RESEARCH PAPER

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

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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 MIC activity and 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.

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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. Randomly use of antibacterial take place in distribution resistant bacteria because increase ability of resistance different types of these antibacterial [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.* There are different types of bacteria (Gram negative and Gram positive) became multidrug resistance and extensive drug resistance [4,5,6].

P. aeruginosa, a common Gram-negative rod

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found in nature, can infect immunosuppressed and critically ill people, leading to different clinical diseases. 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].

The antibacterial capacity of silver and Ag+ increase of unstable form of electron's lead to occur structural damage in cell wall of bacterial cell and die of cell [8–10]. The efficiency of Ag+ as an antibacterial agent is strongly influenced by interactions with DNA, according to the available data. The Ag+ is commonly provided externally as a therapeutic. The substances silver (silver sulfadiazine) may be use in treatment skin infection [11–13]. The substances use in treatment different types of bacterial and parasite infection and limit distribution of infection because high activity and easy penetration of bacterial cell membrane [14-16]. These compounds may be use in different methos to reduce bacterial infection [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. All samples were identified by routine work and certainly identification with antibiogram by VITEK® 2 Compact. 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

Dissolved amount (0.2 Sulfadiazine 99.9%) in amount of water (100 mL of deionized water) and place it in an ultrasonic cleaner for half an hour at room temperature; it is shown in the reaction Fig. 1.

Bacterial culture

Bacterial samples were cultured in enrichment media (BHIB) and incubated (37 °C 24 hours). Then measured density of growth with standard McFarland 0.5 (1.5 × 108 CFU/ml). The optical density (OD 600) between 0.08 and 0.1 to ensure suitable amount of tested bacterial cells.

Test MIC

Different concentration (8-16-32-64-128-256-512-1024µg/ml) were done from stock of test substances (Sulfadiazine NPs). Then used microplates (contain 96 wells) with stock of antibacterial (Mueller-Hinton broth and bacterial suspension) with multiple replication, positive & negative control to ensure precise test. Technique ELIZA 630nm used in second day of end incubation period [20,21].

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)

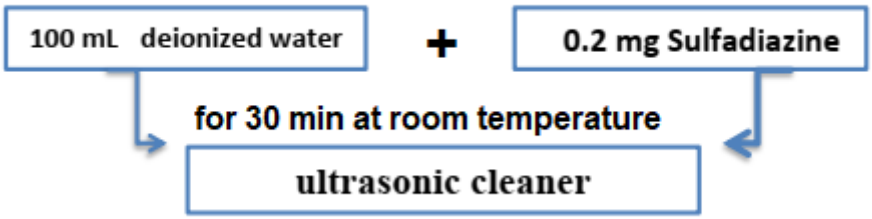

Fig. 1. Reaction diagram (1) converting sulfadiazine antibody to nanoscale

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

Characterization by energy-dispersive X-rays

EThis analytical technique was done to explain fraction of elements in Sulfadiazine (Fig. 4). According this figure Silver 41.4% and carbon 32.5%, nitrogen 6.9 %, and oxygen 5.1% Sulfadiazine NPs showed high purity .

Characterization by scanning electron microscope

Used (SEM scanning electron microscope) to detect precise structure details of this nanoparticles. The details in Fig. 5 refer to preparation of sulfadiazine in the nanometer. Scanning electron microscope results refer to composition of nanoparticles separated from each other. Also, most of them it found in a lumpy form. 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

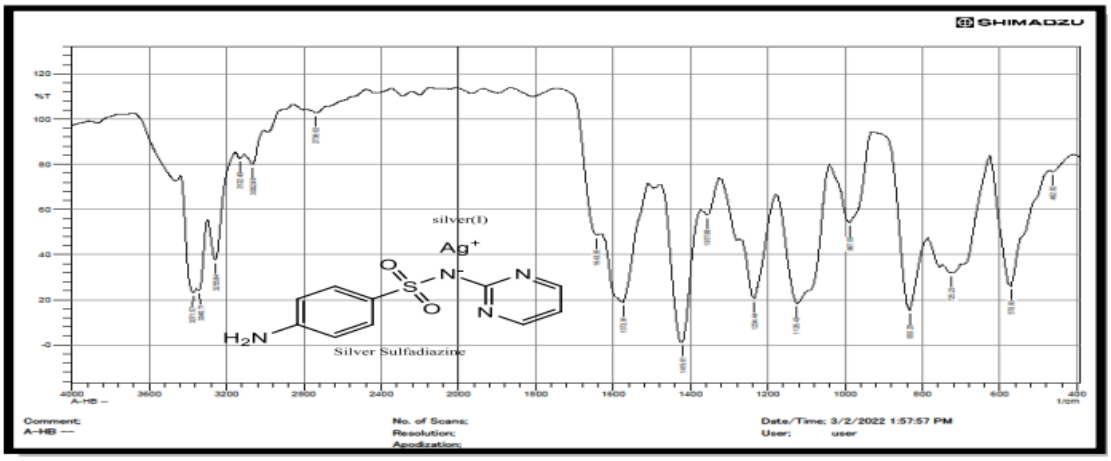

Fig. 2. Infrared spectrum of a compound Nano- Sulfadiazine

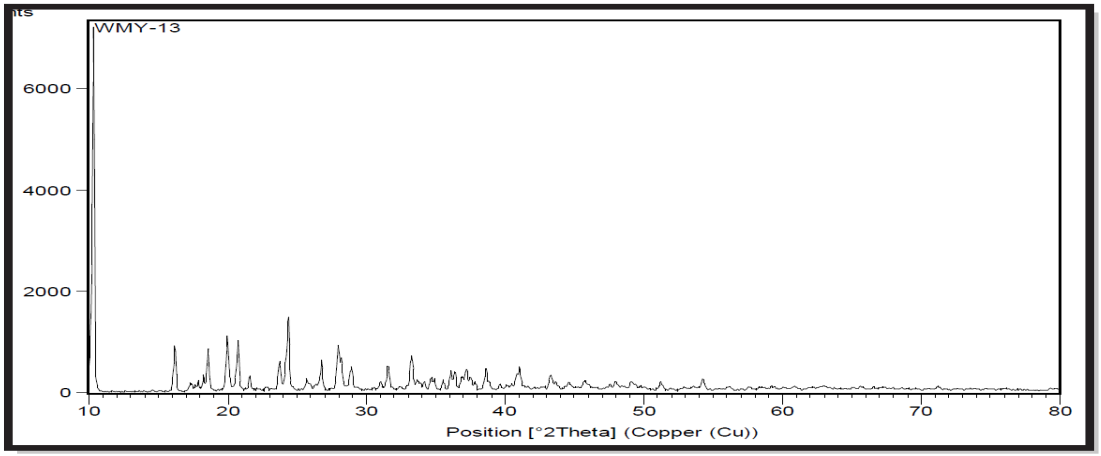

Fig. 3. shows the X-ray diffraction spectrum of an antibody Nano Sulfadiazine

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).

Antibacterial susceptibility test

The results of antibiogram of the tested pathogenic bacteria (P.aeruginosa , S.aureus) isolated from skin infection (burn infection) showed that P. aeruginosa exhibited high resistance to different types of antibiotics (Cefotaxime, Amikacin, Gentamicin, Ticarcillin-Clavulanate, Piperacillin, Cefepime, Ciprofloxacin, Tobramycin, Ceftazidime, Levofloxacin, Polymyxin, and Meropenem). Other pathogenic bacteria S.aureus was resistant to the antibiotics (Oxacillin, Levofloxacin, Vancomycin, Benzyl penicillin, Gentamicin, Tobramycin, Linezolid, Teicoplanin, Tetracycline, Tigecycline).

Detection activity of MIC

It is concentration that inhibit bacterial growth [23]. Table 1 and Fig. 7 showed results of MIC which some isolates 40% of P. aeruginosa with MIC

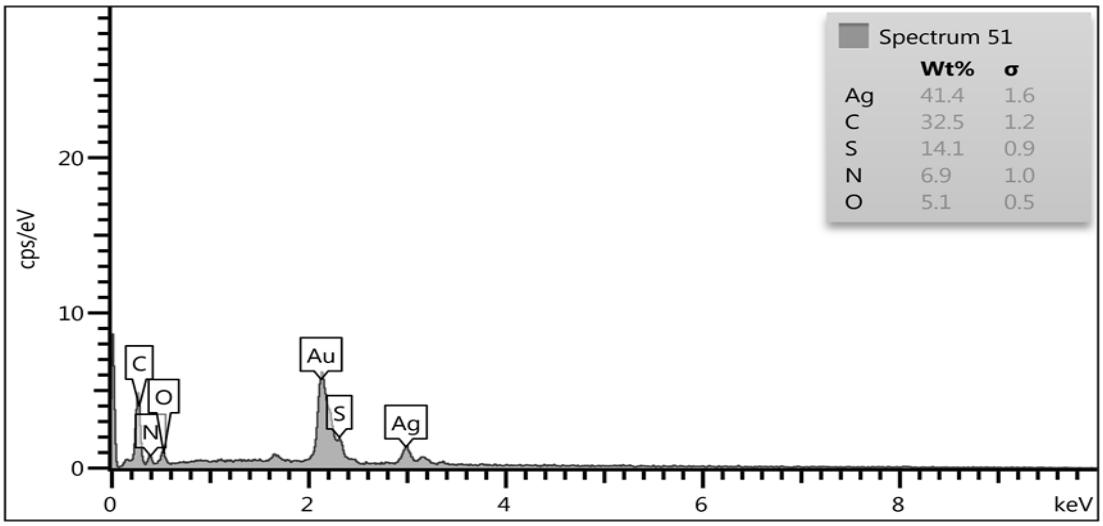

Fig. 4. Energy-dispersive X-ray spectrum of Nano Sulfadiazine

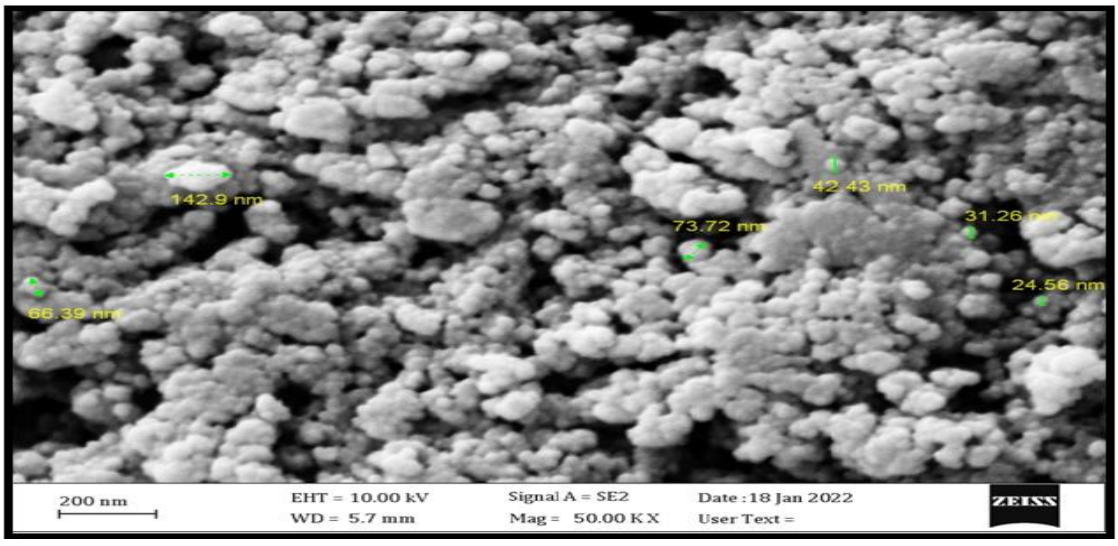

Fig. 5. SEM Nano Sulfadiazine SEM image.

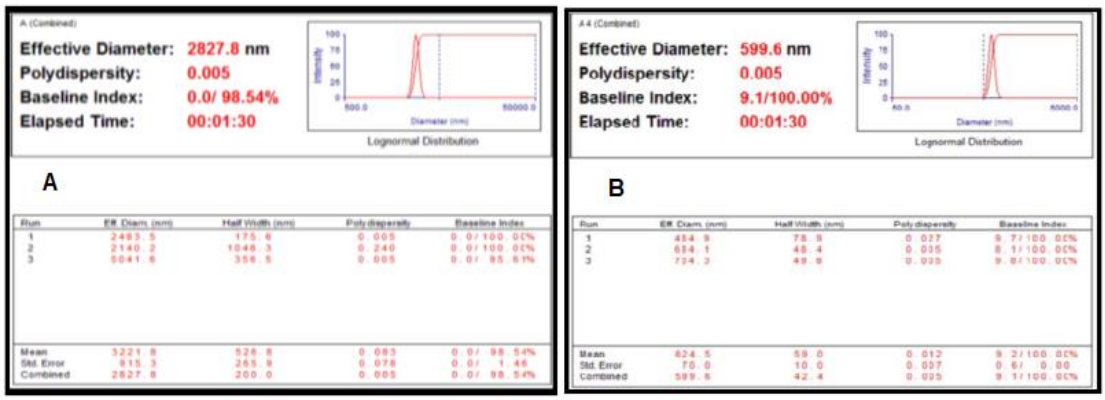

Fig. 6. the granular size in the liquid of sulfadiazine.

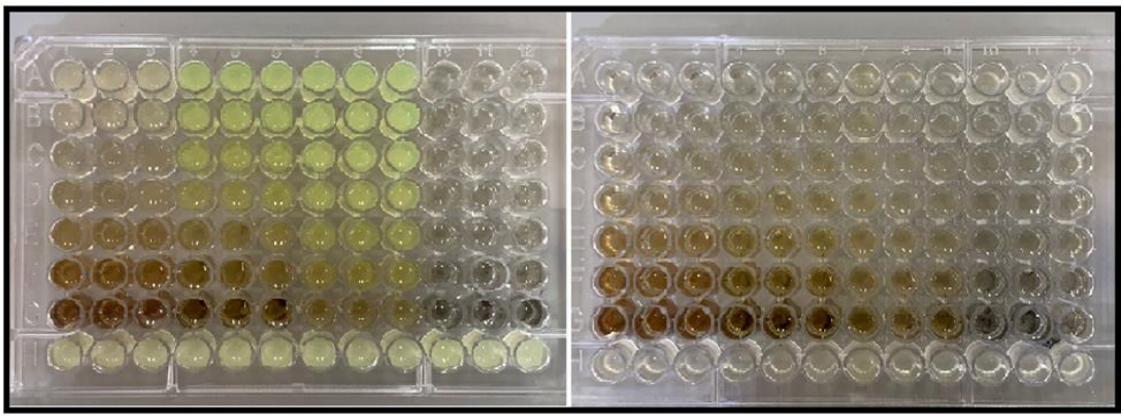

Fig. 7. MIC test using microplates

Table 1. Minimum Inhibitory Concentration of sulfadiazine and sulfadiazine NPs

compounds	*Pseudomonas aeruginosa* MIC µg/ml						*Staphylococcus aureus* MIC µg /ml					
sulfadiazine	64	128	64	64	128	64	64	64	64	128	64	64
sulfadiazine NPs	32	32	16	16	16	32	32	32	32	32	32	32

128 µg/ml but other 60% with MIC 64 µg/ml. The nano-sulfadiazine MIC results of these bacteria 40% (MIC 32 µg/ml) and other isolates 60% (MIC 16 µg/ml). The standard sulfadiazine results showed that some S. aureus 20% (MIC 128 µg/ml) but other isolates 80% (MIC 64 µg/ml).

The increase in the effectiveness of Nano-sulfadiazine against P. aeruginosa and S. aureus compared to the standard sulfadiazine indicate that these nanoparticle with high antibacterial activity. These activity increase with penetration large amount from nanoparticle through membrane and reduce role of efflux pump mechanisms. Also, these particles may be considered antibacterial agent to other types of multidrug resistance pathogenic bacteria and it use alone or mixed with different types of antibiotics in different relationship like synergism effect.

CONCLUSION

Nano-sulfadiazine is few toxic and useful as antibacterial substance especially Pseudomonas aeruginosa and Staphyllococcus aureus comparison with standard sulfadiazine. This substance can pass through bacterial cell membrane. Then this process led to increase

level of antibacterial substance. So, bacterial cell cannot use mechanisms of resistance because it lost activity of efflux pump system and other mechanisms. In future may be use this substance in treatment infection that cause with these bacteria and decrease using of antibiotics

CONFLICT OF INTEREST

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

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