria. Brevibacterium are Gram-positive bacteria which is ubiquitously present on the human skin, and causes foot odour. Escherichia coli is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in humans. Antibacterial screening was done by the well diffusion method against these two bacteria at the dilution 4,8,12,16,20 mg /ml ,using Ab as standard. The petri dishes were incubated for required time and the antibacterial zone was measured by HI anti biotic Scale are illustrated in the figure 2. It was observed synthesized nano compounds showed better activity for Brevibacterium than Escherichia coli.

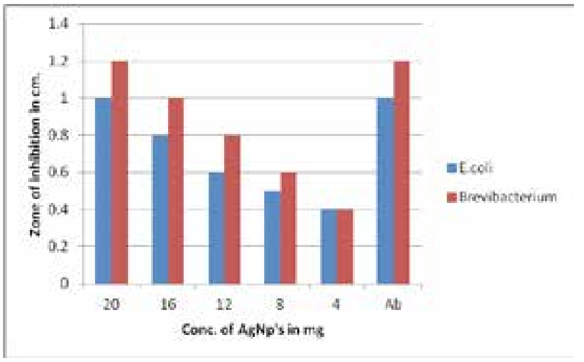

Fig 2: Comparative studies: Zone of Inhibition in E. coli & Brevibacterium

Conclusion:

Nanoparticles extended their origin due to its vast features through manipulation of matter on miniscule scale involving many interdisciplinary areas for their contribution in retrieving interesting features. An attempt in realizing the study of antibacterial activity is performed involving silver nitrate, bovine serum albumin, water and ammonium nitrate. All these were characterized and interpreted with various techniques in predicting the responsible molecular constitution leading to agglomeration of particles. The absorbance is high at low wavelength in composite in contrast with individuals signifies that electronic, vibrational and rotational levels play crucial role in formation of composite that lead to agglomeration. Antibacterial studies reveal that silver nano particles shows significant inhibitor effect on Gram negative bacteria (E. coli and Brevibacterium) than Gram positive bacteria (streptococci & Bacillus Subtilis) tested with nanoparticle of silver at 26nm were obtained at low p^H.

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