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Evaluation Profile of Silver Nanoparticle Synthesized By Aloe Vera Extract

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Abstract: Silver nanoparticles have unique properties which are used in molecular diagnostics,therapies and several medical procedures. Silver nanoparticles produced have problems with Toxicity and Stability. To overcome this, the biological method provides a feasible alternative. The Silver nano-particles of size 15nm were synthesized by using Aloe vera extract has been characterized and evaluated for its anti microbial activities, stability and toxicity.The Aloevera gel has been isolated and used in the extraction process. The synthesized Nanoparticles found to be bactericidal and had maximum anti microbial activity at 250µl concentration of plant extract. The Zeta Potential was found to be -18.3 mV, which uniquely proved to be stable.FTIR studies had revealed possible decrease in the size of silver metal with its corresponding peaks. Toxicity analysis shown with hemolysis percentage of 9.67 and hence low low toxic effect. Silver nanoparticles were synthesizing parameters are mostly depends the amount of plant extract and directly influence the stability, toxic effect of the particles. Thus aloe vera gel extract was key material in synthesizing the silver nanoparticles with above proven results and its effectiveness in other therapeutic applications.
Keywords: Nanoparticles, Aloe vera, stability, Toxicity

Introduction

Silver nanoparticles have tremendous anti-microbial activities. Silver nanoparticles shows very strong bactericidal activity against gram-positive as well as gram-negative bacteria.Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics [1], antimicrobials and therapeutics [2]. Fundamental studies carried out in the 1980s and1990s showed that silver NP exhibit unique optical properties such as surface plasmon resonance (SPR), well-developed surfaces, catalytic activity, high electrical double layer capacitanceSilver is more reactive than gold; hence, first of all, the methods for the synthesis and effective stabilisation of NP with narrow size distributions. However there is always a hype for production of nanoparticle economically, user friendly and also commercially viable as well as environment clean synthesis.Silver nanoparticles have the ability to bind to bacterial cell wall and subsequently penetrate through it, thereby causing structural changes in the cell membrane The second mechanism involves inhibition of DNA synthesis by silver ions.

Many diverse approaches have been carried out to synthesize silver nanoparticles via Chemical and Physical methods such as sonochemical, microwave assisted process [8], microbial assisted[9] and recently via green chemistry route[10]. The problem with physical and chemical methods are that there is no proper stabilising agent to keep the Silver nanoparticles stable. The Nano silver particles if produced at higher concentrations have a greater chance of killing the Synthesizing microbe itself. Microbe assisted synthesis involves intracellular accumulation of Silver nano particles. Microbe assisted synthesis takes longer reaction times and

also demands subsequent extraction and recovery steps. On the other hand, plant extract mediated synthesis does not involve intracellular accumulation and the reaction time is reported to be very short compared to that of microbial assisted synthesis[14].In earlier reports, natural polymers like starch and chitosan[14] were shown to stabilize silver nanoparticles and separate reducing agents were used.Aloe vera is a stemless plant with rosettes of thick fleshy leaves. The plant is used because of its theraupetic properties.Aloe vera is opted for the synthesis of silver nano particles because of the presence of natural phytochemicals which provide natural capping and reducing agents.[15] The various phytochemicals present in Aloevera are AloinA and AloinB, Flavanoids, Lupeol, Mannose, Resveratol, Emodin. Mainly the gel which is found in the central part of the plant is used in extraction.The Green coloured gel close to the Skin contain Aloin, which is the main stabilising agent in our research.It also contains emodin which is a phytochemical reducing agent.This plant extract is expected to synthesise a stable AgNP.

Materials and Chemicals Required

0.01M AgNO3 stock solution,0.001M AgNO3 working solution,Aloe vera extract,Distiller water Whatman No.1 Filter paper,Magneticstirrer,bead,Conical flask and beaker.

Preparation of Plant Extract

Fresh Aloe vera leaves were collected.The gel was extracted from the leaves using traditional Hand filleting procedure.25g of gel was chopped into pieces and grinded using Mortar and Pestle[15].The gel was mixed with equal volume of distilled water and heated at 85^0C for 10 minutes. The mixture was filtered by using Whattman No.1 filter paper.The extract was stored at 4^0C.[16]

Synthesis of Silver Nanoparticles

20 ml of the working solution was taken in a glass beaker and kept in magnetic stirrer for 15 minutes at 65^0C.Plant extract of 250µl and 500µl concentrations were added in drop.The mixture is kept for stirring for 15 minutes to observe color change to reddish brown[7,9].

Figure 1: Synthesis of silver nano particles

(A) Aloe vera extract

(B) 0.001M Silver nitrate

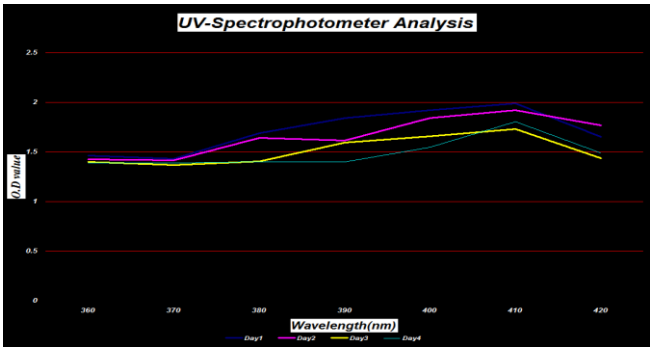
(C)Silver nitrate solution after adding 250µl of aloe vera extract and keeping at incubation in the dark for 24

Characterization Studies

UV VIS Spectrometer

The bioreduction of reaction mixture of pure silver ions were observed using Uv vis spectrophotometer at different wavelength taking 3ml of sample and 3ml of distilled water as blank. The progress of silver nitrate reduction reaction within metal ions and leaf extract were evaluated at different wavelength from 360- 420nm.

Figure 2: UV visible Spectrometer Analysis



Zeta Potential and Size

A particle size analyzer was used to determine the particle size distribution of the silver nano particle sample.In order to find out the particle size and charge distribution, the nanoparticles sample was dispersed in water.1ml of sample was diluted with 9 ml distilled water and the sample was taken for AnalysisThe analysis was carried out in computer controlled particle size analyzer [(ZETA SizersNanoseries (Malvern Instrument Nano ZS)] to find out the particles size distribution.

FTIR Analysis

FTIR analysis was used to characterize the nature of Compounds that stabilizes the silver nanoparticles by bioreduction process. The infrared spectra are recorded on Fourier Transform Spectrometer in the mid–infrared region (MIR) within the range of 400 cm^{-1} to 4500 cm^{-1}). Due to the complex interaction of atoms within the molecule, IR absorption of the functional groupsvaries over a wide range.[17]

Antibacterial Assay

Disc Diffusion Method

Silver nanoparticles bactericidal effect was studied against two gram-positive (*Bacillus subtilis*ATCC-6633 and *Streptococcus pneumonia* ATCC 49619) and two gram-negative (*Escherichia coli* ATCC-25922 and *Pseudomonas aeruginosa* ATCC-27853) bacterial pathogens.Overnight culture inoculum (100 µl) of bacteria(E. coli) was spread on to LB agar plates. Sterile paper discs (Whatman filter paper) of 5 mm diameter (containing 20 µg/ml silver nanoparticles) along with control containing Silver nitrate Working solution were placed. The cultured agar plates were incubated at 37^0C for 24hours.After 24 hours of incubation the zone of inhibition is measured.[16]

Toxicity Assay

Hemolysis Test

Silver nanoparticles Cytotoxic effect was studied by performing Hemolysis test.10 ml of blood sample was taken out of which 4ml was taken and added with 8ml of 0.2M D-PBS(pH 7.0) [20].The mixture is taken in a centrifuge tube and centrifuged at 10000rpm for 10 minutes.The pellet containng RBC is further washed 5 times by D-PBS at 10000rpm for 3 minutes.The RBC obtained was diluted with 40 ml D-PBS.From this the Test Sample, Positive and Negative controls were Prepared.The Samples were kept for incubation at room temperature for four hours.It is vortexed and cantrifuged at 10000rpm for 3minutes.The supernatent was collected and the OD valued were observed at 577 nm.[18,19].Hemolysis % was calculated.

$$\text{Hemolysis\%} = \frac{Mean\ OD\ of\ sample\ to\ be\ tested - Mean\ OD\ of\ Negative\ control}{Mean\ OD\ of\ Positive\ control - Mean\ OD\ of\ Negative\ control} * 100$$

Results

Silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon resonance in silver nanoparticles. The change in color is due to the reduction of silver ions which indicates the formation of silvernano particles.

UV Absorption spectra of silver nanoparticles formed in the reaction media had absorbance peak at 410 nm.

Figure (3): Nano silver Particles exhibiting

Anti-bacterial activity

Silver nanoparticles exhibited antibacterial activity against *E. coli*,*Pseudomonas aeruginosa*, *Bacillus subtilis*and *Streptococcus pneumoniae* as it showed a clear inhibition zone(Figure- 3). The Zone of inhibition is higher at 250µl concentration kept for 18 hours of incubation.

Figure 4(a): Zeta potential distribution

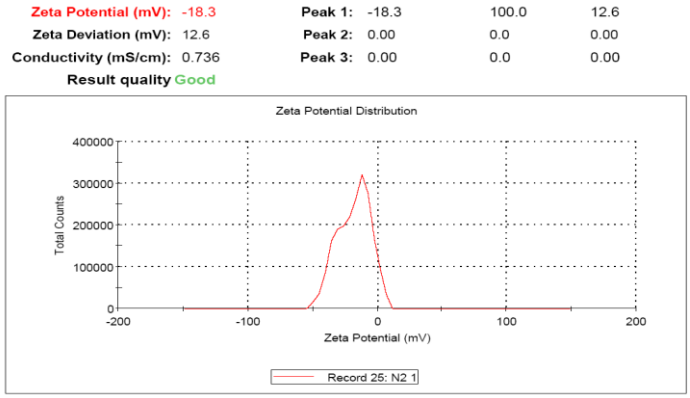


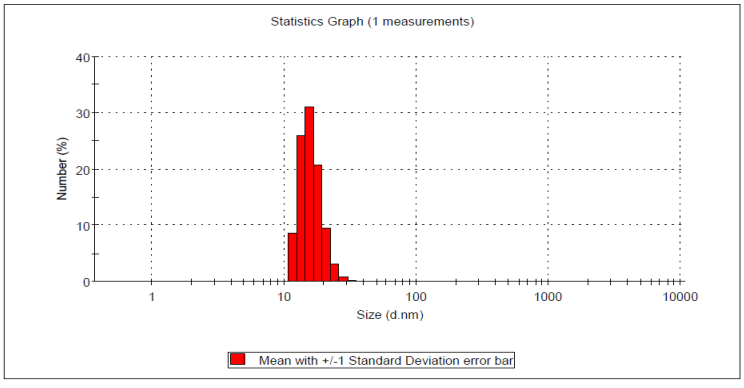
Figure 4(b): Zeta Sizer and Standard deviation error bar

Size d.nm	Mean Number %	Std Dev Number %
0.4000	0.0	
0.4632	0.0	
0.5365	0.0	
0.6213	0.0	
0.7195	0.0	
0.8332	0.0	
0.9649	0.0	
1.117	0.0	
1.294	0.0	
1.499	0.0	
1.736	0.0	
2.010	0.0	
2.328	0.0	
2.696	0.0	
3.122	0.0	
3.615	0.0	
4.187	0.0	
4.849	0.0	

Size d.nm	Mean Number %	Std Dev Number %
5.615	0.0	
6.503	0.0	
7.531	0.0	
8.721	0.0	
10.10	0.0	
11.70	8.6	
13.54	26.0	
15.69	31.1	
18.17	20.7	
21.04	9.4	
24.36	3.1	
28.21	0.7	
32.67	0.1	
37.84	0.0	
43.82	0.1	
50.75	0.1	
58.77	0.0	
68.06	0.0	

Size d.nm	Mean Number %	Std Dev Number %
78.82	0.0	
91.28	0.0	
105.7	0.0	
122.4	0.0	
141.8	0.0	
164.2	0.0	
190.1	0.0	
220.2	0.0	
255.0	0.0	
295.3	0.0	
342.0	0.0	
396.1	0.0	
458.7	0.0	
531.2	0.0	
615.1	0.0	
712.4	0.0	
825.0	0.0	
955.4	0.0	

Size d.nm	Mean Number %	Std Dev Number %
1106	0.0	
1281	0.0	
1484	0.0	
1718	0.0	
1990	0.0	
2305	0.0	
2669	0.0	
3091	0.0	
3580	0.0	
4145	0.0	
4801	0.0	
5560	0.0	
6439	0.0	
7456	0.0	
8635	0.0	
1.000e4	0.0	



The graphical representation of particle size distribution of silver nanoparticles synthesized by Aloevera extract was 15nm (Figure- 4(a)).The zeta Potential was found to be -18.3 mV.The size of particles were determined to lie between 10-20 nm. The peak was found to be at 15 nm(Figure 4(b)).Hence the Size of the particle was determined as 15 nm.

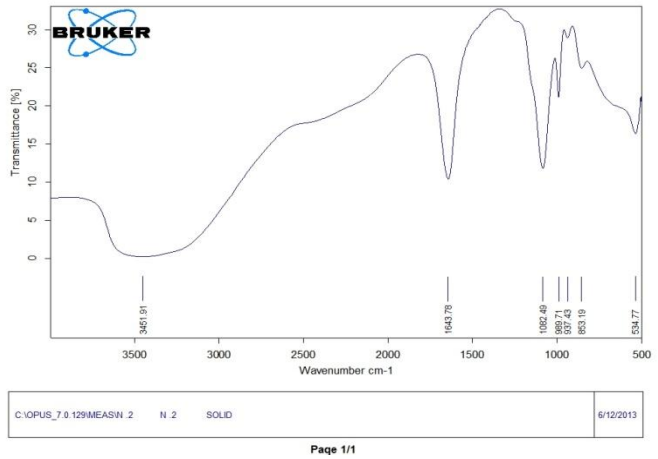
The FTIR Absorbance and transmittance graphs were interpreted to determine the bond stretching and compounds that were actually reduced during Nano silver synthesis.Peak at 3454.91cm $^{-1}$ showed O-H bond streching.Peak at 1643.78cm $^{-1}$ showed C=O bond stretching(Figure- 5 (a,b)).

The Hemolysis % was found to be 9.67.The hemolysis % is relatively very low when compared to other methods of Synthesis thus stating that the Silver nano particles synthesised by using Aloevera was relatively less toxic.

Discussions

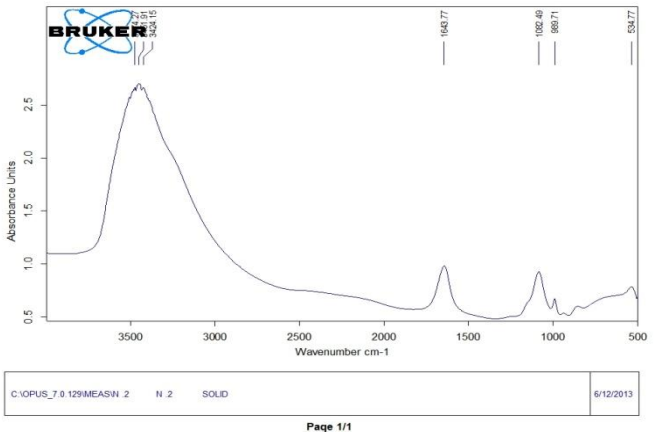
There is always a hope that synthesising the metal nanoparticles without the use of any toxic chemicals have high impact factor. This biosynthesis of silver nanoparticles using Aloevera extract have paved way in production of cost effective, less toxic miniature particles called as nanoparticles. The presence of Aloin,emodin,flavonoids and tannins have proved the Aloevera to have antibmicrobial and antioxidant activity and thus the present study highlights the utility of silver nanoparticles synthesised from this plant and its stability without the addition of any chemical compounds. There were no drastic changes in the stability of the silver nanoparticles present in the various concentrations of the mixture clearly stating the highly stable nature of silver nanoparticles. The maximum absorbance of the silver nanoparticles varied with respect to both concentration of the extract added and also with wavelength. Silver nanoparticles exhibited antibacterial activity against *E. coli,Pseudomonas aeruginosa, Bacillus subtilis*and *Streptococcus pneumoniae* as it showed a clear inhibition zone(Figure- 3). The Zone of inhibition is higher at 250µl concentration kept for 18 hours of incubation.The graphical representation of particle size distribution of silver nanoparticles synthesized by Aloevera extract was 15nm.The zeta Potential was found to be -18.3 mV (Figure- 4(a)).The nano silver particles were found to be of a smaller size.

Figure 5(a): FTIR Transmittance graph



The spectra were obtained in the wavelength range between 400 and 4000cm^{-1}. The FTIR Absorbance and transmittance graphs were interpreted to determine the bond stretching and compounds that were actually reduced during Nano silver synthesis.Peak at 3454.91cm $^{-1}$ showed O-H bond streching .Peak at 1643.78cm $^{-1}$ showed C=O bond stretching (Figure- 5 (a,b)). The Phytochemical containing these compounds were reduced during nano silver production. Hemolysis % of 9.67 showed that the Silver Nano particles synthesised by Aloevera extract had low toxicity to human cells.

Figure 5(b): FTIR Absorbance graph



Conclusion

The present study concluded that Aloevera can be used as an excellent source for biosynthesis for the silver nanoparticles in aqueous solution. The reduction of the metal ions through gel extracts leading to the formation of silver nanoparticles of fairly well-defined dimensions. The major advantage of synthesizing silver nanoparticle using aloe vera is that they are easily available, safe, and nontoxic. They have a broad spectrum of metabolites that can aid reduction of silver ions, and are quicker than microbe assisted synthesis.Thus it paves way for several other applications which can be developed from the synthesized silver nanoparticles from aloevera extract.

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