Antibacterial Assessment of Zinc Sulphide Nanoparticles Against *Streptococcus pyogenes* and *Acinetobacter baumannii*

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In this study, the antibacterial assessment of zinc sulphide nanoparticles (ZnS NPs) against *Streptococcus pyogenes* and *Acinetobacter baumannii* is investigated. ZnS NPs were synthesized through a co-precipitation method using Polyvinylpyrrolidone (PVP), Polyvinyl alcohol (PVA), and Polyethylene Glycol (PEG). Size and morphology of the synthesized ZnS NPs are followed by a scanning electron microscope (SEM), and it is found that the size of the applied nanoparticles is about 20 nm. In order to evaluate the antibacterial effect of the synthesized ZnS NPs, various concentrations (50 µg/mL, 100 µg/mL and 150 µg/mL) were prepared. Antibacterial assessments are performed through the disc diffusion method in Mueller–Hinton agar culture medium, and the optical density (OD) method is performed by a UV-Vis spectrophotometer in Trypticase™ Soy Broth (TSB) medium. Then, in order to compare the antibacterial effects of the applied nanoparticles, several commercial antibiotics including Penicillin, Amikacin, Ceftazidime and Primaxin antibiotics are used. The achieved results indicate that the antibacterial effect of ZnS NPs has a direct relation against the concentrations, and the concentration of 150 µg/mL shows the highest antibacterial effect in comparison with others. In addition, the nanoparticles are more effective on *Acinetobacter baumannii*. The findings
of this research suggest a novel approach against antibacterial resistance.

У цьому дослідженні вивчається антибактеріальна оцінка наночастинок сульфіду цинку (НЧ ZnS) проти *Streptococcus pyogenes* і *Acinetobacter baumannii*. НЧ ZnS було синтезовано за допомогою методу спільного осадження з використанням полівінілпірролідона, полівінілового спирту та поліетиленгліколю. Розмір і морфологію синтезованих НЧ ZnS було простежено сканувальним електронним мікроскопом, і встановлено, що розмір застосовуваних наночастинок становить близько 20 нм. Для оцінки антибактеріального ефекту синтезованих НЧ ZnS було підготовлено різні концентрації (50 мкг/мл, 100 мкг/мл і 150 мкг/мл). Антибактеріальні оцінки виконуються методою дискової дифузії у середовищі культури на основі агару Мюллера–Хінтон, а метод оптичної густини виконується спектрофотометром у видимій і ультрафіолетовій областях світла в живильному середовищі на основі бульйону соєвого триспіна. Потім для порівняння антибактеріальних ефektів застосовуваних наночастинок широкомасштабно використовуються кілька комерційних антибіотиків, включаючи антибіотики пеницилін, амікацин, цефтақазіп і прімаксін. Досягнуті результати свідчать про те, що антибактеріальний ефект НЧ ZnS має прямий зв’язок із концентрацією, а концентрація у 150 мкг/мл показує найвищий антибактеріальний ефект у порівнянні з іншими. До того ж, на НЧ ZnS більш ефективно на *Acinetobacter baumannii*. Результати цього дослідження свідчать про новий підхід до антибактеріальної резистентності.

В этом исследовании изучается антибактериальная оценка наночастич сульфид цинка (НЧ ZnS) против *Streptococcus pyogenes* и *Acinetobacter baumannii*. НЧ ZnS были синтезированы с помощью метода совместного осаждения с использованием поливинилпирролидона, поливинилового спирта и политетиленгликоли. Размер и морфология синтезированных НЧ ZnS прослежены сканирующим электронным микроскопом, и установлено, что размер применяемых наночастиц составляет около 20 нм. Для оценки антибактериального эффекта синтезированных НЧ ZnS были подготовлены различные концентрации (50 мкг/мл, 100 мкг/мл и 150 мкг/мл). Антибактериальные оценки выполняются методом дисковой диффузии в среде культуры на основе агара Мюллера–Хинтон, а метод оптической плотности выполняется спектрофотометром в видимой и ультрафиолетовой областях света в питательной среде на основе бульона соевого триспина. Затем для сравнения антибактериальных эффектов применяемых наночастиц используются несколько коммерческих антибиотиков, включая антибиотики пенициллин, амиказин, цефтақазіп и прімаксін. Достигнутые результаты свидетельствуют о том, что антибактериальный эффект НЧ ZnS имеет прямую связь с концентрацией, а концентрация 150 мкг/мл показывает самый высокий антибактериальный эффект по сравнению с другими. К тому же, наночастицы более эффективны на *Acinetobacter baumannii*. Результаты этого исследования свидетельствуют о новом подходе к антибактериальной резистентности.

**Key words:** zinc sulphide nanoparticles, antibacterial effects, *Streptococ-
coccus pyogenes, Acinetobacter baumannii.

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1. INTRODUCTION

Antibiotics react against bacteria in different ways to eliminate and reduce diseases growth [1, 2]. The recognized platforms for the fight are including DNA destruction, cell wall degradation, effects on the cytoplasmic membrane, prevent of protein synthesis and antimetabolites [3, 4]. The bacterial resistance when is occurred that mutations be enabled in bacteria and they found resistance against antibiotic drugs and new generations of them will be appear that cannot be fought against them [5]. The main cause for this type of drug resistance are self-treatment or excessive use of antibiotics [6]. This phenomenon endangers the entire human society so that this risk has been likened to terrorism [7]. Bacterial resistance against antibiotics is one of the biggest challenges in the modern age that threatens whole of human health [7]. *Streptococcus pyogenes* is a gram positive cocci; it is thought that about 700 million infections is caused by this bacteria annually and 650 thousands of these infections are severe and harmful (Fig. 1, b) [8, 9]. The mortality rate is about 25% for caused infections by it [10]. This bacterium creates important diseases in humans. Pharyngitis, impetigo,

![Microscopic images](image)

**Fig. 1.** Microscopic images: a) *Acinetobacter baumannii*; b) *Streptococcus pyogenes* [15].
erysipelas, cellulitis and necrotizing fasciitis are some diseases that caused by this pathogen. [11]. So far, penicillin and ampicillin are used as the most effective medical treatments against *Streptococcus pyogenes*. Compared with gram-positive bacteria, gram negative bacteria are more resistant against antibiotics due to their impenetrable walls [12]. Acinetobacter is species of gram-negative bacteria that be seen as bacilli or coccobacillus [13]. These bacteria are non-motile, oxidase negative and have not ferment sugars activity. These bacteria are resistant to most antibiotics such as penicillin but often sensitive to quinolones. *Acinetobacter baumannii* is an opportunistic pathogenic bacterium that leads to serious infections especially in hospitalized patients [13]. The mortality rate is high in blood infections for this bacterium and has been reported between 30–52% [14] (Fig. 1, a).

Additionally, *Acinetobacter baumannii* cause severe infections in susceptible individuals. This bacterium is one of the main causes of nosocomial infections that can cause serious infections such as bacteremia, respiratory infections, urinary tract infections, pneumonia, endocarditis and wound and skin infections [16]. Many factors such as enzymes, exotoxins, siderophore and outer membrane proteins are involved in the pathogenesis role of this bacterium. Clinical isolates types of *Acinetobacter baumannii* are showed significant increase in resistance against existing antibiotic treatments that has led to a challenge against this bacterium [14, 17]. In recent decades, antibiotic resistance among strains of *Acinetobacter baumannii* is increased alarmingly. Resistance against antibiotics has led to limited treat against caused infections by this bacterium in patients [18].

Nanotechnology as a new knowledge in different scientific fields such as medical microbiology has been entered [19]. In recent years, nanotechnology researchers have achieved great success in production of new antimicrobial drugs against a variety of microorganisms and even for resistant strains against conventional drugs [20–26]. Nanomaterials have been found much attention due to their special features including their large surface to volume ratio and high reaction activity that can have more captured bacteria. Properties of nanoparticles are dependent on their size; the size controls in nanoparticles is performed for achieving different properties in various biomedical applications [27, 28].

ZnS NPs are one of important semiconductor material from group II–VI and have a deep gap between their full and empty electron balances [29, 30]. The reactivity role of materials in nanoscale is significantly increased, and so far, numerous researches have been conducted to investigate antibacterial properties of nanomaterials [31–34].
In this study, the antibacterial property of ZnS NPs against *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria were investigated *in vitro* environment and offer a promising approach to fight versus bacterial resistance.

2. MATERIALS AND METHODS

2.1. Materials

Polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), polyethylene glycol (PEG), zinc acetate dehydrate, sodium sulphide, deionized water (capacity 1500 mL), solid culture medium (Mueller–Hinton agar, 17.0 g/L), Trypticase™ Soy Broth (TSB) double strength bottled broth and nutrient broth medium (D (+)-glucose, 1 g/L) were purchased from Sigma-Aldrich (USA). *Streptococcus pyogenes* (PTCC 1762) and *Acinetobacter baumannii* (PTCC 1797) bacteria were purchased from scientific and industrial research organization (Iran). Petri dishes and other used materials and reagents were provided through standard sources with the highest available purity and quality.

2.2. Apparatus

An incubator (Binder-Model BD 115, China) was used to provide a bacterial culture medium and growth condition. In order to provide the sterilization, an autoclave was used (Pars mehr, Iran). A UV-Vis spectrophotometer (MACHEREY-NAGEL GmbH & Co. KG, Germany) was used to investigate and compare the antibacterial effects of ZnS NPs in TSB culture medium. Morphology of synthesized nanoparticles was investigated through scanning electron microscopy (SEM) (SU3500 Premium VPSEM, Japan).

2.3. Synthesis and Preparation of ZnS NPs

ZnS NPs were synthesized according to previously used protocol [35]. The details for the used protocol were summarized as below. In this protocol, ZnS NPs were synthesized through co-precipitation method using PVP, PVA and PEG [36–38]. In this technique, precipitation occurred in involved metal ions with sulphide ions within the production solution. Firstly, Zinc acetate dihydrate (0.1 M) and Sodium sulphide (0.1 M) were mixed with together through capping agent solutions like PVP, PVA and PEG. Then, other production steps were done, and it was achieved solution inserted in 80°C within 4 hours to obtain ZnS NPs in powder state.
2.4. Preparation Bacterial Culture Medium

2.4.1. Preparation Mueller–Hinton Agar

Firstly, according to the protocol, the specified amount of agar powder was dissolved in distilled water to reach pH 7.3; the temperature was 25°C [39]. Then, they were heated and a homogeneous solution was obtained. In the next step, the achieved solution was placed in an autoclave for sterilizing. Finally, the solution was poured in Petri dishes near the flame and they were incubated at 37°C for 24 hours.

2.4.2. Preparation TSB Double Strength Bottled Broth

Firstly, according to the protocol, the specified amount of related powder was dissolved in distilled water to reach pH 7.3; the temperature was 25°C. Then, they were heated and a homogeneous solution was obtained [40]. In the next step, the achieved solution was placed in several culture tubes in an autoclave for sterilizing. Finally, the prepared culture tubes were stored in the refrigerator until use.

2.4.3. Preparation Nutrient Broth

Firstly, according to the protocol, the specified amount of powder was dissolved in distilled water to reach pH 7.3; the temperature was 25°C. Then, they were heated and a homogeneous solution was obtained. In the next step, the achieved solution was placed in ten culture tubes in an autoclave for sterilizing. Finally, *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria were grown in these prepared tubes [41]. In this research, the nutrient broth culture medium was used as a bacterial growth medium and bacteria were cultured at 37°C for 48 hours.

2.5. Methodology for Investigation of the Antibacterial Effects of ZnS NPs in Mueller–Hinton Agar Culture Medium

In this method, the antibacterial effects of ZnS NPs were investigated against *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria in Mueller–Hinton agar medium. Various concentrations of ZnS NPs (50 µg/mL, 100 µg/mL and 150 µg/mL) were used. A culture medium with distilled water and without any ZnS NPs or antibiotic was used as the control group. In order to compare the antibacterial effects of ZnS NPs, some commercial antibiotics including
Penicillin, Amikacin, Ceftazidime and Primaxin were used in a fixed concentration (200 µg/mL). The bacteria were grown at 37°C for 24 hours. Then, disc diffusion for various concentrations of ZnS NPs, control test and used antibiotics were investigated. In addition, the bacterial colonies were counted.

2.6. Methodology for Investigation of the Antibacterial Effects of ZnS NPs in TSB Culture Medium

In this method, the antibacterial effects of ZnS NPs were investigated against *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria in TSB medium.

Various concentrations of ZnS NPs (50 µg/mL, 100 µg/mL and 150 µg/mL) were applied. A culture medium without any ZnS NPs or antibiotic was used as the control group. In order to compare the antibacterial effects of ZnS NPs, some antibiotics including Penicillin, Amikacin, Ceftazidime and Primaxin were used in a fixed concentration (200 µg/mL). The bacteria were grown in culture tubes at 37°C during 48 hours. A UV-Vis spectrophotometer was used to measure the concentration of bacteria (OD was 600 nm).

2.7. Data Analysis

All analyses based on the achieved data was performed using Excel software (2010, Microsoft). In order to evaluate the antibacterial effects of ZnS NPs and some antibiotics including Penicillin, Amikacin, Ceftazidime and Primaxin on *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria in the TSB culture medium, the one-way ANOVA ($\alpha = 0.05$) was applied.

It should be noted that the Kolmogorov–Smirnov test was performed to evaluate the normality of data. The t-test analysis ($p < 0.05$) was used to determine the colony forming units per millilitre (CFU/mL) and to calculate the viability of *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria versus the highest concentration of ZnS NPs (150 µg/mL).

3. RESULTS

3.1. SEM Investigation

In order to find the exact size and morphology of synthesized ZnS NPs, a SEM microscope was used. After this experiment, the result showed that the particles size of synthesized ZnS NPs was about 70 nm. Figure 2 shows related images of ZnS NPs with different mag-
nification. In Figure 2, a, magnification was \( \times40000 \) and the scale bare was 200 nm, and in Fig. 2, b, magnification was \( \times4000 \) and the scale bare was 100 nm. The synthesized ZnS NPs showed dumbbell-shaped and spherical shape in microscopic studies. Nanoparticles size and their morphology were effective on the intensity of their antibacterial properties.

3.2. Antibacterial Effects of ZnS NPs in Mueller–Hinton Agar Culture Medium

The antibacterial effects of ZnS NPs in Mueller–Hinton agar culture medium were investigated in existence of various concentrations of ZnS NPs (50 \( \mu \)g/mL, 100 \( \mu \)g/mL and 150 \( \mu \)g/mL). To compare the antibacterial effects of ZnS NPs, Penicillin, Amikacin, Ceftazidime and Primaxin antibiotics were used (Table 1).

The achieved results showed that the antibacterial effects of ZnS NPs had direct relation to their concentrations. The highest used concentration of ZnS NPs (150 \( \mu \)g/mL) showed the stronger inhibition effects against *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria.

3.3. Antibacterial Effects of ZnS NPs in TSB Culture Medium

The antibacterial effects of ZnS NPs in TSB culture medium were investigated in existence of various concentrations of ZnS NPs (50 \( \mu \)g/mL, 100 \( \mu \)g/mL and 150 \( \mu \)g/mL). The results of this experiment
were achieved through UV-Vis spectrophotometer and OD was 600 nm. To compare the antibacterial effects of ZnS NPs, Penicillin, Amikacin, Ceftazidime and Primaxin antibiotics were used. The obtained results in this experiment were shown in Diagrams 1–4.

According to Diagram 1 and Diagram 2, it was concluded that the antibacterial effects of ZnS NPs were more effective on *Acinetobacter baumannii*; also, these results confirmed the successful antibacterial effects of ZnS NPs against both bacteria.

In next study, the antibacterial effects of ZnS NPs were compared with some commercial antibiotics (Penicillin, Amikacin, Ceftazidime, and Primaxin) in TSB culture medium. Besides, the antibacterial effects of ZnS NPs were compared with distilled water. This experiment was performed for each bacterium separately, and the found results were provided in Diagram 3 and Diagram 4.

The statistical analysis of the results was performed based on

| Bacterium type       | Effect of ZnS NPs, 50 µg/mL | Effect of ZnS NPs, 100 µg/mL | Effect of ZnS NPs, 150 µg/mL | Effect of Penicillin, 200 µg/mL | Effect of Amikacin, 200 µg/mL | Effect of Ceftazidime, 200 µg/mL | Effect of Primaxin, 200 µg/mL | Effect of distilled water |
|----------------------|-----------------------------|-----------------------------|-----------------------------|-------------------------------|-----------------------------|-------------------------------|-----------------------------|---------------------------|
| *Streptococcus pyogenes* | +                           | ++                          | +++                         | ++                            | +                           | ++                            | +                           | −                         |
| *Acinetobacter baumannii* | ++                         | +++                         | +++                         | −                             | +                           | +                             | +                            | −                         |

*Streptococcus pyogenes* inhibition Zone Diameter [mm] versus: Effect of ZnS NPs (50 µg/mL) is of 6; Effect of ZnS NPs (100 µg/mL) is of 11; Effect of ZnS NPs (150 µg/mL) is of 17; Effect of Penicillin is of 10; Effect of Amikacin is of 5; Effect of Ceftazidime is of 4; Effect of Primaxin is of 4; Effect of distilled water is of 1. *Acinetobacter baumannii* inhibition Zone Diameter [mm] versus: Effect of ZnS NPs (50 µg/mL) is of 8; Effect of ZnS NPs (100 µg/mL) is of 13; Effect of ZnS NPs (150 µg/mL) is of 18; Effect of Penicillin is of 1; Effect of Amikacin is of 7; Effect of Ceftazidime is of 5; Effect of Primaxin is of 6; Effect of distilled water is of 1.
one-way ANOVA ($\alpha = 0.05$). The details have been provided in Table 2. As shown in this Table, the maximum effect was found for ZnS NPs (150 µg/mL) on Acinetobacter baumannii. In addition, other comparable details presented clearly.

According to obtained results in Diagram 3 and Diagram 4, it is clear that there was a high bacterial resistance against evaluated antibiotics (Penicillin, Amikacin, Ceftazidime, and Primaxin). In addition, the effects of antibiotics on bacteria were different. In comparison with nanoparticles, bacteria in the presence of antibiotics showed more growth.

In the next study, the total bacterial colony/mL were obtained for Streptococcus pyogenes and Acinetobacter baumannii and related results shown in Diagram 5. The results of this experiment were analysed via t-test ($p < 0.05$), and the mean for Streptococcus pyogenes and Acinetobacter baumannii was $78500$ colony/mL and $88875$ colony/mL, respectively.
CFU/mL was calculated for each bacterium in treatment with various agents.

The results showed that the highest used concentration of ZnS NPs (150 µg/mL) was more effective in order to prevent and inhibit activity of *Streptococcus pyogenes* and *Acinetobacter baumannii* (Most Inhibition Effect). In last experiment, the viability of *Streptococcus pyogenes* and *Acinetobacter baumannii* bacteria were investigated versus highest concentration of ZnS NPs (150 µg/mL). The viability of *Streptococcus pyogenes* was reached to zero at 24 hours after treatment with ZnS NPs (150 µg/mL). This time for *Acinetobacter baumannii* was 34 hours (Diagram 6). The mean of viability based on t-test analysis for *Streptococcus pyogenes* and *Acinetobacter baumannii* was 1.32logCFU/mL and 1.77logCFU/mL, respectively ($p < 0.05$).
| Groups                                                   | Total time of evaluation, hour | Sum of absorbance | Average of absorbance | Variance of absorbance |
|----------------------------------------------------------|-------------------------------|-------------------|-----------------------|------------------------|
| ZnS NPs (50 µg/mL) on *Streptococcus pyogenes*           | 15                            | 15.57             | 1.038                 | 0.173346               |
| ZnS NPs (100 µg/mL) on *Streptococcus pyogenes*         | 15                            | 8.74              | 0.582667              | 0.04215                |
| ZnS NPs (150 µg/mL) on *Streptococcus pyogenes*         | 15                            | 5.11              | 0.340667              | 0.006392               |
| ZnS NPs (50 µg/mL) on *Acinetobacter baumanii*          | 15                            | 13.48             | 0.898667              | 0.135484               |
| ZnS NPs (100 µg/mL) on *Acinetobacter baumanii*         | 15                            | 7.18              | 0.478667              | 0.025098               |
| ZnS NPs (150 µg/mL) on *Acinetobacter baumanii*         | 15                            | 3.88              | 0.258667              | 0.003841               |
| Distilled water on *Streptococcus pyogenes*             | 15                            | 17.93             | 1.195333              | 0.110084               |
| Penicillin (200 µg/mL) on *Streptococcus pyogenes*      | 15                            | 8.11              | 0.540667              | 0.017164               |
| Primaxin (200 µg/mL) on *Streptococcus pyogenes*        | 15                            | 14.65             | 0.976667              | 0.07281                |
| Amikacin (200 µg/mL) on *Streptococcus pyogenes*        | 15                            | 14.66             | 0.977333              | 0.075078               |
| Ceftazidime (200 µg/mL) on *Streptococcus pyogenes*     | 15                            | 15.31             | 1.020667              | 0.078535               |
| Distilled water on *Acinetobacter baumanii*             | 15                            | 19.2              | 1.28                  | 0.1447                 |
| Penicillin (200 µg/mL) on *Acinetobacter baumanii*      | 15                            | 18.06             | 1.204                 | 0.133454               |
| Primaxin (200 µg/mL) on *Acinetobacter baumanii*        | 15                            | 8.805             | 0.587                 | 0.023821               |
| Amikacin (200 µg/mL) on *Acinetobacter baumanii*        | 15                            | 7.66              | 0.510667              | 0.016864               |
| Ceftazidime (200 µg/mL) on *Acinetobacter baumanii*     | 15                            | 12.76             | 0.850667              | 0.059092               |
4. DISCUSSION

Despite the fact that we live in an era of full of new and advanced technologies, with these technologies, we can realize the mechanisms of the disease, and we are able to design new drugs in the molecular forms. Infectious disease are considered as one of the worldwide health challenges [42, 43]. At the beginning of the 20th century, infectious diseases were the leading cause of mortality [44, 45]. Reduced mortality and infectious diseases in the past century were primarily due to the using of antimicrobial agents [46]. Yet today, resistance against antibiotics has been reached to a high level; this resistance makes antimicrobial drugs inapplicable [18, 47].

Unfortunately, there is no certainty that the growth and development of new antimicrobial drug can have a timely manner to rapid and permanent improvement antibacterial effects against micro-
bial pathogens [48]. For example, drug-resistant infections in hospitals and in communities, which are caused by pathogenic gram-positive and gram-negative bacteria, are growing, and the steadily development of antimicrobial resistance will threaten human health due to the inability of human to treat these infections [49]. The dynamics pattern of infectious disease and the occurrence of resistant bacteria types against a number of antibiotics are raised as a challenging issue [48, 50, 51].

One of the recent efforts to eliminate this challenge is discovering antimicrobial effects of nanomaterials that, in present of them, the microbial pathogens may not be able to raise bacterial resistance

**TABLE 3.** Different types of ZnS nanostructures with various synthesis method that used as an antibacterial material.

| Nano type                      | Size of achieved nanoparticles | Synthesis method      | Target microorganism                                                                 | References |
|-------------------------------|--------------------------------|-----------------------|-------------------------------------------------------------------------------------|------------|
| ZnS nanocomposite             | 15–30 nm                       | chemical deposition   | *Peptostreptococcus anaerobius*  
*Streptococcus pyogenes*  
*Bacteroides fragilis*  
*Escherichia coli*  
*Klebsiella pneumonia*   | [61]          |
| ZnS NPs                      | 100 nm                         | sonochemical precipitation | *Candida albicans*                                        | [62]            |
| Copper doped ZnS (Cu:ZnS)      | ≥ 63 nm and ≥ 85 nm             | solvothermal          | *Escherichia coli*  
*Bacillus subtilis* | [63]            |
| PANI coated ZnS nanocomposite | 15.7 nm and 15.4 nm             | microwave-assisted solvothermal | *Escherichia coli*  
*Proteus* | [64]            |
| ZnS nanocrystals              | 3–5 nm                         | co-precipitation      | *Escherichia coli*  
*Klebsiella pneumonia*  
*Bacillus subtilis*  
*Staphylococcus aureus*   | [65]          |
| ZnS nanospheres              | 100–400 nm                      | chemical              | *Escherichia coli*                                       | [22]        |
| ZnS–cellulose nanocomposite   | 10 nm                          | co-precipitation      | *Escherichia coli*  
*Streptococcus pyogenes*  
*Acinetobacter baumannii* | [66]        |
| ZnS NPs                      | 70 nm                          | chemical              | *Acinetobacter baumannii*                     | this work  |
Preparation antimicrobial nanoparticles compared to synthesis of antibiotics are affordable and also stable for long-term storage with long half-life sufficiently [58, 59]. In addition, many nanoparticles can be stable against unstable conditions [60].

So far, the ZnS nanostructures have been used to fight against several bacteria that the size and production methods of them have been applicable and useful in their antibacterial effects (Table 3).

The high ratio of surface area to volume and physical & chemical characteristics of various nanomaterials contribute to their effective antimicrobial activity [67, 68]. Destroying the cell wall and subsequent degradation of cell membrane, damage cell membranes and caused disruptions in the activities of proteins and DNA are probable mechanisms for antibacterial effects of ZnS NPs against Streptococcus pyogenes and Acinetobacter baumannii nanoparticles.

4. CONCLUSION

Here, ZnS NPs with the size of 70 nm were synthesized and applied as an antibacterial agent against Streptococcus pyogenes and Acinetobacter baumannii in the presence of several major antibiotics including Penicillin, Amikacin, Ceftazidime and Primaxin. Based on UV-Vis investigations (OD 600 nm), the antibacterial effect of ZnS NPs showed a direct relation along with the applied concentrations, and the maximum effect was obtained when the concentration of nanostructure was of 150 µg/mL. Total bacterial colony/mL (CFU) of Streptococcus pyogenes and Acinetobacter baumannii bacteria in the maximum concentration of ZnS NPs (150 µg/mL) was 10000 colony/mL and 8000 colony/mL, respectively. It should be noted that the maximum antibacterial effects of antibiotics (200 µg/mL) was related to Primaxin where the total bacterial colony/mL (CFU) of Streptococcus pyogenes and Acinetobacter baumannii bacteria were 70000 colony/mL and 45000 colony/mL, respectively, and was not comparable against ZnS NPs. The viability of Streptococcus pyogenes and Acinetobacter baumannii bacteria in the presence of ZnS NPs (150 µg/mL) was 24 hours and 24 hours, respectively. The findings of this research can be used as the novel anti-resistance antibacterial agent in the future of medical treatment platforms in order to reduce the occurred infectious diseases by Streptococcus pyogenes and Acinetobacter baumannii.

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