IJBPAS, September, 2023, 12(9): 4008-4014

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

SYNTHESIS AND SPECTROPHOTOMETRIC ESTIMATION OF SOME NOVEL SILVER NANOPARTICLES WITH BSA PROTEIN S

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Received 23rd June 2022; Revised 26th Aug. 2022; Accepted 4th Dec. 2022; Available online 1st Sept. 2023

https://doi.org/10.31032/IJBPAS/2023/12.9.7361

ABSTRACT

Silver nanoparticles (AgNPs) were successfully developed by a simple chemical reduction method. The particles were synthesized via the reduction of silver nitrate by trisodium citrate and citrate is used as capping agent, stabilizing agent and reducing agent. The resulting AgNPs were characterized by UV-visible spectrophotometer andtransmission electron microscopy (TEM). The result of TEM shows that AuNPs with sodium citrate have particle size of 44.04 nm (diameter) and have developing stability. The Result of UV- VIS shows that AgNPs with sodium citrate gives peak at 444 nm and single peak shows monodispercity. Interaction of BSA protein with AgNPs shows that the concentration of BSA decreases the absorbance increases.

Keywords: Silver nanoparticles, chemical reduction, sodium citrate, TEM, spectroscopy, BSA Protein

INTRODUCTION

Metal particles have established great attention due to their unusual properties differ from bulk metal and also they have ultrafine size. Properties of the metal nanoparticles (chemical and physical) are dependent on their size, structure, shapeand size distribution. Therefore, control over the size and size distribution is essential and is frequently achieved by varying the synthesis methods, reducing agents and stabilizers **[1]**. However, chemical reduction is simple method so it is most commonly used. In

order to produce silver nanoparticles with controlled particle sizes, shapes and particle size distribution, reduction method also permits variation in the molar concentration of the reactant, dispersant and accelerate rate of reactant. The size, shape and particle size distribution powerfully depend on the nature of the reducing agent so the selection of suitable reducing agent is also a crucial factor, as. During the synthesisprocess, if the reaction rate is too fast, rapid formation of a large amount of metal nuclei will occur and result in particles that are too small **[2]**. Silver nanoparticles (Ag-NPs or nanosilver) have attracted increasing interest because of their distinctive physical, chemical and biological properties compared to their macro-scaled counterparts **[3]**. Silver nanoparticles are the theme of researchers thanks to their distinctive properties (e.g. size, form and antimicrobial properties). Ag-NPs have distinctive physico-chemical properties, with a high electrical and thermal conduction, surface-enhanced Raman scattering, chemical stability, catalytic activity and nonlinear optical behaviour **[4]**. Ag-NPs exhibit wide-ranging spectrum bactericidal and fungicidal activity **[5]**. The idea behind the useof Ag-NPs in the water filtration membrane is based on the utilization of their proven antimicrobial properties and slow release rate of Ag-NPs from the membrane **[6]**. The interaction of proteins with Ag NPs may change the natural properties of both NPs and proteins. BSA is used as a carrier for intracellular transportation and abundantly present in blood plasma. In present work, study of silver nanoparticles interaction with BSA at different concentrations is done. Interaction studies at different concentration of BSA with nanoparticles are characterized by UV-visible spectroscopy.

EXPERIMENTAL

Materials

Silver nitrate ($AgNO_3$) and Trisodium citrate were purchased from Sigma-Aldrich. Aqueous solutions and stock solutions were prepared by using double distilled water.

Synthesis and characterization of Ag NPs

The citrate stabilized AgNPs were prepared via chemical reduction method **(Figure 1)** in which 1 mM $AgNO_3$ is reduced by 1 % trisodium citrateand citrate is act as reducing agent, capping agent also stabilizing agent. The colour of AgNPs solution is observed from colourless to yellow **(Figure 2)**. Here the citrate ions reduce the Ag^{+1} ion to neutral Ag atoms. The colour observed of solution after the procedure from colourless to yellow colour which confirm the presence of silver nanoparticles. Characterization of silver

nanoparticles was done via UV-visible spectrophotometer and transition electron microscope (TEM).

Synthesis of AgNPs with BSA

Distilled water was added to 0.1 g of BSA to produce 10 ml of stock solution. Further take 5 ml from stock solution to make 50 ml 38.8µM solution. The procedure andamount of BSA were determined by research and testing. In the next step, take 5 ml solution from 38.8µM BSA and then it was mixed with 5 ml of Ag-citrate solution while stirring at room temperature. The stirring was continued for 1 h. This obtained solution was incubated for 24 h at 4˚C to simplify BSA-AgNPs conjugation. After that the solution was centrifuged for 30 min at 15,000 rpm, and the resultant pellet was re-distributed in distilled water. A similar procedure was repeated for different concentration of BSA with gold nanoparticles. Different concentration containing BSA solutions were made by diluting 38.8µM solution with double distilled water.

RESULTS AND DISCUSSION

The solution of silver nanoparticles which were made by adding 1mM silver nitrate and 1% citrate was characterised by UV-visible spectroscopy and TEM. In our work we prepared silver nanoparticles solution which is colourless at time after synthesis. Citrate containing AgNPs solution has COO^- negatively chargedgroup on the surface of Ag.We analysed this solution by UV-visible spectroscopy and there was no peak observed so we considered there was no formation of silver nanoparticles. After 2 days we observed peak at 422 nm and colour was light yellow, then after 5 days there was a peak at 444 nm then after 8 days absorbance peak was at 439 nm and colour became more yellowish then after 14 days the absorbance peak observed at 433 nm and colour became greenish yellow then there was no change observed in absorbance after some days. So here we can see that the absorbance become higher when days go but absorbance is higher on 8^{th} days **(Figure 3)**. Further we analysed the formation of silver nanoparticles. Increase in wavelength indicated that particles size also increased. TEM analysis gives the particles size and shape and from TEM analysis we considered the silver nanoparticles were in 44.04 nm in diameter (size) and spherical in shape **(Figure 4)**. An interaction of AgNPs with BSA is shown in **Table 1**.

Interactions of AgNPs with BSA

Interaction of BSA with silver NPs were characterised by UV-visible spectroscopy. We found that the absorption intensity of BSA decreases with decreases in

concentrations of BSA (38.8 µM, 31.06 µM, 21.27 µM, 10.36 µM, 5.27 µM) with NPs, where concentration of NPs are constant, which indicated that the interactions of BSA with citrate-AuNPs, citrate-AgNPs and citrate-MixNPs formed complexes. The spectral changes of BSA within the presence of AgNPs are shown in **Figure 5**. The absorption peak of silver nanoparticle was found to be at 433 nm. For the AgNPs to BSA ratio 5:5 the absorbance decreases at 471 nm and the peak become broader. For AgNPs to BSA ration 5:4.5 the absorbance is slightly increase but there is no change in wavelength compare with ratio 5:5. For ratio of AgNPs:BSA 5:3.5 the absorbance is slightly increase at wavelength 468 nm compared to ratio 5:5 and 5:4.5. For AgNPs to BSA ration 5:2.5 and 5:1.5 the absorbance slightly increases at 469 nm and 471 nm compared to all other ratios. The absorbance of ratio 5:5 is less than ratio 5:1.5 but wavelength are same and this we can see in **(Figure 5)**. The broader peaks indicate the formation of aggregates due to larger size or instability of silver nanoparticles. Here the absorbance increases as the concentration of BSA decreases.

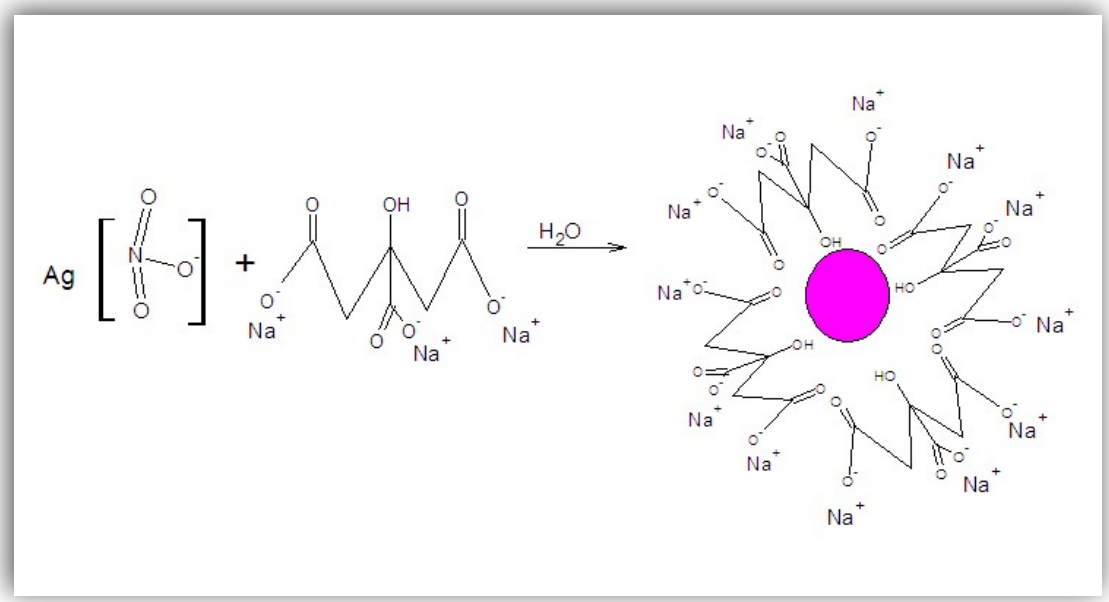

Figure 1: Synthesis of citrate capped silver nanoparticles

Figure 2: Colour changes of citrate capped silver nanoparticles

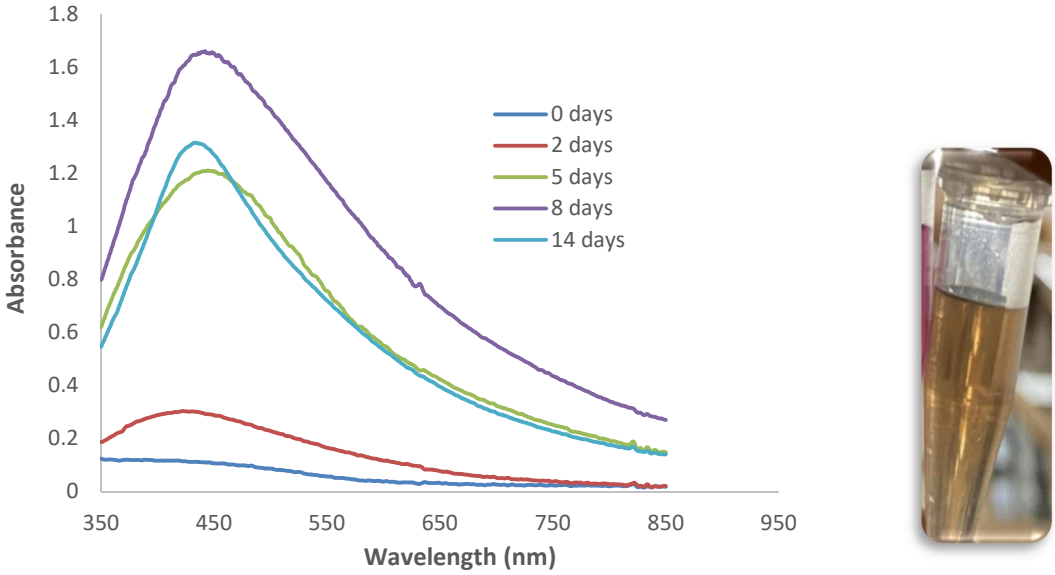

Figure 3: Absorbance peak of citrate capped silver nanoparticles with time

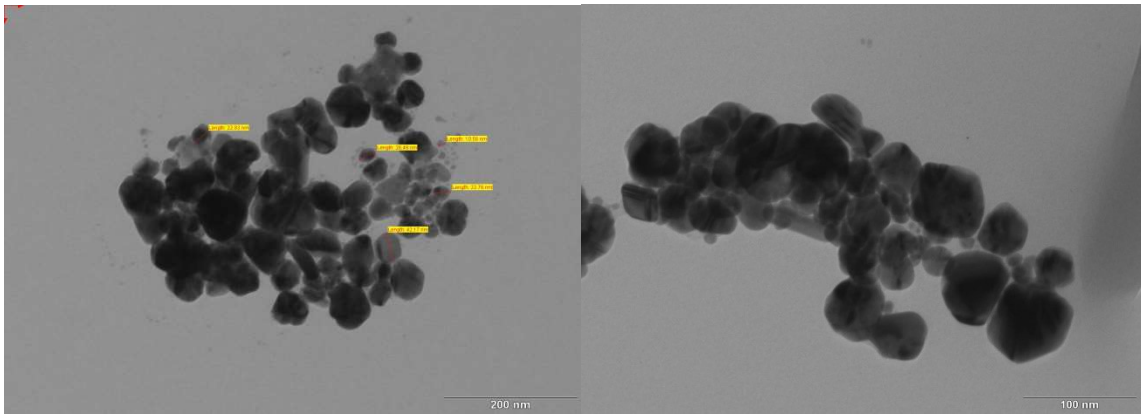

Figure 4: TEM images of citrate capped silver nanoparticles made by adding 1mM silver nitrate and 1 % citrate

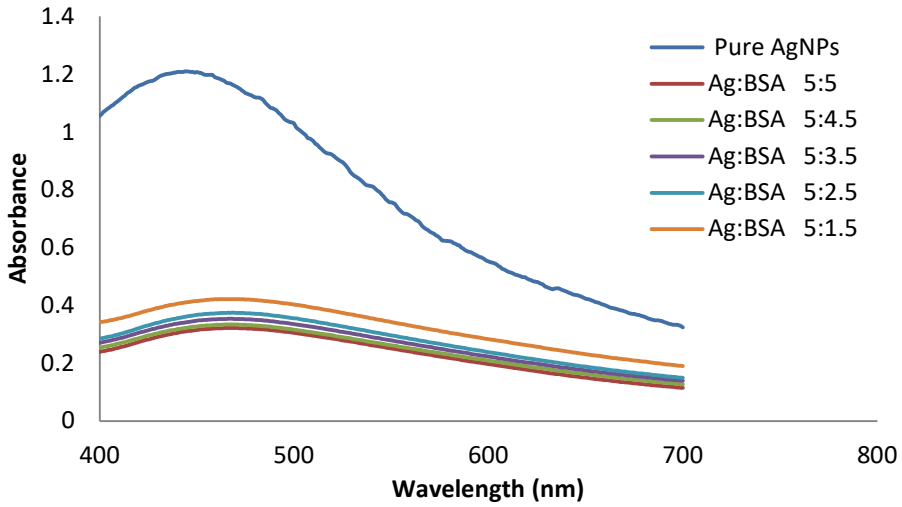

Figure 5: Absorbance spectra of BSA with citrate capped AgNPs

Table 1: Spectrophotometrically estimation of the interaction of AgNPs and BSA

BSA : AgNPs	Absorbance
5:5	0.322
4.5:5	0.334
3.5:5	0.354
2.5:5	0.375
1.5:5	0.422

CONCLUSIONS

Silver nanoparticles in were synthesized by the citrate reduction method. Preparing Silver nanoparticles takes more time to develop. In our work it was 15 days to prepared silver nanoparticles. The morphology, size and shape of citrate capped AgNPs were characterised using TEM and UV-visible spectrophotometer. The TEM shows diameter 44.04 nm and UV-visible give maximum absorbance at 444 nm of 1mM gold and 1 % citrate. This data was used in our further study. Such environmental responsive synthesis method for AgNPs has excessive potential in huge scale manufacturing to match the increasing marketable and industrial demand. Interaction of BSA protein with AgNPs shows the strong binding according to data which we obtained from spectrophotometer.

This data is used for further study in medical field or biotechnology filed or many filed which are related with this.

ACKNOWLEDGEMENT

The authors are highly thankful to the chemistry department of Shri M. M. Patel Institute of Sciences & Research, Gandhinagar and also thankful to chemistry department of R. R. Mehta college of Science, Palanpur for infrastructural support and use of experimental facilities.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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