

Synthesis of Dithizone Capped Silver Nanoparticles Via Chemical Reduction Method & Study of Their Biological Properties

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Abstract: Silver nanoparticle aggregates were synthesized using dithizone to obtain an assembly of silver clusters. Stable dispersion of the cluster in aqueous medium has been examined out of dithizone-capped silver nanoparticle assemblies. The UV-vis spectroscopy during the particle evolution has been studied in detail. From the SEM image and XRD pattern it was confirmed that the particles are made of pure silver only. The capping action of dithizone has been authenticated from the FTIR spectra. UV-vis spectroscopy and SEM images reveal that the time-dependent particle evolution has unique bearing on the stability and surface properties of the clusters. The concentrations of silver nitrate, dithizone, and NaBH₄ have important influence on the particle evolution and its size. Further the Ag-NPs were examined for bioevaluation, were the particles showed good to better activity.

Keywords: Silver NP's, Dithizone, SEM, bioevaluation, better activity.

Introduction:

Chemical reduction is the most frequently applied method for the preparation of silver nano particles (Ag-NPs) as stable, colloidal dispersions in water or organic solvents.^{1,2} Commonly used reductants are borohydride, citrate, ascorbate, and elemental hydrogen.³⁻¹¹ The reduction of silver ions (Ag⁺) in aqueous solution generally yields colloidal silver with particle diameters of several nanometers.² Initially, the reduction of various complexes with Ag⁺ ions leads to the formation of silver atoms (Ag⁰), which is followed by agglomeration into oligomeric clusters.¹² These clusters eventually lead to the formation of colloidal Ag particles.¹² When the

colloidal particles are much smaller than the wavelength of visible light, the solutions have a yellow color with an intense band in the 380–400 nm range and other less intense or smaller bands at longer wavelength in the absorption spectrum. This band is attributed to collective excitation of the electron gas in the particles, with a periodic change in electron density at the surface (surface plasmon absorption).^{13–15}

Previous studies showed that, use of a strong reductant such as borohydride, resulted in small particles that were somewhat monodisperse, but the generation of larger particles was difficult to control.^{16,17} Use of a weaker reductant such as citrate, resulted in a slower reduction rate, but the size distribution was far from narrow.^{3,4,18} Controlled synthesis of Ag-NPs is based on a two-step reduction process.¹⁷ In this technique a strong reducing agent is used to produce small Ag particles, which are enlarged in a secondary step by further reduction with a weaker reducing agent.³ Different studies reported the enlargement of particles in the secondary step from about 20–45 nm to 120–170 nm.^{19–21} Moreover, the initial sol was not reproducible and specialized equipment was needed.⁵ The syntheses of nanoparticles by chemical reduction methods are therefore often performed in the presence of stabilizers in order to prevent unwanted agglomeration of the colloids.

Synthesis of Silver nanoparticles via chemical reduction method:

In the present work, we report the formation, morphology and biological activity of the Dithizone-capped silver nanoparticles. The silver nanoparticles with uniform shapes and sizes can be obtained by the method proposed in this work. The method utilize here is a simple chemical reaction of silver iodide and sodium borohydride. The advantages of the method is ease of preparation, convenience in use and especially, that the obtained silver nanoparticles are uniform in their shapes and sizes. The last point is important for bioevaluation measurements because the shapes and sizes of the metal nanoparticles are significant parameters. Furthermore, we employed UV-visible (UV-vis) spectroscopy to determine the optimum conditions for the preparation of stable silver colloids. Time-dependent UV-vis spectroscopy and Scanning electron microscopy (SEM) are employed to monitor the silver formation process of the nanoparticles. Specifically, we observed changes in the shapes of the silver nanoparticles during the formation of the nanoparticles. This may be helpful in understanding the growth of the nanoparticles and creates a new dimension in controlling the shapes of the nanoparticles.

Reagents and Instruments:

All the reagents used were of AR grade. Dithizone was purchased from Aldrich. Silver nitrate was obtained from National Refinery Pvt Ltd, and a 0.1 M aqueous solution was used as stock solution. Sodium borohydroxide was obtained from Merck, India. Organic-free water was used throughout the experiment.

The UV-visible spectra were recorded on a Shimadzu UV-vis spectrophotometer, and the solutions were taken in a 1 cm well-stoppered quartz cuvette. Fourier transform infrared (FTIR) spectral characteristics of the samples were collected on a Shimadzu FTIR spectrometer with the samples as KBr pellets. The FTIR spectrum was recorded over 45 scans of each sample, and the background spectrum was automatically subtracted. The formation of single-phase compound was checked by X-ray diffraction (XRD) technique. The XRD pattern was taken with X-ray diffractometer (XPRT-PRO) at room temperature, using CuK_α radiation $\lambda=1.5406 \text{ \AA}$ over a wide range of Bragg angles ($30^\circ \leq 2\theta \leq 85^\circ$). SEM micrograph of dithizone capped Ag-NP was obtained on a NOVA-230 with an operating voltage of 10 KV. For SEM analysis, the specimen was suspended in distilled water, dispersed ultrasonically to separate individual particles, and one or two drop of the suspension deposited onto holey-carbon coated copper grids and dried under Infrared lamp.

Synthesis of the Silver Nanoparticle Assembly:

Dithizone-Capped Ag Nanoparticles:

A total of 2.5 mL of 10^{-2} M AgNO_3 was added to 75 mL of triply distilled organic-free water. A total of 5 mL of 10^{-2} M dithizone (dissolved in hot water) was added as stabilizer to the solution with stirring. After 10 min of mixing, 2.5 mL of 10^{-2} M NaI was dropped into the solution slowly, yielding a green yellow AgI colloid. A total of 20 mg of NaBH_4 was added to the AgI colloidal solution, and the reaction mixture was continually stirred for about 20 min. The silver colloid was finally obtained. During the whole reaction, the color of the colloidal solution changed from green-yellow to nut-brown at the beginning, then to brown, and finally to black.

Results and discussion:

Evolution and Characterization of Silver Nanoparticle Aggregates:

UV-vis spectroscopy Study:

The aggregates of silver nanoparticles have been synthesized using silver nitrate as the precursor salt and dithizone as the capping agent. The successive changes of the absorption spectra of synthesized silver nanoparticles aggregates are shown in Figure 1. After the addition of AgNO_3 to the sodium iodide solution, the color of the solution changes from colorless to light yellow indicates the nucleation of the silver particles at their infancy. The UV absorption measurements of the synthesized nano particles were studied by dissolving them in solvents like DMSO, THF & CHCl_3 .

Figure 1 shows the UV-vis spectra of silver colloids obtained. The surface Plasmon resonance (SPR) band is broad indicating poly-dispersed nanoparticles. A smooth and narrow absorption band at 421 nm is observed in DMSO solvent of dithizone capped Ag-NP, whereas 452 nm is observed for dithizone in DMSO which clearly indicates the formation of silver colloids. UV-visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles. The optical absorption spectra of metal nanoparticles are dominated by surface Plasmon resonances (SPR), which shift to longer wavelengths with increasing particle size. The position and shape of plasmon absorption of silver nanoclusters are strongly dependent on the particle size, dielectric medium, and surface-adsorbed species. The surface plasmon absorption of silver nanoparticles have the short wavelength band in the visible region around 409 nm is due to the transverse electronic oscillation.

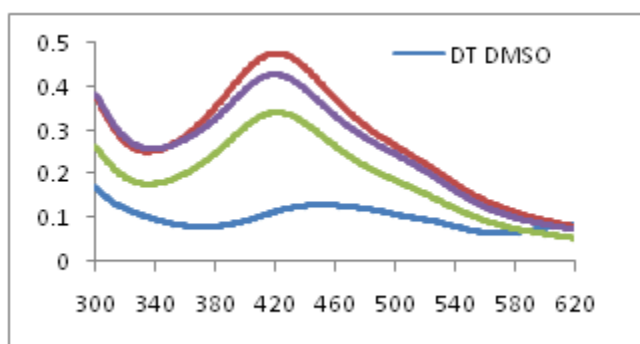


Fig. 1: UV-vis absorbance spectra of Dithizone & Dithizone capped AgNP in DMSO.

Scanning electron microscopy study:

The SEM images obtained for colloid is shown in figure 2. It is clear from the SEM images in figures 2, that the particles are nearly crystalline.

The Scherrer rings, characteristic of fcc silver is clearly observed, showing that the structure seen in the SEM image are nano crystalline in nature. It is observed that the silver nanoparticles are scattered over the surface and no aggregates are noticed under SEM. The difference in size is possibly due to the fact that nanoparticles are being formed at different times.

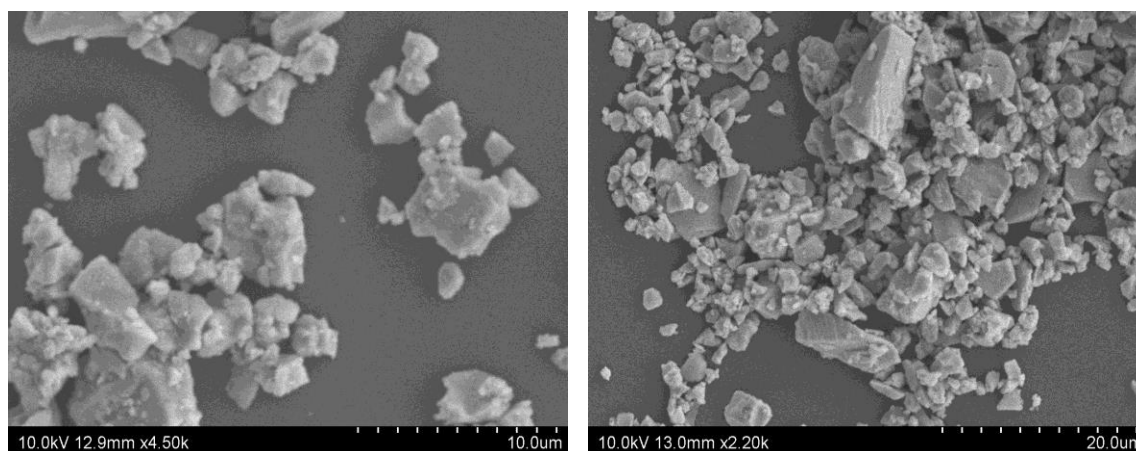


Figure 2: SEM images of synthesized Dithizone capped silver nanoparticles.

X-ray diffraction study:

XRD spectra of Ag-NPs measured in the small and wide angle region are shown in Fig.3. The XRD spectrum confirms the tendency of nano particles to form the organized structures, as seen from the peaks in the small angle XRD spectrum in Fig. 3. The peaks are broadened because of the nano crystalline nature of silver nanoparticles. By comparing with standard database values, all the peaks can be indexed to face-centered cubic (fcc) silver crystal structure. Three peaks at 2θ values of 38.099, 64.483 and 77.442, correspond to the (1 1 1), (2 0 0) and (2 2 0) planes of silver nanoparticle, respectively.

The XRD of different particle sizes of Ag-NPs capped with dithizone prepared in water as solvent. From this figure, it can be noticed that the particles appeared basically amorphous and abroad. Size-dependent and structure-specific features in diffraction patterns can be quite striking

The FTIR spectrum of the nano crystals provides additional information about the local molecular environment of dithizone. The FTIR spectra of the free dithizone and Ag nanoparticle capped by dithizone thiol are represented in Fig. 4. From the spectrum of free dithizone thiol, it can be noticed that the azo group appeared at $1580 - 1650 \text{ cm}^{-1}$, respectively. The weak band appeared at 2360 cm^{-1} is attributed to the S-H stretching vibration mode. The two bands appeared between 1380 and 1240 cm^{-1} are assigned to the stretching vibration of C-N group. The band appeared at $1627 - 1690 \text{ cm}^{-1}$ is attributed to the N-H primary stretching group. The band appeared at 756 cm^{-1} is assigned to C-S stretching mode. Generally, the vibrational spectrum of Ag-NPs capped with dithizone thiol molecule is similar to the spectrum of dithizone thiol indicating that, the organic molecules have indeed become a part of the nanoparticles.

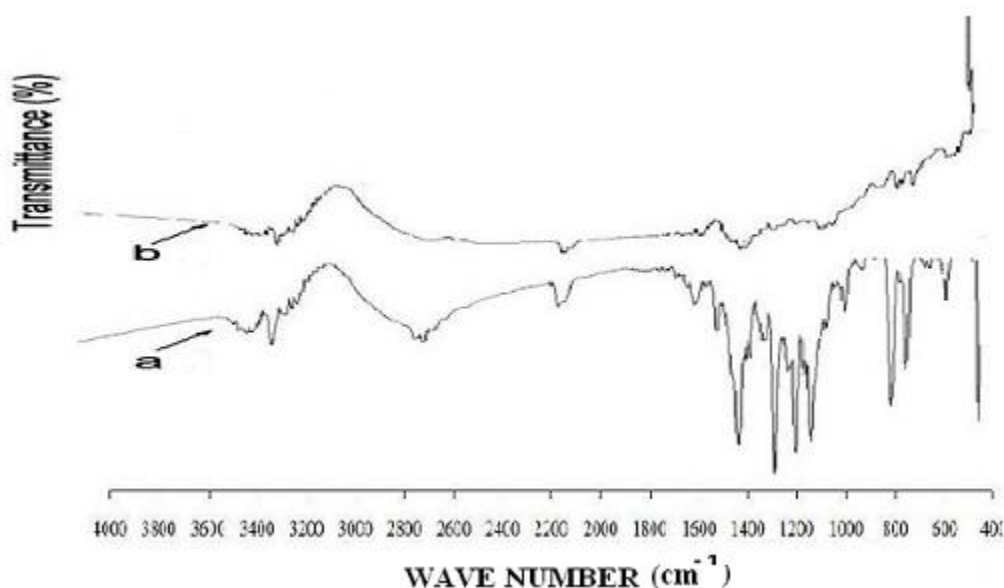


Figure 4: FT-IR spectra of (a) Dithizone; (b) Dithizone capped Ag-NP.

Pharmacology Protocol:

Human pathogenic bacteria species *Salmonella typhi*, *Vibrio cholera*, *Shigella dysenteriae*, *Staphylococcus aureus* are used in this study. These were collected from Department of Microbiology, Andhra Medical College, Visakhapatnam, Andhra Pradesh (India).

Preparation of bacterial inoculum:

The microorganisms were inoculated into Muller Hinton broth and incubated at $35 \pm 2^\circ\text{C}$ for 4 h. The turbidity of the resulting suspensions was diluted with MH broth to obtain a transmittance of 25.0 % at 580 nm. That percentage was found spectrophotometrically comparable to 1 McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0×10^8 CFU/ml. **The Bausch & Lomb® spectrophotometer, Model Spectronic 20** was used to adjust the transmittance of the working suspensions. This suspension used as inoculum.

Agar well diffusion assay:

The modified agar well diffusion method of Perez *et al.*, was employed. Each selective medium was inoculated with the microorganism suspended in Muller Hinton broth. Once the agar was solidified, it was punched with a six millimetres diameter wells and filled with required concentration of compounds and Ciprofloxacin (antibiotic) used as standard for positive control while Pure Solvents were used as negative control. Results were determined based on size of the inhibitory zone surrounding the wells containing the extract comparing with standard and blank. The diameter of zones of inhibition was measured in mm using HiMedia zone reader.

Minimum Inhibitory Concentration:

The minimum inhibitory concentration of compounds was determined using broth dilution assay. The medium containing different concentrations of compounds viz., 100mg - 100µg per ml prepared by serial dilution (10^{-1} dilution). After inoculation of culture, the tubes were incubated for 24 hours at 37°C . The MIC of each sample was determined by measuring the optical density in the spectrophotometer (Electronics India) at 580nm and compared the result with those of the non-inoculated broth used as blank. Control was prepared with media and inoculum only without compounds. The experiment was conducted according to NCCLS standards (Now as CLSI).

The synthesized Ag-NPs showed significant inhibitory activity against various human pathogenic bacteria species, like *S. typhi*; *V. Cholera*; *S. dysenteriae*; *E. Faecalis*. *E. faecalis* showed resistance to Ag-NPs while *V. cholerae* showed sensitivity to compounds when compared with compounds inhibitory potential against *S. typhi* and *S. dysenteriae*. From Table 1, synthesized compound showed dose dependent inhibitory activity. Zone of inhibition was increases with concentration of compound. The Minimum Inhibitory Concentration range found to be between 1 to 100mg/ml (Table 1). 1mg/ml is the lowest MIC of compound against *V. Cholera*. The results are comparable with antibiotic Cprofloxacin.

Table 1: Anti bacterial activity of compounds against human pathogens.

	Zone of inhibition(mm)*			
	<i>S. typhi</i>	<i>V. Cholera</i>	<i>S. dysenteriae</i>	<i>E. faecalis</i>
Ag-NPs ⁺	10	11	10	9
DMSO	8	7	7	7
Ciprofloxacin	17	17	15	15

⁺ 50 μ g of compound (1 μ g/ μ l concentrated), * 6mm is the well size

Table 5.3: Minimum Inhibitory Concentration of compounds determined by Broth Dilution Assay.

Compound ⁺	MIC(mg/ml)			
	<i>S. typhi</i>	<i>V. Cholera</i>	<i>S. dysenteriae</i>	<i>E. faecalis</i>
Ag-NPs ⁺	10	100	100	\geq 100
DMSO	ND	ND	ND	ND
Ciprofloxacin	1	1	1	10

'ND' Not Determined

Conclusion: In summary, the work reported here has revealed several key features on the aggregate formation of silver colloids via their adsorptive interactions with the organic compound Dithizone, containing thiol functional groups. The physicochemical properties of

silver nanoparticles prepared by chemical reduction are strongly determined by the nature of the used chemical reductant. In particular, silver nanoparticles prepared by reduction with Dithizone and sodiumborohydroxide show significant differences in physical (morphology, plasmon resonance) and chemical (aggregation) properties. This methodology can be applied to the gram level synthesis of the monodispersed silver nanoparticles that are extremely stable. Thus, the method is advantageous in the sense that metallic particles can be separated from the free ligands, obtained in the form of powder, and redispersed in the aqueous phase without any kind of size and shape deformation of the particles.

References:

1. Tao A, Sinsermsuksaku P, Yang P, *Angew Chem Int Ed*, 2006, **45**, 4597.
2. Wiley B, Sun Y, Mayers B, Xi Y, *Chem-Eur J*, 2005, **11**, 454.
3. Lee P. C, Meisel D, *J Phys Chem*, 1982, **86**, 3391.
4. Shirtcliffe N, Nickel U, Schneider S, *J Colloid Interface Sci*, 1999, **211**, 122.
5. Nickel U, Castell A. Z, Poppl K, Schneider S, *Langmuir*, 2000, **16**, 9087.
6. Chou K-S, Ren C-Y, *Mater Chem Phys*, 2000, **64**, 241.
7. Evanoff Jr D, Chumanov G. J, *J Phys Chem B*, 2004, **108**, 13948.
8. Sondi I, Goia D. V, Matijević E, *J Colloid Interface Sci*, 2003, **260**, 75.
9. Merga G, Wilson R, Lynn G, Milosavljevic B. H, Meisel D, *J Phys Chem C*, 2007, **111**, 12220.
10. Creighton J. A, Blatchford C. G, Albrecht M. J, *J Chem Soc Faraday Trans*, 1979, **75**, 7902.
11. Ahmadi T. S, Wang Z. L, Green T. C, Henglein A, El-Sayed, *M. Science*, 1996, **272**, 1924.
12. Kapoor S, Lawless D, Kennepohl P, Meisel D, Serpone N, *Langmuir*, 1994, **10**, 3018.
13. Henglein A, *Chem Rev*, 1989, **89**, 1861.
14. Gutiérrez M, Henglein A, *J Phys Chem*, 1993, **97**, 11368.
15. Ershov B. G, Janata E, Henglein A, *J Phys Chem*, 1993, **97**, 339.
16. Creighton J, Blatchford C, Albrecht M, *Photochem Photobiol*, 1994, **60**, 605.
17. Schneider S, Halbig P, Grau H, Nickel M, *J Chem Soc Faraday Trans*, 1979, **75**, 790.

18. Emory S, Nie S, *Anal Chem*, 1997, **69**, 2361.
19. Schneider S, Halbig P, Grau H, Nickel U, *Photchem Photobiol*, 1994, **60**, 605.
20. Schirtcliffe N, Nickel U, Schneider S, *J Colloid Interface Sci*, 1999, **211**, 122.
21. Rivas L, Sanchez-Cortes S, Garcia-Ramos J. V, Morcillo G, *Langmuir*, 2001, **17**, 574.