RESEARCH PAPER

Development of Sulfadiazine by Nano Method and Study Its Effect Against Multi-Drug Resistant Bacteria

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ARTICLE INFO

Article History:
Received 03 July 2024
Accepted 21 September 2024
Published 01 October 2024

Keywords:
EDX
Nano-Antimicrobial
Pseudomonas aeruginosa
SEM
Staphylococcus aureus

ABSTRACT

Nanotechnology-based antibiotic synthesis is one of the most crucial contemporary strategies for preventing antibiotic resistance. Synthesis of nano sulfadiazine antibiotic was nanoscale made by using standard sulfadiazine in this study, Physically, without using any chemicals. The resulting nanocomposite was examined using XRD, EDX, and SEM methods, and their characteristics were contrasted with those of nano sulfadiazine, whose average crystal size was 48.32 nm. The ability of nano sulfadiazine to prevent bacteria growth was examined by measuring the minimum inhibitory concentration of two species of bacteria using an ELISA technique; it was compared to regular sulfadiazine particles. The results of the broth microdilution method with standard sulfadiazine gradient (concentration) ranges of 8-1024µg/ml show the MIC ranging 64-128µg/ml among five MDR *P. aeruginosa* isolates and five MDR *S. aureus* isolates. While the results of Nano-sulfadiazine MIC ranged from 16-32µg/ml for *P. aeruginosa*, isolates and 32µg/ml for *S. aureus* isolates.

How to cite this article
Saleem A., Hamzah A., Hammadi M. Development of Sulfadiazine by Nano Method and Study Its Effect Against Multi-Drug Resistant Bacteria. J Nanostruct, 2024; 14(4):1023-1029. DOI: 10.22052/JNS.2024.04.003

INTRODUCTION

One of today's biggest health system concerns is antibiotic resistance, which poses a significant health risk to the general public. Multi-Drug Resistance (MDR) is a problem that affects health care negatively on a global scale. Due to constant exposure to antimicrobial medications, bacteria are resistant to antibiotic treatments. Microbial infections have significantly grown during the last ten years, which has resulted in a rise in resistance [1,2]. Pathogenic organisms resistant to numerous chemotherapeutic treatments are said to exhibit multi-drug resistance [3]. The development of MDR, which increases mortality and morbidity rates, is an extremely common occurrence among microorganisms. This procedure is becoming more prevalent for several reasons. The usage of unidentified antimicrobial agents is the most significant one [2]. Among the most common causes of severe nosocomial infections are *Pseudomonas aeruginosa and Staphylococcus aureus. The ESKAPE pathogens (Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and*

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Enterobacter species) are a collection of bacteria that are able to "escape" antibiotic therapy due to rising multi-drug resistance [4,5]. The World Health Organization (WHO) has included ESKAPE infections among the germs for which the development of new antibiotics should be given top priority [6].

P. aeruginosa, a common Gram-negative rod found in nature, can infect immunosuppressed and critically ill people, leading to high rates of mortality and morbidity. It is linked to a number of infections, including bloodstream infections, urinary tract infections, burn-wound infections, and respiratory infections. Gram-positive *S. aureus* is a common pathogen that primarily affects the skin and soft tissues in the general population, but it can also cause serious infections like pneumonia, respiratory tract infections, sepsis, infections at surgical sites, infections in prosthetic joints, and infections of the heart and blood vessels in hospitals [7].

Silver and, more specifically, ionic silver Ag^{+} have been employed for their antibacterial properties from the dawn of time. For broad-spectrum antibacterial activity, Ag+ forms covalent connections with electron-donating groups like the sulfhydryl group of cysteine or electrostatic bonds with negatively charged molecules. Most Ag+-targeted bacterial sites are proteinaceous, where alterations in amino acid residues lead to structural damage, compromise metabolic and replicative activities, and other effects [8–10]. The efficiency of Ag+ as an antibacterial agent is strongly influenced by interactions with DNA, according to the available data. When Ag+ is commonly provided externally as a therapeutic, silver compounds, such as silver sulfadiazine, are utilized to administer it. The majority of bacteria that result in burns and chronic wound infections are susceptible to the amounts of sulfadiazine used topically. Fortunately, exposure to therapeutic levels of Ag+ typically does not threaten human health, despite the vast range of reactivity [11–13]. The U.S. Food and Drug Administration (FDA) has given the antibacterial medication sulfadiazine its approval for use in treating and preventing certain bacterial illnesses, such as ulcers, toxoplasmosis encephalitis, urinary tract infections, and other diseases [14,15]. The pure antibiotics made at the nanoscale can pass through bacterial cell membrane barriers more precisely and steadily than conventional antibiotic compounds [16].

Nanoparticles have drawn a lot of interest due to their distinct physical, chemical, optical, and mechanical properties. [17]. New methods for treating bacterial infections, creating alternative antimicrobial medications, reducing biofilm development, medication delivery, and cell therapy are anticipated to result from recent advances in nanotechnology [18]. Numerous benefits may be associated with nanoparticles. The majority of today's antibacterial substances are natural compounds that have been chemically changed [19].

MATERIALS AND METHODS

Bacterial Identification and Antibiotic Susceptibility

Patients resting in the Baquba Teaching Hospital in Iraq with burns and wounds were used to collect clinical samples. Microscopical, cultural, morphological diagnosis, and biochemical tests were first used to identify bacteria isolates. However, the confirmatory identification test was based on the VITEK® 2 Compact device, and testing for antibiotic susceptibility was also done using this device. The device has unique data that converts the outcome of bacterial metabolism into numbers and provides a quick response in just eight hours.

Converting sulfadiazine antibiotic to nanoscale

Sulfadiazine 99.9% obtained from Pharmaceutical Company Samarra Iraq. Dissolve 0.2 gm of Sulfadiazine in 100 mL of deionized

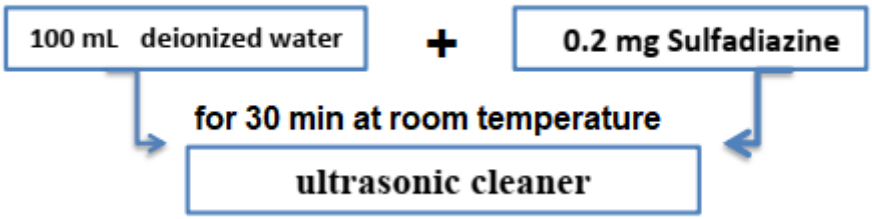

Fig. 1. Reaction diagram (1) converting sulfadiazine antibody to nanoscale

water and place it in an ultrasonic cleaner for half an hour at room temperature; it is shown in the Fig. 1.

Preparation of bacterial inoculum

The overnight BHI broth medium, which is similar to the McFarland 0.5 standard and yields turbidity comparable to that of a bacterial suspension containing 1.5 × 108 colony forming units (CFU)/ml, was used to prepare bacterial suspensions for inoculation. In reality, an OD 600 between 0.08 and 0.1 matches a McFarland 0.5 standard match.

Minimum - Inhibitory Concentration

Depending on references [20,21], the stock solution of Sulfadiazine and NPs Sulfadiazine were prepared. Serial concentrations with twice the progressive value rate (8-16-32-64-128-256-512-1024µg/ml) were then prepared and loaded into defined rows of 96 well microplates from the antibiotic stock solution. Three replicates' wells were assigned to each treatment with a negative control and a positive control. The three wells of microplates for tests with equal conditions were used for each bacterial isolates testing. The test wells were composed of serial dilutions of antibiotics, Mueller-Hinton broth and bacterial suspension. The negative control wells for each case consisted of the serial dilutions of antibiotics, Mueller-Hinton broth and no bacterial suspension. The positive control wells consisted of Mueller-Hinton broth and the bacterial suspension without antibiotic. After inoculation and incubation at 37°C

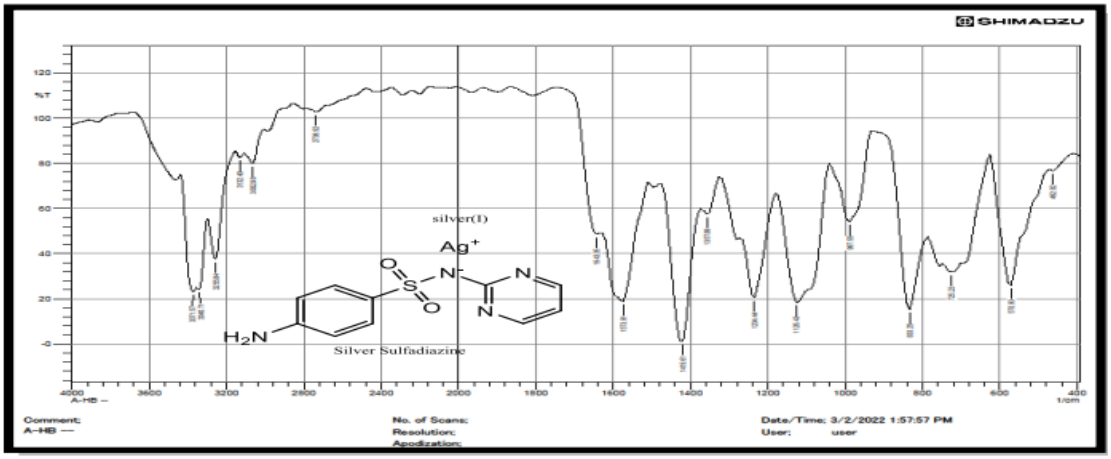

Fig. 2. Infrared spectrum of a compound Nano- Sulfadiazine

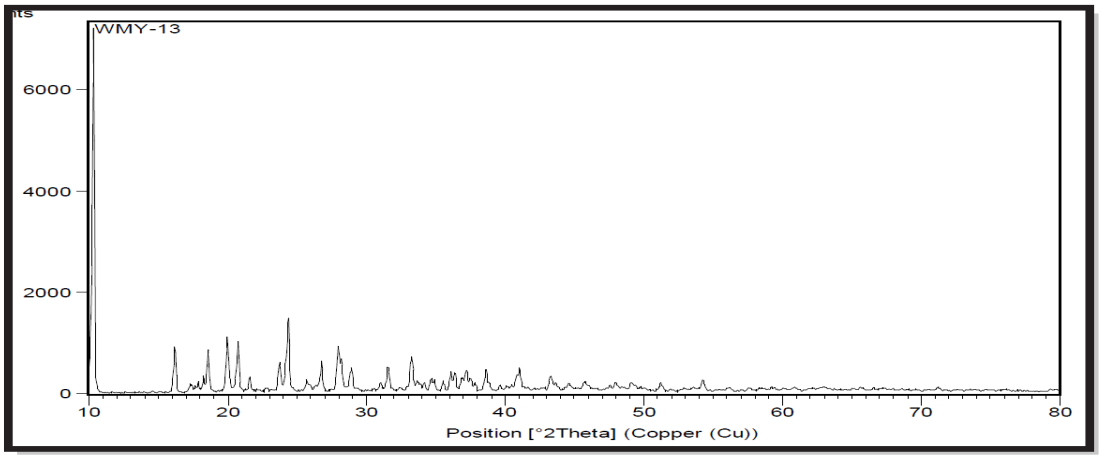

Fig. 3. shows the X-ray diffraction spectrum of an antibody Nano Sulfadiazine

for 24 hours, the plates were subjected to scanning at 630 nm of wavelength ELISA reader.

RESULTS AND DISCUSSION

Nano Sulfadiazine Characterization by (FTIR)

The results shown in Fig. 2 were compared with the standard sulfadiazine obtained from the Samarra Pharmaceutical Laboratory. It was confirmed that the peaks are identical and that conversion to nanoscale will not lead to any change. Where a band appeared at a frequency (3371, 3340 cm^{-1}) belonging to the primary amino group (NH_2), a band at a frequency (3255 cm^{-1})) belonging to the secondary amine group (NH), a band at a frequency (3062.cm^{-1}).) returns (C-H) of the aromatic ring, another band at frequency (1126, 1419 cm^{-1}) group (SO_2), and a band at a frequency (1643 cm^{-1}) due to the expansion (C = C) of the aromatic ring, and a band at a frequency (1573.91 cm^{-1}) returns to (C = N), and the beam at a frequency (1234 cm^{-1}) returns to (C-N) This agrees with the scientific literature[22].

Characterization of Nano- Sulfadiazine by X-ray diffraction

The X-ray spectra of Nano Sulfadiazine are in

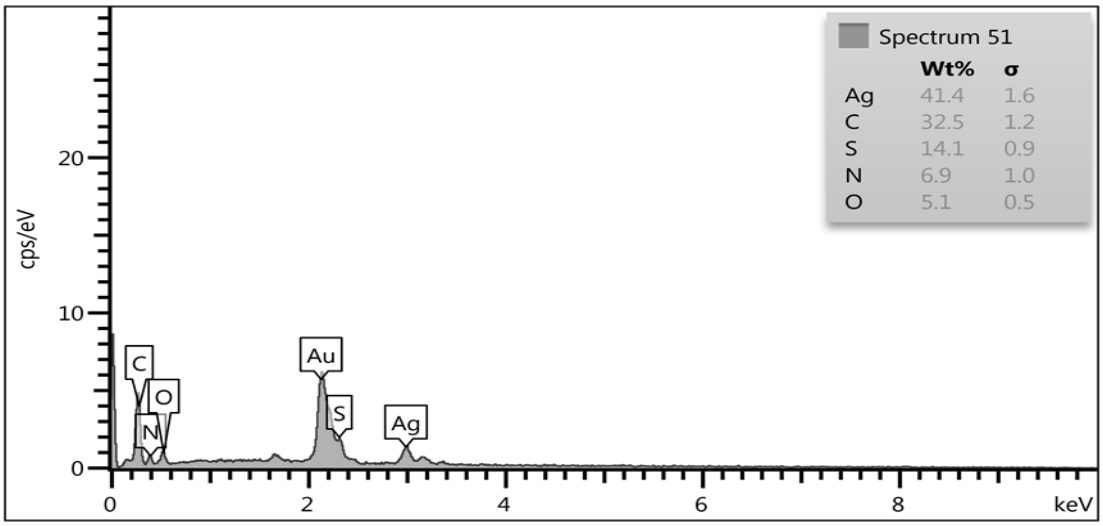

Fig. 4. Energy-dispersive X-ray spectrum of Nano Sulfadiazine

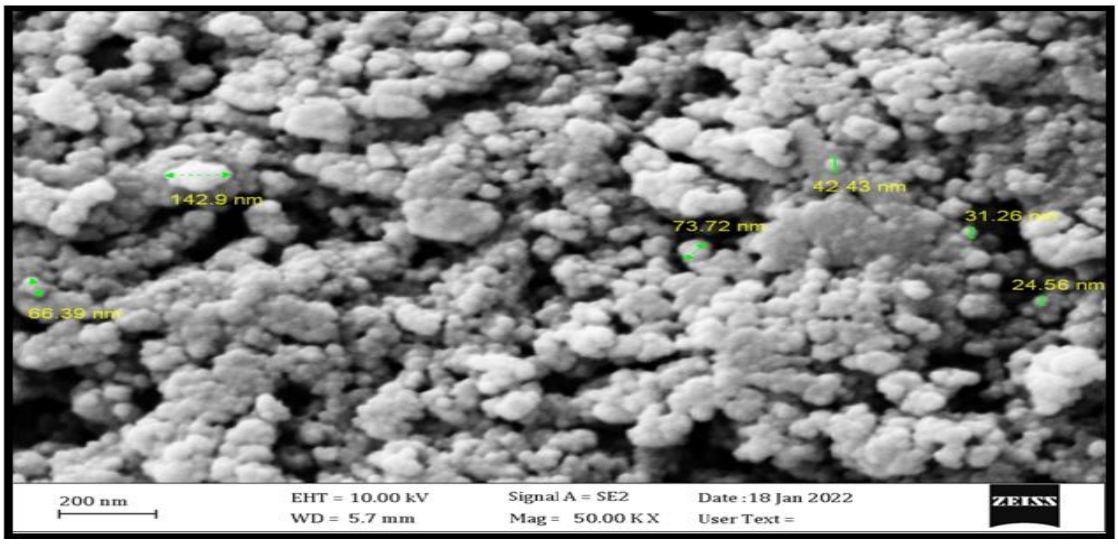

Fig. 5. SEM Nano Sulfadiazine SEM image.

Fig. 3. The average size of the crystals by using the Debye-Scherrer equation was 48.32nm.

Characterization by energy-dispersive X-rays

Energy-dispersive X-ray was used to determine the fraction of elements contained in Sulfadiazine NPs, as shown in Fig. 4. The results revealed the presence of Silver 41.4% and carbon 32.5%, nitrogen 6.9 %, and oxygen 5.1% Sulfadiazine NPs showed high purity.

Characterization by scanning electron microscope

The morphological and structural structures of Nano Sulfadiazine were studied using an SEM scanning electron microscope. Fig. 5 shows that the nanoparticles were prepared in the nanometer range and the SEM images showed that some of the nanoparticles are well separated from each other while most of them are present in a lumpy form due to This agglomeration due to electrostatic effects and the average diameter of these particles is 63.54 nm.

Measurement of the particle size of Nano sulfadiazine in liquid

A particle size meter was used to determine the size of the sulfadiazine. The size of the standard sulfadiazine was compared. Fig. (6-A) shows its size equal to 2827.8 nm, and after conducting treatments on it, it was converted into a nano-antibody, where its size became 599.6 nm, as shown in Fig. (6-B).

Diagnosis of bacterial isolates and antimicrobial resistance

Twenty-five isolates of *Pseudomonas aeruginosa*, as well as eighteen isolates of *Staphylococcus aureus* were identified from clinical samples of burn and wound patients. It was diagnosed and its resistance to antibiotics was detected by the Vitek 2 device, where five most resistant isolates of each type were selected. The five isolates of *P. aeruginosa* (P1-P5) showed resistance pattern against Cefotaxime, Amikacin, Gentamicin, Ticarcillin-Clavulanate, Piperacillin, Cefepime, Ciprofloxacin, Tobramycin, Ceftazidime, Levofloxacin, Polymyxin, and Meropenem. While the five *S. aureus* isolates (S1-S5) were resistance to Oxacillin, Levofloxacin, Vancomycin, Benzyl penicillin, Gentamicin, Tobramycin, Linezolid, Teicoplanin, Tetracycline, Tigecycline.

Determination of Minimal Inhibitory Concentration (MIC)

MIC is the lowest concentration of an antibacterial agent expressed in mg/L (µg/mL) which, under strictly controlled in vitro conditions, completely prevents visible growth of the test

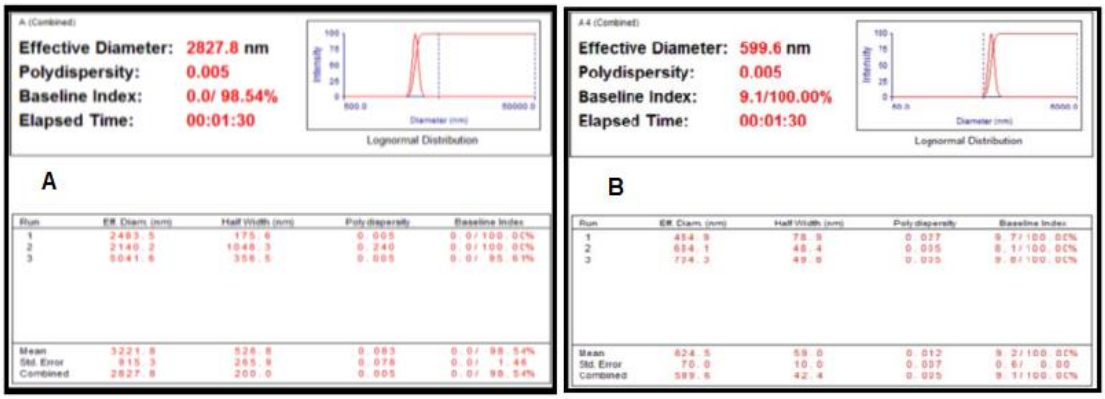

Fig. 6. the granular size in the liquid of sulfadiazine.

Table 1. Minimum Inhibitory Concentration of sulfadiazine and sulfadiazine NPs

compounds	Pseudomonas aeruginosa MIC µg/ml					Staphylococcus aureus MIC µg /ml				
	P1	P2	P3	P4	P5	S1	S2	S3	S4	S5
sulfadiazine	64	128	64	64	128	64	64	64	64	128
sulfadiazine NPs	32	32	16	16	16	32	32	32	32	32

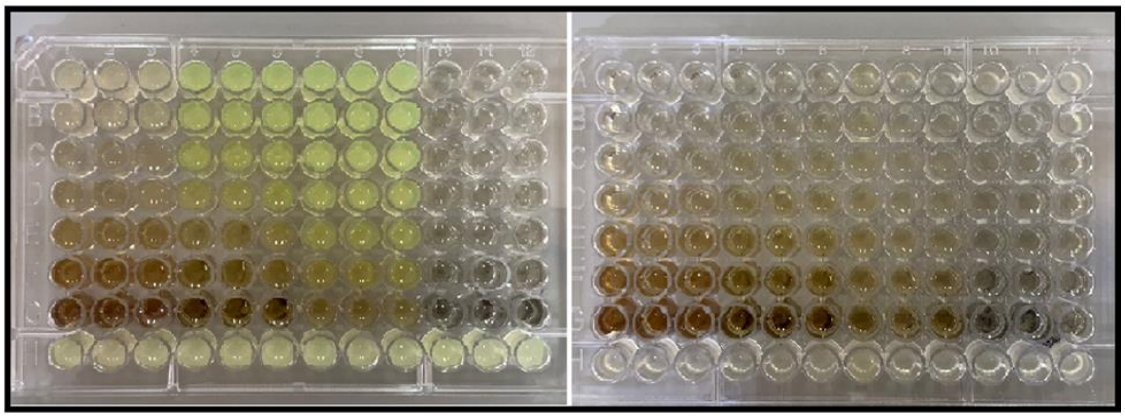

Fig. 7. MIC test using microplates

strain of an organism [23].The results of broth micro dilution method (Fig. 7) with standard sulfadiazine gradient (concentration) ranges of 8-1028µg/ml revealed that two *P. aeruginosa* isolates (P2, P5) (40%) with MIC 128µg/ml were obtained, while three isolates(P1, P3, P4) (60%) with MIC 64µg/ml were detected. The results of Nano-sulfadiazine MIC determined for these isolates were detected that two isolates (P1, P2) (40%) with MIC 32 µg/ml and three isolates (P3-P5) (60%) with MIC 16µg/ml (Table 1). The results of standard sulfadiazine gradient for *S. aureus* shown one isolate (S5) (20%) with MIC 128µg/ml and four isolates (S1-S4) (80%) with MIC 64µg/ml, while all five isolates (100%) shown same MIC 32µg/ml for Nano-sulfadiazine (Table 1).

The increase in the effectiveness of Nano-sulfadiazine against P. *aeruginosa* and *S. aureus* compared to the standard sulfadiazine indicate that the small size of the antibiotic increased its surface area, which plays an important role in increasing the effectiveness of it. This may be due to its ease of permeability from membrane of bacteria cells, as well as accumulating inside the cell in greater concentrations, which makes it difficult for bacterial cells to get rid of them through the resistance system using flow pumps responsible for the disposal of harmful substances outside the bacterial cell.

CONCLUSION

Based on our findings, we can conclude that increase in the effectiveness of Nano-sulfadiazine against *P. aeruginosa* and *S. aureus* compared to the standard sulfadiazine indicate that the small size of the antibiotic increased its surface area, which plays an important role in increasing its effectiveness of it. This may be due to its ease of permeability from the membrane of bacteria cells, as well as accumulating inside the cell in greater concentrations, which makes it difficult for bacterial cells to get rid of them through the resistance system using flow pumps responsible for the disposal of harmful substances outside the bacterial cell. Furthermore, these nanoparticles have no toxicity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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