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Synthesis of 4-Amino-5- Mercapto-3-Methyl Triazole Capped Silver Nanoparticles and Study of Their Biological Properties

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Abstract:

Silver nanoparticles capped with 4-amino-5-mercapto-3-methyl triazole (AMMT) are synthesized by chemical reduction method and are characterized by UV-Visible, XRD, TEM, SEM studies. The nanoparticles showed maximum optical density at 419nm.The size and shape of synthesized nanoparticles obtained by TEM analysis are spherical and uniform with an average size of 12±2nm. The size of particles obtained by TEM is in good agreement with XRD studies. The anti bacterial impact of synthesized nanoparticles is assessed at various doses 25µg, 50µg, 75µg, 100 µg. They showed potent inhibitory activity against human pathogenic bacteria compared to standard antibiotic ciprofloxacin.

Key words: Silver nanoparticles, 4-amino-5-mercapto-3-methyl triazole (AMMT), Capping agent, Antibacterial activity

Introduction:

Chemical reduction is the generally applied method for synthesis of silver nanoparticles[1,2]. The commonly used reductants are sodium borohydride, ascorbate, elemental hydrogen and citrate[3-11]. Sodium borohydride is a very strong reducing agent to produce small sized nanoparticles. The synthesis of nanoparticles by chemical reduction methods is generally performed in the presence of stabilizer to prevent agglomeration of colloids.[12-16] 4-amino-5-mercapto-3-methyl triazole is an important triazole derivative having broad spectrum of

biological activity. Due to its structural feature, it can also act as good capping agent hence stabilizes the size of synthesized silver nanoparticles and enhances the biological activity of silver nanoparticles.

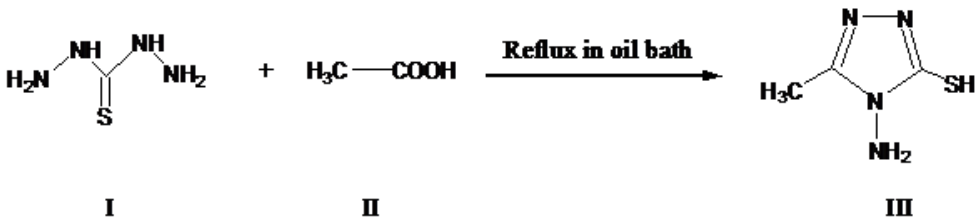
Reagents and Instruments:

All reagents used were AR grade. Silver nitrate was obtained from National refinery pvt .Ltd and 0.1 molar aqueous solution was used as a stock solution. Sodium borohydride was obtained from Merck, India. Organic free water was used throughout the experiment.

UV-Visible spectra were recorded on Shimadzu UV-Visible spectrophotometer and solutions were taken in a 1cm well stoppered quartz cuvette. The formation of single phase compound was checked by X-ray diffraction (XRD) technique. The XRD pattern was taken with X-ray diffractometer (XPERT-PRO) at room temperature using CuK_{α} radiation $\lambda = 1.5406\ A^0$. Over a wide range bragg angles ($30^0 \leq 2\ \theta \leq 85^0$). F20 Tecnai High Resolution microscope (Philips, Netherlands) was used to obtain TEM micrograph of Ag-NP's at 20K data. SEM micrograph of Ag-Np's was obtained on NOVA-230 microscope with an operating voltage of 10kv.

Synthesis of 4-amino – 5-mercapto – 3-methyl triazole:

4-amino -5-mercapto-3-methyl triazole was prepared by conventional as well as microwave methodologies. A mixture of thiocarbohyrazide (2.5g, 5m mol) and glacial acetic acid (15ml) was refluxed on an oil bath for 5 hrs. The reaction mixture was cooled to room temperature and excess solvent was distilled off under reduced pressure. The residual solid was crystallized from methanol and shining yellow crystals were obtained. The reaction was also carried out with domestic microwave oven. The time taken for this was only 5 mins.



Scheme 1: Synthesis of 4-amino-5-mercapto-3-methyl triazole

The ^{1}H NMR spectrum of 4-amino -5-mercapto -3-methyl triazole (Fig: 1) was recorded in DMSO (d_6) and compared authenticate spectra. Peaks at δ 12.2, 7.4, 2.2 were assigned to SH, NH_2, CH_3 respectively.

The ^{13}C NMR spectrum of 4-amino – 5-mercapto -3-methyl triazole (Fig: 2) was recorded in acetone. Peaks at 169.22, 155.55, 23 ppm were assigned to (-N=C-S), (-N=C-C), (CH_3) respectively.

Major IR spectrum bands of 4-amino -5-mercapto-3-methyl triazole showed 3459 cm^{-1} υ ($-NH_2$), 2940 cm^{-1} υ, (CH_3) , 1541 cm^{-1} υ (C=N of ring).

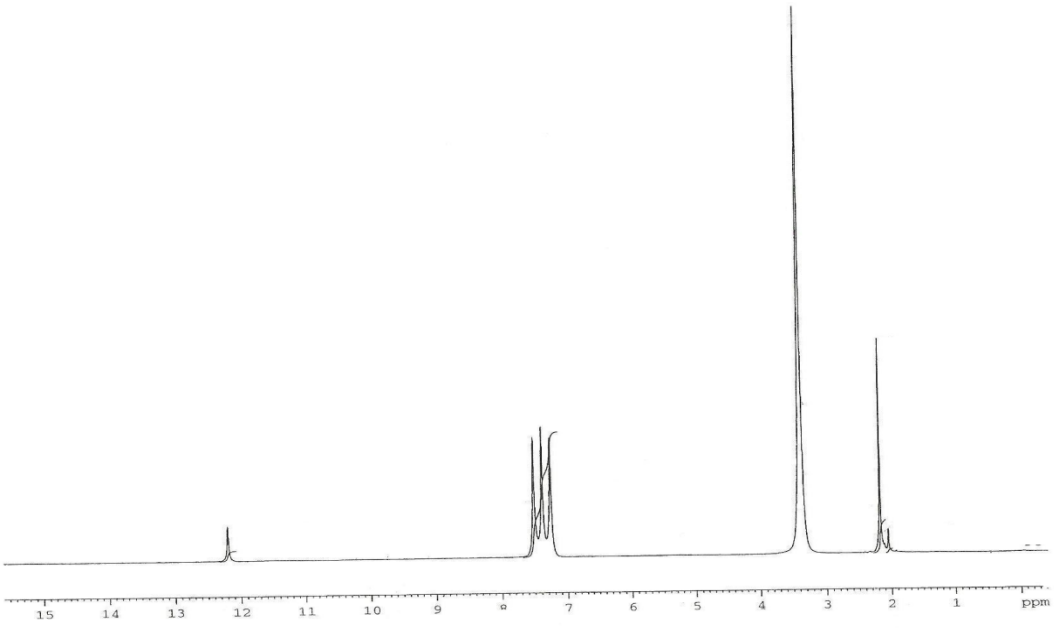


Fig 1: ^{1}H Spectrum of Compound III

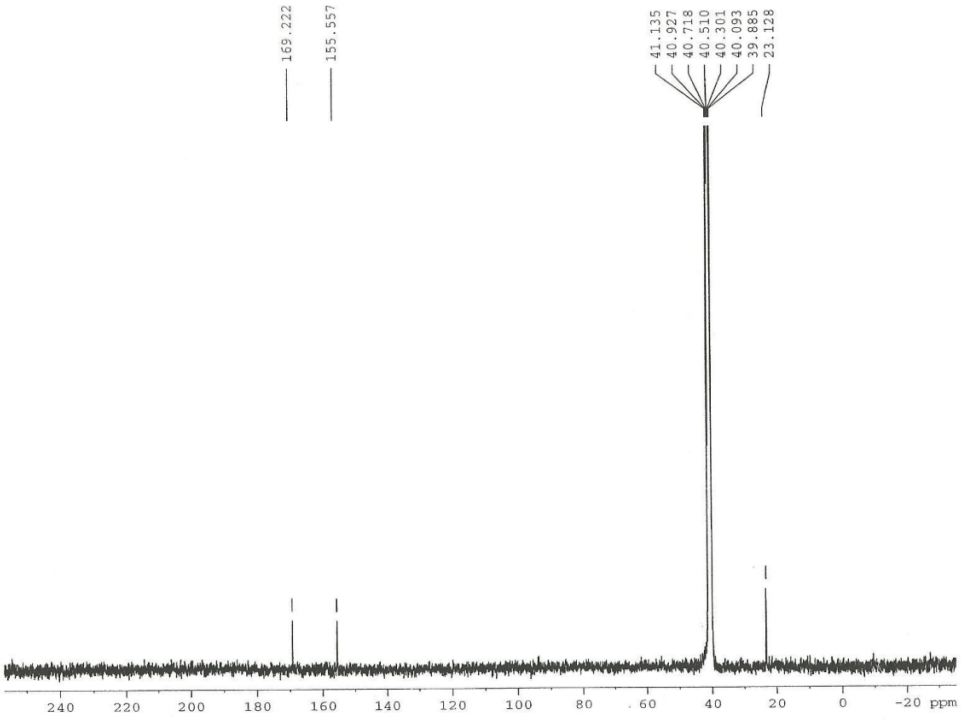


Fig 2: ^{13}C Spectrum of Compound III

Synthesis of Silver Nanoparticles:

The silver nanoparticles were synthesized from silver nitrate salt precursor and stabilized by a capping agent, 4-amino-5-mercapto-3-methyl triazole. Yellow colour colloidal silver is obtained by treating the silver nitrate with ice - cold sodium borohydride[17]. The optimal set of conditions required to synthesize silver nanoparticles is described in the following section. The chemical reaction is the reduction of silver nitrate with sodium borohydride. Excess sodium borohydride is needed for both the purposes to reduce ionic silver and to stabilize the silver nanoparticles form that.

$$AgNO_3 + NaBH_4 \rightarrow Ag^0 + 1\ H + B\ H_3 + NaNO_3$$

The particle size of silver nanoparticles obtained is 12±2nm. The surface Plasmon absorbance is near 400nm and peak width at half maximum (PWHM) is 50-70nm. The relationship between aggregation and optical properties is investigated along with a method to protect the particles using 4-amino-5-mercapto-3-methyl triazole.

Protocol

Reactions are carried in clean dry 125 mL Erlenmeyer flasks. A 10mL volume of 1.0 mM silver nitrate was added drop wise to 30mL of 2.0 mM sodium borohydride solution that had been cooled in ice – bath. The reaction mixture was stirred vigorously on a magnetic stir plate. After addition of 2ml of silver nitrate, the colour of the solution turned light yellow and turned to brighter yellow when all the silver nitrate had been added. The addition process took about three minutes, then stirring was stopped and stir bar removed. The clear yellow colloidal silver is stable at room temperature stored in a transparent vial. Upon aggregation the colloidal silver solution turns yellow, violet and then grayish.(Fig:3).

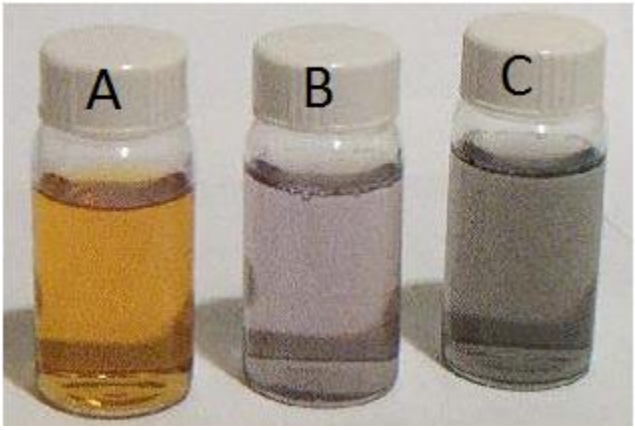


Fig 3: Colloidal silver in various stages of aggregation A) dark yellow B) Violet and C) Grayish as aggregation proceeds.

Result and Discussion:

Reaction conditions including relative quantities of reagents (both the absolute number of moles of each reactant as well as their relative molarities) and stirring time must be carefully controlled to obtain stable yellow colloidal silver. If stirring is continued once all of the silver

nitrate has been added, aggregation begins as the yellow sol first turns a darker yellow, then violet, and eventually grayish, after which the colloid breaks down and particles settle out. Similar aggregation may also occur if the reaction is interrupted before all of the silver salt has been added. It was also found that the initial concentration of sodium borohydride must be twice that of silver nitrate: $[NaBH_4]/ [AgNO_3]$ =2.0. When concentration was varied from 2.0mM while using 1.0 mM silver nitrate, breakdown of the product took place in less than an hour.

Characterization of silver nanoparticle:

Silver nanoparticles were examined using UV-VIS spectroscopy (Shimadzu UV-Visible spectro photometer).

Optical Characterization:

The distinctive colors of colloidal silver are due to a phenomenon known as Plasmon absorbance. Incident light creates oscillations in conduction electrons on the surface of the nanoparticles and electromagnetic radiation is absorbed. The spectrum of the clear yellow colloidal silver from the synthesis above is shown in Fig: 4. The Plasmon resonance produces a peak near 419nm, with PWHM of 50 to 70nm.

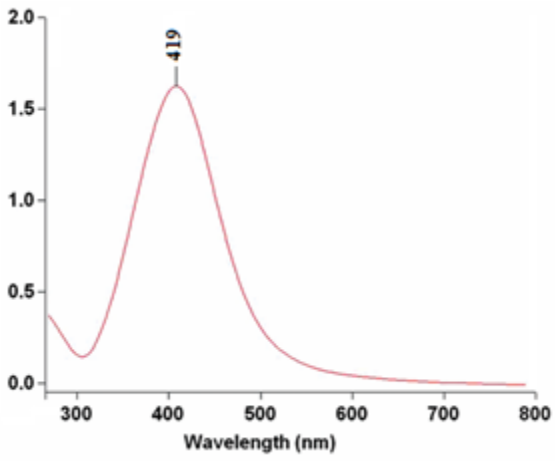


Fig 4: UV-Vis absorbance spectra of 4-aino-5-mercapto-3-methyl triazole capped AgNP

XRD Study:

Fig: **5** shows the XRD pattern of Capped Ag nanoparticles obtained. By comparing with standard database values, all the peaks can be indexed to face-centered cubic (fcc) silver crystal structure. Three peaks at 2y values of 39.79, 44.483 and 47.442, correspond to the (1 1 1), (2 0 0) and (2 2 0) planes of silver nanoparticle, respectively. The average crystallite size according to Scherrer equation calculated using the highest peak of the 39.79 is found to be 12.61 nm.

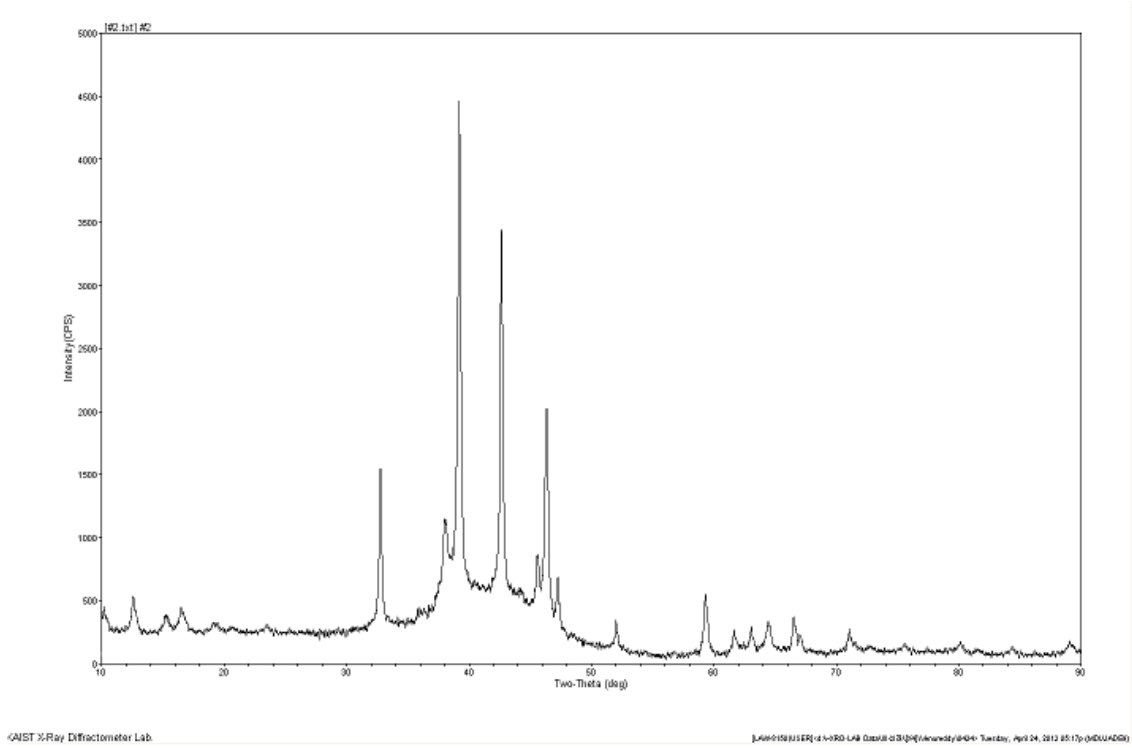


Fig 5: XRD patterns 4-amino-5-mercapto-3-methyl triazole capped silver nanoparticles.

Particle size measurement using TEM:

Silver nanoparticles that produced were examined using transmission electron microscopy (Phillips, Netherland Model: Technai20). A sample of silver nanoparticles from a freshly synthesized clear yellow sol was prepared by drying a small drop on a carbon – coated 200 mesh copper grid. The TEM image of one region of the sample is shown in Fig 6. The TEM image shows the silver particles are spherical with sizes of 12±2nm.

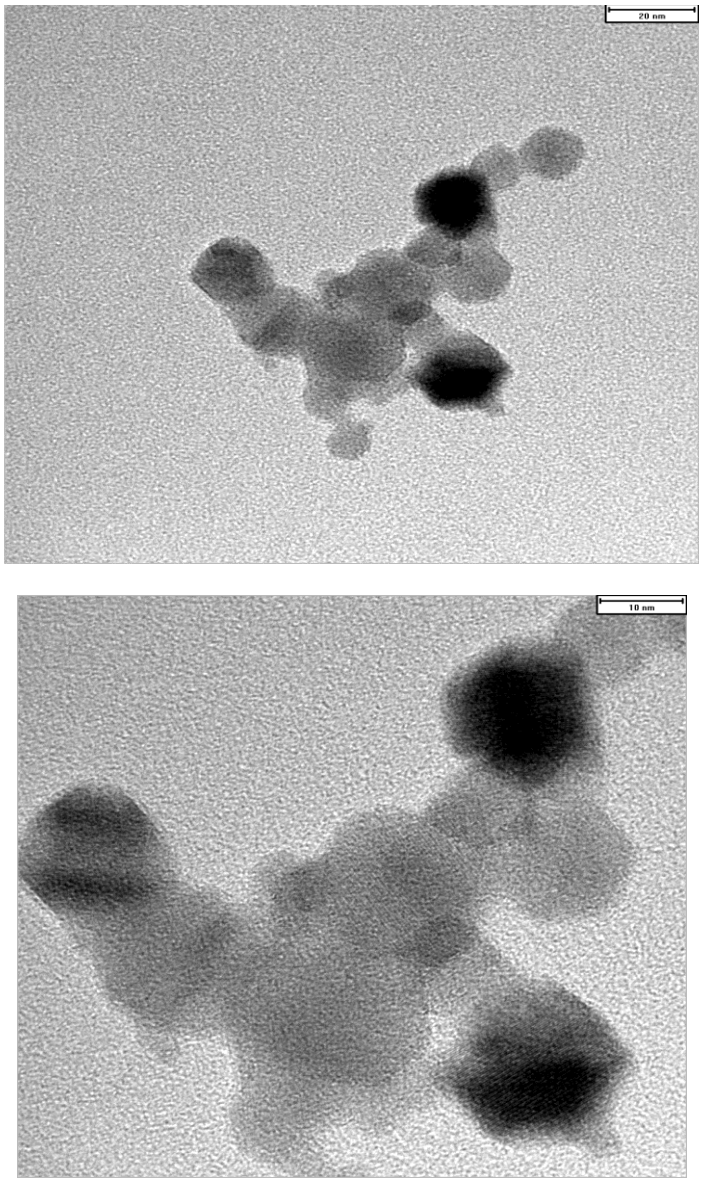
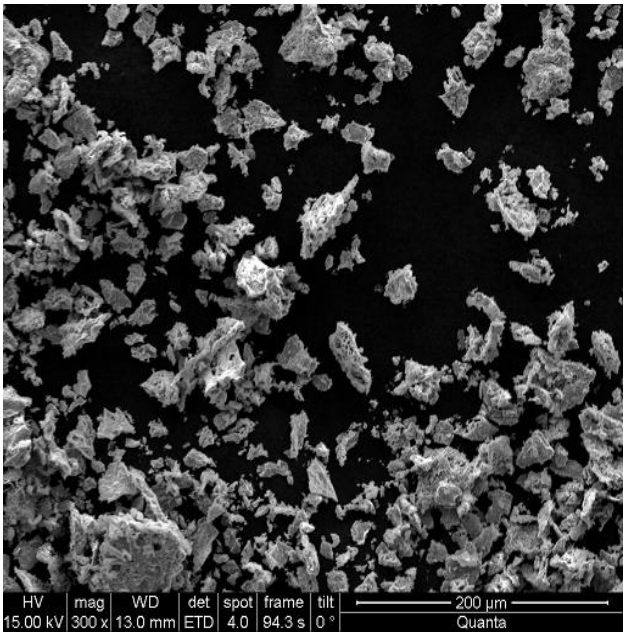


Fig 6: Transmission electron micrographs of the silver nanoparticles used in this work (a) The bar marker represents 20nm (b) 10 nm

Scanning electron microscopy study:

Fig: 7 shows SEM image of synthesized silver nanoparticles. SEM image shows that particles are nearly crystalline in nature. It is noticed that nparticles are dispersed on the surface without any aggregation. The variation in the size of particles is probably due to the fact that, the nanoparticles might have formed at different times.



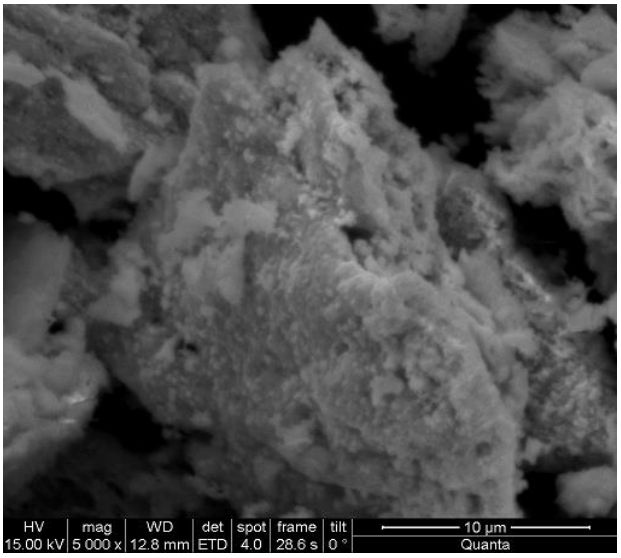


Fig 7: SEM images of 4-amino-5-mercapto-3-methyl triazole onto a surface of silver nanoparticles

Antimicrobial studies of 4-amino-5-mercapto-3-methyl triazole capped silver nanoparticles:

Human pathogenic bacteria:

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 inoculums:

The microorganisms were inoculated into Muller Hinton broth and incubated at 35 ± 2^0 C for 4h.The turbidity of the resulting suspensions was 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 x 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 Hi-Media zone reader.

Minimum inhibitory concentration:

The minimum inhibitory concentration of synthesized Ag-NP compound was determined using broth dilution assay[18,19]. 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^{0} 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 inoculums only without compounds. The experiment was conducted according to NCCLS standards (Now as CLSI).

Results:

The synthesized Ag-NP s showed significant inhibitory activity against various human pathogenic bacteria species, like *S.Typhi; V.Cholera ; S. dysenteriae; E.Faecalis*. It was found that the synthesized Ag-NP compound was potent inhibitory agent when compared with standard antibiotic.12mm was the highest zone of inhibition showed by compound against *V.Cholerae* (Table I). *E. faecalis* showed resistance to Ag-Nps while *V. Cholera* showed sensitivity to compounds when compared with compounds inhibitory potential against *S.typhi* and *S.dysenteriae*. From table III, synthesized Ag-NP compounds 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 II). 1mg/ml is the lowest MIC of compound against *V.Cholera*. The results are comparable with antibiotic Ciprofloxacin.

Table I: Antibacterial activity of compounds against human pathogens.

	Zone of inhibition(mm)*			
	S. typhi	*V. Cholera*	*S. dysenteriae*	*E. faecalis*
$AgNPs^{+}$	11	12	11	10
DMSO	8	7	7	7
Ciprofloxacin	17	17	15	15

+50 µg of compound (1 µg/µl concentrated), * 6 mm is the well size

Table II: Minimum in Concentration of compounds determined by Broth Dilution Assay.

$Compound^{+}$	MIC(mg/ml)			
	S. typhi	*V. Cholera*	*S. dysenteriae*	*E. faecalis*
$AgNPs^{+}$	100	10	100	100
DMSO	ND	ND	ND	ND
Ciprofloxacin	1	1	1	10

'ND' Not Determined

Table III: Dose dependent inhibitory effect of AgNP+ on various human pathogenic bacteria species.

Type of Human Pathogenic Bacterial sps.	Causing Disease	Zone of inhibition(mm) at different Doses			
		25µg	50µg	75µg	100µg
Salmonella typhi	Typhoid fever	12	15	17	18
Vibrio cholera	Cholera	14	16	17	18
Shigella dysenteriae	Dysentery	12	13	15	16
Enterococcus faecalis	Gastrointestinal infections	10	12	13	15

Conclusions:

4-amino – 5-mercapto – 3-methyl triazole capped silver nanoparticles were synthesized by chemical reduction method. Their morphology, photo physical and biological activities were studied. Their photo physical activities showed that the produced nanoparticles are in uniform size and spherical shape. The microbial studies of these compounds showed good activity against human pathogenic bacteria like *S. typhi; V. Cholera; S. dysenteriae; E. Faecalis.* 12mm was the highest zone of inhibition showed by compound against *V. Cholera*. This compound showed Dose dependent inhibitory activity. Zone of inhibition was increased with concentration of compound. The microbial activity of synthesized Ag-NP Compound is comparable with that of standard antibiotic Ciprofloxacin.

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