Antibacterial Activity Emphasized Sulphamerazine Capped Silver Nanoparticles With Their Synthesis & Characterization

U. Nagababu, Govindh Boddeti, Bhagavathula. S. Diwakar and Anindita Chatterjee

Abstract: The research paper describes a simple one-pot and single phase synthesis of silver nanoparticles (AgNP) coated with sulfamerazine (SM) through chemical reduction approach by using $NaBH_4$ at room temperature. The structural and morphological analysis was performed by ultraviolet-visibile spectroscopy (UV-Vis), infrared spectroscopy (FTIR), powder X-ray diffraction (PXRD), scanning electron microscope (SEM) and high resolution transmission electron microscope (HRTEM) techniques. The obtained sulfamerazine encapsulated silver nanoparticles (SMAg-NPs) with an average size of 29.6 nm in spherical shape. The synthesized SMAg-Nps were further screened for antibacterial evaluation against gram positive and gram negative bacteria employing disc diffusion method. The results attested the enhancement in antibacterial properties of SMAg-NPs.

Index Terms: Antibacterial, SMAg-NP's, Sulphamerazine, PXRD, SEM, HRTEM, Diffusion method.

1. INTRODUCTION

The interest in developing the metal based nanoparticles has been increasing day to day due to potential applications in many research fields such as contact action, [1a,b] sensors,[2] physics, and optics.[3,4] But there are some limitations like particle aggregation, unit cell reactions and poor solubility which influence the properties of metal nanoparticles over a period of time. The literature reports attested the self assembly of organic molecules on the surface will improve the solubility and stability of metal nanoparticles. Also, the encapsulation of metal nanoparticles with organic compounds will have an effect on the gross properties and found enhanced applications in asymmetric catalysis,[5] medical specialty, and drug delivery.[6]Lately, silver has been found applications in medical fileds[7] but the solubility of silver and silver salts (e.g., $AgNO_3$) render it impractical in several clinical applications. Nanometer size silver particles have attracted the researchers due to the ease in synthesizing with desired size and shapes in aqueous/ organic phases [11-15] and also the flexibility to create composite thin films with several applications in the medical diagnostic fields. Furthermore, Ag-Nps were used with improved performance in semiconductors; article of clothing with material modification is an incontestable statement. For example, AgNPs were successfully coated on medical devices which were used in infection free transplantation.[16,17]

Sulfa drugs were well known antibacterial drugs with wide applications in veterinary fields. Sulfa drugs were frequently used in treating infections particularly for patients who are hypersensitive to antibiotics. The chemistry of sulfonamides in medicinal agents became a large & important research area in pharmaceutical sciences. In the present work, it was reported the formation, characterization and biological screening of the sulfamerazine (Figure-1) encapsulated silver nanoparticles (SMAg-NPs). The silver nanoparticles were resulted with uniform shapes and size through a chemical reaction with sodium borohydride. This methodology utilizes the benefits of convenient and eases preparation, particularly to get AgNPs with uniform shape and size. Time-dependent UV-vis analysis and TEM are used to identify the formation of SMAg-NPs.

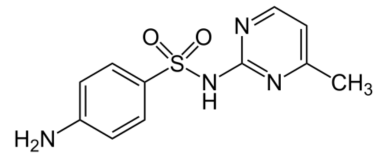

***Figure**-1: Sulfamerazine*

2 EXPERIMENTAL SECTION:

2.1 Materials: Silver nitrate (>99%), Sodium borohydride ($NaBH_4$), Sulfamerazine were procured from SigmaAldrich. All materials were used without further purification.

Instruments: The samples were characterized by powder X-ray diffraction (PXRD) recorded on XPERT-PRO diffractometer by using copper Kα (λ=1.54059Å) radiation. Absorbance spectra were measured by a Shimadzu UV-3100 photometer. TEM measurements were taken on Hitachi H-8100 IV operated at two hundred kilovolt. All the measurements were taken at room temperature.

2.2 Preparation of Sulfamerazine capped Silver nanoparticles: In a typical synthesis method, 2.5 mL of $AgNO_3$ (10^{-2} M) was mixed to 75 mL of DI water. In the next step, 5 mL of Sulfamerazine drug (10^{-2} M) was added as stabilizer under stirring. Further, 20 ml of $NaBH_4$ solution was added drop wise to the above reaction mixture, and stirred for about 20 min. The obtained silver colloid was observed by the change of the color initially from colorless to white, then to brown, and finally to gray color.[Figure-2]

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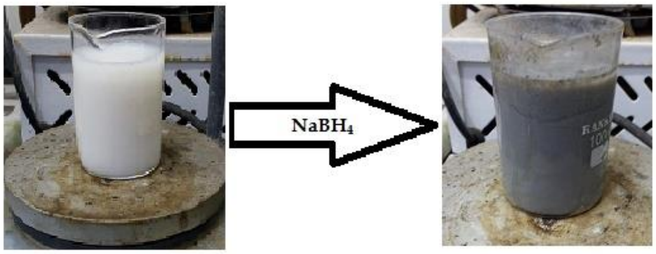


Figure-2: *Formation of AgSM-NPs*

2.3 Antibacterial studies:

The microorganisms were initially inoculated into Muller Hinton broth and incubated at 35 ± 2°C for four hours. The murkiness of the ensuing suspensions was diluted with MH broth to get a coefficient of 25.0% at 580 nm. That % was found spectrophotometrically akin to 1 McFarland murkiness normal standard. This level of murkiness is akin to more or less 3.0 × 10^8 CFU/ml. The Bausch & Lomb® photometer, Model Spectronic 20 was used to modify the coefficient of the operating suspensions. This suspension used as substance/ inoculum.

2.4 Agar- well diffusion assay:

Perez et al., introduced the modified agar well diffusion method was utilized in this study.[18] Every selective medium was inoculated with the organism suspended in Muller Hinton broth.[19,20] Results were tabulated based on size of the repressive zone of inhibition.

3 Results and Discussion:

Initially, SMAg-NPs were achieved by chemical reduction technique. The formation of Sulfamerazine-stabilized SMAg-NPs was characterized by UV-Vis spectra and SEM. Figure 3 shows the absorption spectra of SMAg-NPs. A representative absorption peak about 412 nm was noticed after synthesis. This peak confirms the formation of SMAg-NPs. The absorption spectra of Sulfamerazine drug has been measured separately under similar conditions which forms a distinct absorption band at around 475 nm as depicted in figure 3. The observation of new surface plasmon absorption band at 412 from the mixture confirmed the formation of sulfamerazine capped silver nanoparticles SMAg-NPs by this method.

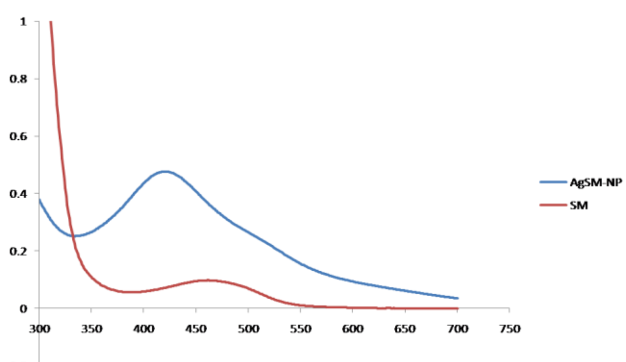


Figure 3: *The UV–Vis spectra of SMAg-NPs*

Figure 4 shows the SEM micrograph of SMAg-NPs. SEM image represents the morphology of the nanoparticles. It is observed that the scattered crystalline nature of silver nanoparticles without aggregated state. The shape of the particles is not clear from SEM microscopy.

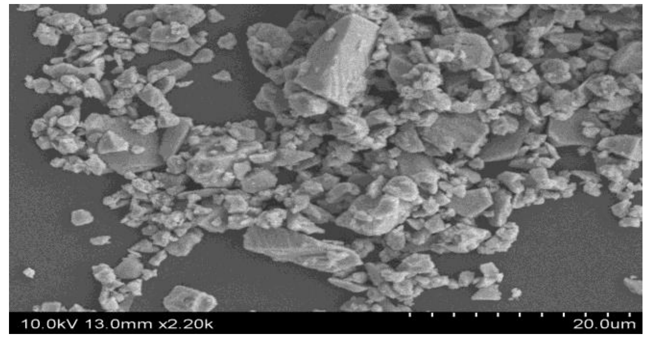


Figure 4: *The scanning electron micrograph of SMAg-NP's*

Transmission microscopy has been carried to analyze the particular size and form of the SMAg-NPs and the resulted images were depicted in Figure 5. It was noticed from the TEM pictures in Figures 5a and 5b, that the obtained nanoparticles are almost spherical in nature having a size of 29 nm. From the figure, it's observed that the SMAg-NPs are spread over the surface and no aggregation noticed. Variation in size is probably due to that the nanoparticles are being fashioned at totally different times.

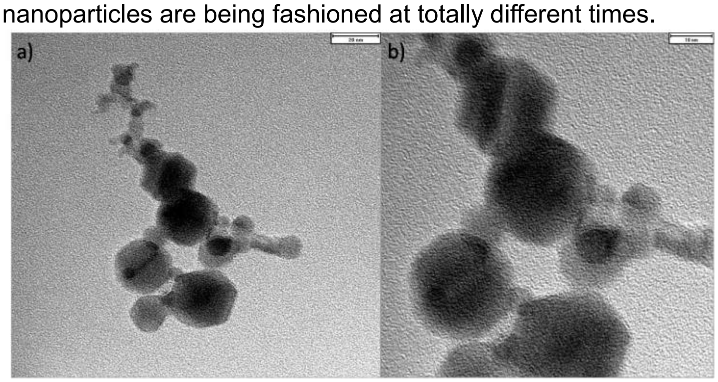


Figure 5: ***a)*** *HR-TEM of SMAg-NPs showing spherical shapes and bar represents 20 nm; b) HR-TEM of SMAg-NPs showing spherical shapes and bar represents 10 nm*

Figure-6 represents the PXRD graph of obtained SMAg-NPs by chemical reduction method. The optical phenomenon peaks appeared at 41.81, 44.79, 51.89, 54.76 and 55.98 represents FCC crystal structure of silver nanoparticles. The common crystallization size per Scherrer's equation using the highest intensity peak of the 41.81 is with 29.6 nm. These results were in concurrence with TEM studies.

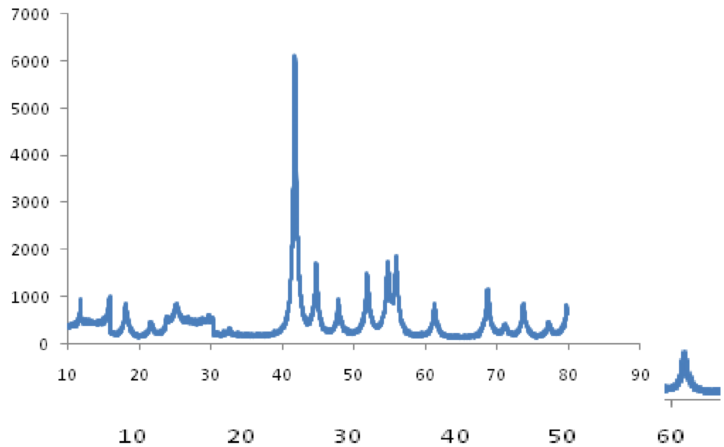


Figure 6: *Powder X-ray diffraction pattern of SMAg-NPs*

The compositional analysis of the SMAg-NPs was performed using the EDX spectrum and is presented in Figure-7. Throughout the scanning range, a clear peak indicating presence of elements like silver including the sulfur, nitrogen and carbon molecules of sulfamerazine. The result attested that the synthesized product consisted of sulfamerazine capped silver nanoparticles SMAg-NPs.

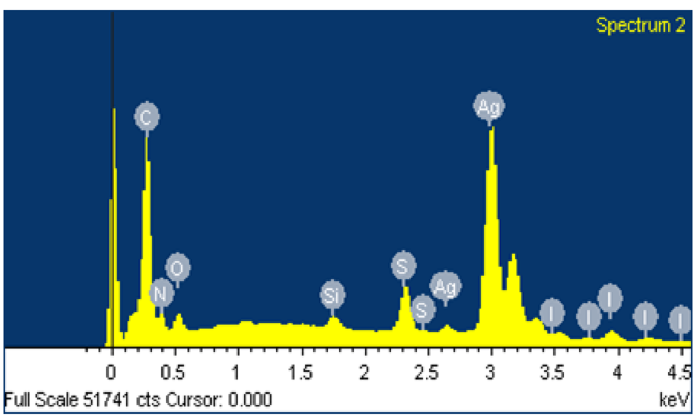
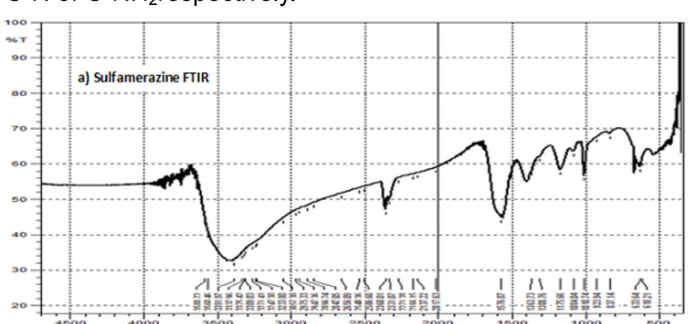


Figure 7: *EDX diffraction pattern of AgSM-NP's*

FTIR study was used to identify the possible mechanism responsible for capping of Ag-NPs synthesized using Sulfamerazine. Figure-8 depicts the FTIR spectrum of obtained SMAg-NPs. In Figure 8, (a) FTIR spectra of Sulfamerazine drug before synthesis and (b) sulfamerazine capped SMAg-NPs. It was observed from Figure 8 (a) only Sulfamerazine drug showed absorptions bands at 3582.90cm^{-1} is due to (NH) and the band observed at 2320 cm^{-1}arise from C-N of C-NH_2respectively.



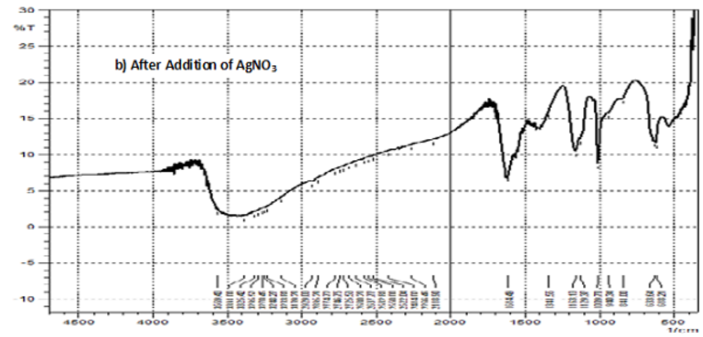


Figure8: *FT-IR spectra of (a) Sulfamerazine; (b) Sulfamerazine capped SMAg-NPs.*

The peak observed at 1399 cm^{-1} is due to S=O stretching mode. Whereas in Figure 8b the strong band at 2320 cm^{-1} was disappeared which indicates the amine group of sulfamerazine is bound with silver Ag(0) to achieve stability.

4 In vitro bactericidal studies:

The bactericidal studies were carried out using Muller Hilton Broth method. The SMAg-NPs showed excellent action against bacterial strains. The minimum inhibitory concentration (MIC) of sulfamerazine and SMAg-NPs was calculated with the lowest concentration at which the bacterial growth was inhibited. The MIC values of naked sulfamerazine for gram-negative strains were found to be ≥ 4.2μg/ml whereas the antibacterial activity of SMAg-NPs decreased considerably. The results are shown in Table 1. The sulfamerazine in conjunction with Ag-NPs is not an potent inhibitor for gram-negative bacteria.

Table 1. The effects of nanoparticles on Gram-positive and Gram-negative Zone of Inhibition in mm (50μl of compound from 1mg/ml)

Compounds	ML	MP	BS	KP	EC	PS
Sulfamerazine	25	22	23	22	21	20
AgSM-NPs	27	29	25	23	21	23
DMSO	13	10	11	10	11	16
Ampicillin	29.4	30	29.1	25.1	30.5	30.1

Micrococcus luteus (ML), Micrococcus proteus (MP), Bacillus subtilis(BS), Klebsiella pneumoniae(KP), Escherichia coli (EC) and Pseudomonas syringae (PS)

In our investigation, the results were showed that, sulfamerazine capped silver nanoparticles SMAg-NPs will enhance medicinal drug activates against gram-positive bacteria, however not on gram-negative bacteria (Fig 9).

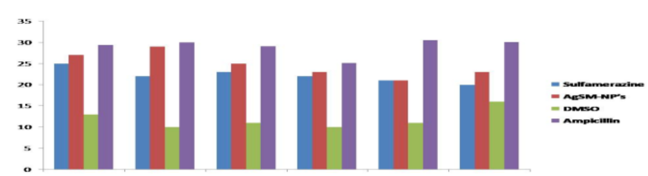


Fig 9: *Antibacterial aactivity of SMAgNPs on different bacterial strains*

From the results, it was noticed that there was an increment in the bactericidal activity of sulfamerazine-encapsulated SMAg-NPs to the pure sulfamerazine. Especially the results obtained for Micrococcus proteus (MP) showed almost equal to that of the ampicillin antibiotic. Whereas, the antibacterial activity on EC & Sulfamerazine are same and comparatively less than reference antibiotic.

5 CONCLUSION:

The present research work is incontestable to sulfamerazine, which can be encapsulated with Ag-NPs for the antimicrobial activity against gram-positive bacterium particularly Micrococcus proteus (MP). In vitro disinfectant experimnts showed that the (SM)-encapsulated Ag-NPs substantial activity. In line with these findings, the synthesized nanoparticle system could offer a replacement of effective antimicrobial agents.

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