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Selenium Oxide Nanoparticles and Their Antibacterial Applications

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Abstract

In the light of committed efficacy of selenium nanoparticles in biomedical implementation, this study used Selenium oxide nanoparticles(SeO_2NPs) to report their antibacterial activity. The synthesis of SeO_2NPs is assured by characterization with XRD, and morphological characters that were observed using SEM and TEM analysis. The crystalline nature of SeO_2NPs is assured by XRD analysis. SEM and TEM images show that SeO_2 NPs are uniform and in spherical shape of size 20-40 nm. SeO_2NPs play a critical role in growth inhibition of Escherichia coli and Staphylococcus epidermidis. Antibacterial activity of SeO_2NPs showed by agar well diffusion and minimum inhibitory concentration (MIC) tests. SeO_2 NPs showed inhibition zone about (23mm) against E.coli and about (18) against Staphylococcus epidermidis. SeO_2NPs showed a MIC value 31.25 µg/mL against both isolates. In briefly, these results suggest that SeO_2NP can be candidates for supplements and antibacterial substitutes against bacteria for topical use like dressing.

Keywords: antibacterial activity, Selenium oxide nanoparticles, MIC, E.coli, Staphylococcus epidermidis

المستخلص

في ضوء الفعالية المثبتة لجسيمات السيلينيوم النانوية في المجال الطبي الحيوي، استخدمت هذه الدراسة جسيمات أوكسيد السيلينيوم النانوية (SeO_2NPs) لتحديد الفعالية المضادة للبكتيريا. تم تأكيد تخليق SeO_2NPs من خلال التوصيف باستخدام XRD والصفات الشكلية التي تمت ملاحظتها باستخدام تحليل SEM وTEM. تم ملاحظة الطبيعة البلورية لـ SeO_2 NPs من خلال تحليل XRD . اظهرت صور TEM, SEM أن SeO_2 NPs منفردة وكروية الشكل بحجم 20-40 نانومتر. أدى SeO_2NPs دورًا واضحا في تثبيط نمو الإشريكية القولونية والمكورات العنقودية الجلدية. درست فعالية SeO_2NPs المضادة للبكتيريا من خلال اختبارات انتشار حفر الآجار والحد الأدنى من التركيز المثبط (MIC). أظهرت SeO_2NPs منطقة تثبيط حوالي (23 ملم) ضد الإشريكية القولونية وحوالي (18 ملم) ضد المكورات العنقودية البشروية. أظهرت SeO_2NPs قيمة MIC (31.25 ميكروجرام/مل)ضد كلا العزلتين. باختصار، تشير هذه النتائج إلى أن SeO_2NPs يمكن أن يكون مرشحًا للمكملات الغذائية والبدائل المضادة للبكتيريا ضد البكتيريا للاستخدام الموضعي مثل الضمادات.

Introduction

There has been increasing attention in the era of antibiotic bacterial resistance rapidly continue to develop adaptive countermeasures against classic antibiotics[1]. Bacteria consider life threating agents, able of supporting infectious diseases. The history of bacteria as occasional agents for infection return to the 14th century. Salvarsan was the first antimicrobial agent introduced in 1910. After that , another antimicrobial agents as nalidixic acid, chloramphenicol, and macrolids were used worldwide. The 20th century experienced temporal comfort to

infectious bacterial pathogens. Therewith, overexposure to antibiotics and development of efficacious countermeasures against antimicrobial agents led to the evolution of antibiotic resistant bacteria[2]. Since then, great efforts were condensed on overcoming the emanation of these resistant strains by evolution of new drugs with chemical diversity and finding of antibiotic producing bacteria as well as new antibiotics from natural unexplored sources. In spite of that, these progressions could not recompense the rapidly growing number of resistant strains of bacteria. Nanotechnology is quite used in the formation of various products in the fields of medicine and biology. Using nanotechnology in biology has extended many occasions in many areas including drug delivery, diagnosis, tissue engineering, imaging and fight against bacterial infections[3]. By needing for new antibacterial agents, nanoparticles have been proposed to treat infections as they use different mechanism to kill bacteria than conventional antibiotics[4] with rather low toxicity in body cells. So nanomaterials can be counted as a promising alternative to conventional antibiotics to rule bacterial infections[5]. Selenium is a nutrient element that has a massive function in biological systems which represent as one of the important compounds to integrate with antibacterial agents. Selenium is an essential element in the diet, desired for maintenance of growth and health [6]. In recent years, many studies have pointed to the ability of selenium nanoparticles to display anticancer[7], antioxidant[8], antibacterial and antibiofilm[9] properties. So notable antibacterial activity of selenium nanoparticles have been proved against pathogenic bacteria, yeast and fungi[10][11][12].

Materials and Methods

1. Synthesis of SeO_2 Nanoparticles

20 ml of sider leaves extract was added drop by drop to a solution containing 100 mL of tween and 500 mg of sodium selenite penta hydrate dissolved in 400 ml of deionized water under vigorous stirring. The mixture was stirred for 1 hour and the color was changed to white (dispersed) Thereafter, the white dispersed solution was centrifuges at 8000 rpm in cooling centrifuge and the pellet was re-dispersed and washed with hot water to remove the excess of any side products. The white solid was then calcined at 400 ^{o}C for 2 hours to get the required nanoparticles[12].

2. Characterization of SeO2 Nanoparticles

2.2 **Scanning Electron Microscopy (SEM) Of Seo2 Nanoparticles**

To study the morphology and size of SeO2 nanoparticles, it was used scanning electron microscope (SEM). Images of the samples were acquired using a conventional secondary electrone detector and a 10kV electron beam. The nanoparticles powder was placed on double sided tape with one side glued to the sample holder and the other to the sample. Under vacuum, the samples were then sputtered with a thin layer of gold.[13]

2.3 X-Ray Diffraction (XRD)

The X-ray diffraction was used for characterization of selenium dioxide , the powder was used for test. The nanoparticle sample was dispersed on a low background noise sample holder and analyzed in a Bruker D8 Advance X-Ray diffractometer equipped with a LynxEYE detector. X ray diffraction analysis was operated at a voltage of 40 kV, with current of 40 mA, with copper radiation of 1.54060 Å. The scanning was performed in the 2θ range of 10° to 40° at 0.02°/min with time constant of 1.2 s. [14]. Average size of NPs was determined by applying Debye–Scherer's equation:

$D = K\lambda/\beta\cos\theta$

where K = shape factor, λ = X-ray wavelength, β = full width in radius at half maximum and θ = Bragg's angle.

2.5 Transmission Electron Microscope (TEM)

The SeO2 samples was centrifuged at 10000 rpm for 10 minutes. For transmission electron microscope (TEM) , drop of solution having selenium nanoparticles was placed on the carbon coated copper grids and kept under vacuum desiccation for an overnight before loading them on to a specimen holder. Study of size and morphology of the nanoparticles was performed by transmission electron microscope Carl Zeiss Libra 120M (Carl Zeiss AG, Germany).[15]

3 Bacterial Strains and Culture Conditions

Escherichia coli and Staphylococcus epidemidis isolates were provided by Al-Yourmok Teaching Hospital (Baghdad/Iraq) in October/2023. The isolates identification were improved by VITEK® 2 Compact system. These isolates were kept as glycerol stock solution in -20 °C until use it.

4 Antimicrobial Activity

The antibacterial activity of selenium oxide nanoparticles were determined by the agar well diffusion method. The well of muller hintone agar plates were seeded with selenium oxide nanoparticle (500 µg /ml) for (E. coli and S. epidermdis), the MHA plates were kept at 4C° for 1 hr to allow the proper diffusion , after that kept at 37 C° for 24 hr . The zones of inhibition were measured[16].

Minimum inhibitory concentrations (MICs) of SeO2NPs against standard microbial strains, were determined by the broth microdilution method [17]. Each sample was diluted with microbial growth media to final concentrations ranging from 250 to 1.95µg/mL and transferred into 96-well polystyrene microtiter plates. The wells were further supplemented with bacterial suspensions (~106 colony-forming unit per mL– CFU/mL), and incubated for 24 h at 37◦C. Mueller-Hinton broth (MHB; HiMedia, India) were used as growth media for bacteria. MICs were determined as the lowest concentrations in which there was no visible growth of microorganisms.

Results and Discussion

SEM Analysis

One of the three parameters that influence on their interaction with biological entitied is the morphology of nanoparticles.SEM analysis was used to investigate the morphologies of SeO2NP and revealed the spherical shape.The average size of SeO2NPs was about 33nm(fig.1). Our study agreed with other study [18], who reported a 35.6 ± 7.5 nm average size. Our results were like to those of [19, 20], who reported spherical SeNP production. Among other parameters(mixing methods, concentrations, temperature, the ratio between dispersion phases), morphology strongly depends on the choice of stabilizers. Spherical particles are most frequent since this morphology provides a lower level of surface energy[20].

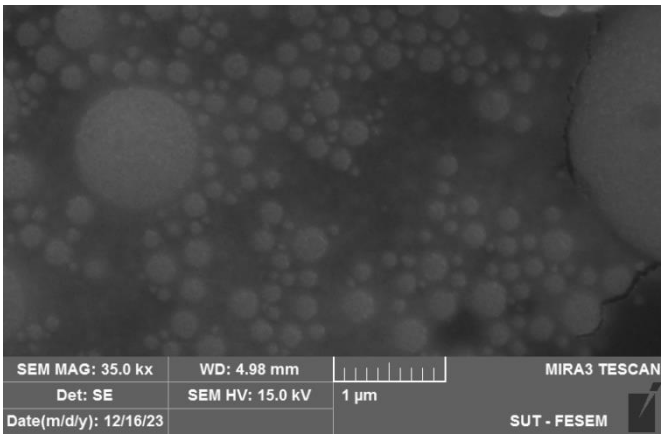


Fig.1: scanning electron microscope analysis of SeO2NPs

XRD Analysis

By using XRD technique, the crystalline structure of SeO_2NPs was reported. The XRD pattern of nanoparticles showed two spectral peaks(Fig.2.a) at 2-theta=23.342, 29.557 with (101), (100) reflections which enhanced and suggested that the SeO_2NPs has been favouring to grow along the (202) direction [21]. XRD analysis revealed the main components of SeO_2NPs (fig.2.b) which include Selenium and oxygen. The crystallinity of SeNPs was proven as an important factor for its bioavailability and bioactivity. Thus, to perform an adequate comparison of antimicrobial activity between samples, all of them needed to be amorphous. [22]Using Debye–Scherer's equation [23], it was possible to determine the average crystal size of SeO_2NPs as 34nm.

a **b**

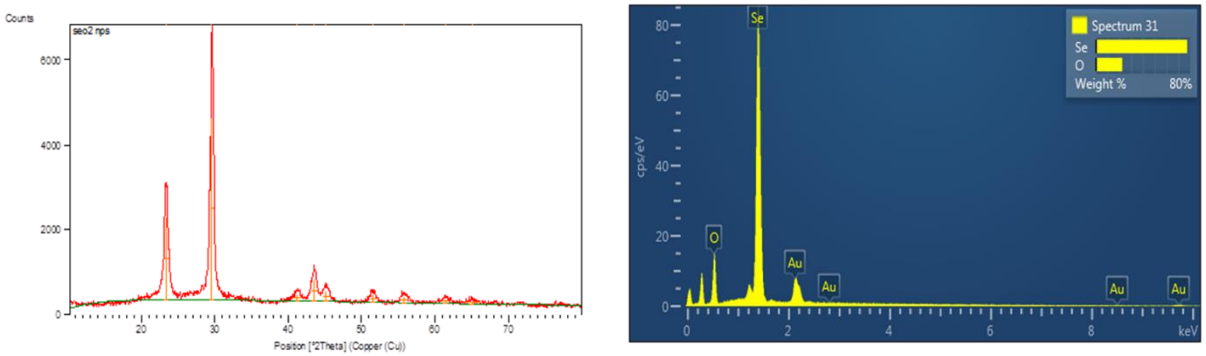


Fig2. XRD pattern analysis of synthesized SeO_2NPs; a:show spectral peaks of SeO_2 b: show the percentage of the SeO_2NPs components

TEM Analysis

TEM images of SeO2NPs showed particle size distribution histogram. Fig.3 explained the related particle size histogram gained after measuring. TEM micrograph shows well-dispersed spherical particles, with diameter range of 20-40 nm (Fig.3) According to previous studies, the spherical shape of the produced nanoparticles confirms the presence of SeNPs[23]. While these results are comparable with a study in which the size of these nanoparticles was observed at 50–150 nm [22], the size of the biosynthesized nanoparticles varies depending on the host producing these particles and also the conditions of production[23].

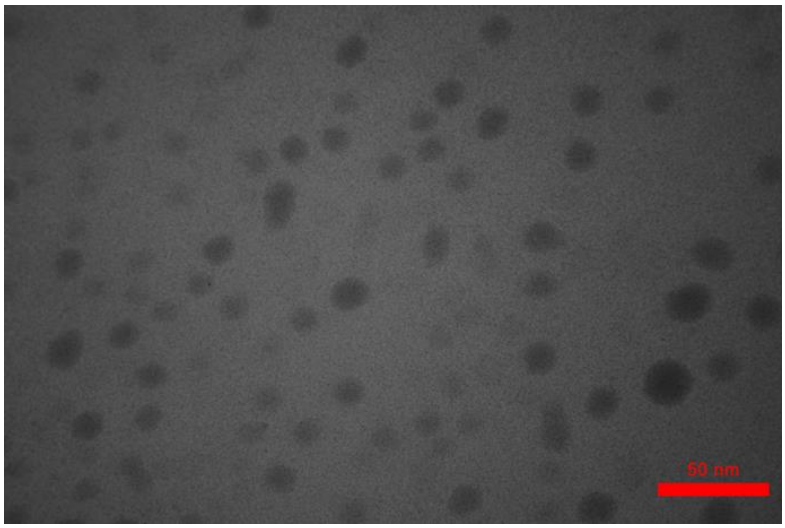
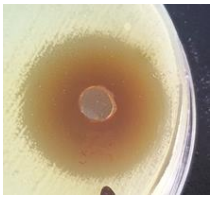


Fig.3: Transmission electron microscopy of Se NPs samples

Antibacterial Activity and MIC

SeO_2NPs is used as alternative to conventional antibiotics to decrease multidrug-resistant pathogens[24]. The synthesis of nanomaterial has been depending on physical and chemical properties that include concentration, surface area, shape, size and stability[25].

In this study, SeO_2NPs exhibited antimicrobial activity against tested bacteria, the antimicrobial activity was shown through measuring the inhibition zone(mm) against E.coli and S.epidermidis (fig.4). At a concentration 250 µg/mL, E.coli has shown 23 mm zone of inhibition and S.epidermidis showed 18 mm. Similarly, the MIC results of SeO_2NPs were observed to be 31..25 µg/mL against both isolates E.coli and S.epidermidis. Once upon time, it was notified that the antimicrobial activity was related with the size on nanoparticles as the smaller has higher stability and excessed surface-to-volume ratio which facilitates effective interactions and intracellular diffusion[26]. In previous reports, SeNPs at concentration of 100 µg/mL exhibited inhibition zone around 22mm against P.aeruginosa and MIC value of 8 µg SeNPs/ 200 µL[27].In other studies, it was reported that SeNPs stabilized with chitosan were efficient against gram-positive bacteria that include S.sanguinis, S.aureus and E.faecalis with MIC ranging from 0.068-0.274 µg/mL[28]. It has been suggested that the SeNPs showd antimicrobial effect by damaging membrane and cell wall, oxidative stress mechanism, and intracellular invasion. Production of ROS(reactive oxygen species) causes oxidative DNA damage and membrane lipid peroxidation that results in cell wall damage and leakage of cytoplasmic contents[29,30]. Furthermore, the morphological changes resulted by SeNPs disturbs respiratory functions and ATP synthesis which stops cell division, damage cell integrity, membrane depolarization and microbial cell death[31,32] .

a  **b** 

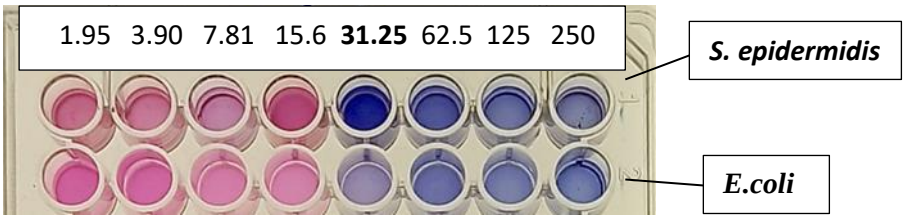
c 

Fig.4: shows antibacterial activity of SeO2NPs; a: nanoparticles against S.epidermidis b: nanoparticles against E.coli, c: MIC of nanoparticles against S.epidermidis and E.coli is in 31.25µg/mL

Conclusions

Using SeO2NPs with an average grain size of 34 nm has exhibited a high potential inhibition of bacterial growth for clinical E.coli and S.epidermidis. SeO2NPs may be considered as an alternative agents to conventional antibiotics to treat some pathogenic bacteria. NPs has the ability to facilitate targeted delivery of antibacterial agents to cells, thereby enhancing treatment efficacy and alleviating adverse effects.

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