PHYSICS AND CHEMISTRY OF SOLID STATE
V. 23, No. 4 (2022) pp. 652-658
Section: Technology

DOI: 10.15330/pcss.23.4.652-658

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ФІЗИКА І ХІМІЯ ТВЕРДОГО ТІЛА
Т. 23, № 4 (2022) С. 652-658
Технічні науки

PACS: 61.46.Df ISSN 1729-4428

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Cold plasma synthesis of Zinc Selenide Nanoparticles for inhibition bacteria

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Zinc Selenide Nanoparticles (NPs) were fabricated by the aqueous technique using cold plasma under atmospheric pressure with an exposure time of 3 min and a gas flow rate of 3 liters per minute. films' structural characteristics and morphological characterization were investigated by X-ray diffract meter, atomic force microscopy (AFM), and scanning electron microscopy (FE-SEM). In addition, parameter like crystal size were calculated. Results showed XRD patterns exhibits structure of polycrystalline of preferential orientation (111) direction. SEM technique shows that the nanoparticles presented are spherical. AFM image verified film formed spherical particles distribute uniformly. The antibacterial disc diffusion property of these Nanoparticles, was performed against Gram-negative bacteria of Escherichia coli and Gram-positive bacteria of Staphylococcus aureus, showing good control of said bacteria. The maximum level of inhibition in coli form bacteria with an average inhibition zone diameter with staphelоscous aureus, implying an increasing trend with increasing/decreasing loading volume of NC volume. Therefore, these nanomaterials, which can be prepared in a simple and cost-effective way, may be suitable for new types of germicidal materials.

Keywords: Zinc Selenide, Nanoparticles, Cold plasma, Escherichia coli, staphelоscous aureus.

Received 10 April 2022; Accepted 09 October 2022.

Introduction

In these days, while the development and civilization of human existence is being developed, the variety of microorganisms (such as the viruses, bacteria and microbes) in natural contact with our body and environment is growing dramatically. For bacteria, anti-bacterial drugs/agents have long been employed to suppress or kill bacteria. Such traditional antibiotics also develop high-grade drug resistance and therefore their effectiveness decreased [1]. Using biotechnology, Nanoparticles and quantum dots, is a great approach to overcome such drug resistances. Due to the specific characteristics and varieties of nanomaterials, various scientific disciplines of bioimages and bio-pharmaceutical requirements have been thoroughly studied, therefore making the use of nanostructure materials in medicine a surprise or a revolution of contemporary medicine today [2]. Today, it is well recognised that nano-agents may destroy the greatest content or even all unwanted micro-organisms without any side effects, whereas traditional antibiotics have a certain percentage success for habitation or bactericidal effects [1]. Several nanomaterials such as silver, gold, copper and copper oxide, ZnS and Chitosan Nanoparticles have recently been found.Strong antimicrobial potential has been established [3]. Zinc Selenide (ZnSe) Nanoparticles are II-IV semiconductors with a direct mass band gap (~2.72 eV) in energy and are useful in many application applications, e.g. LED lighting systems, solar cells and sensors [4]. Different techniques of preparation include soft chemicals, chemical precipitation, soil gel method, cold plasma [5,6].In the areas of generation of ozone, surface modifications, air/water purification, medical facilities and so on, plasma technology is being marketed and industrialised, with promising energy effects and economic advantages

already demonstrated. Nanomaterial synthesis is extensively carried out by techniques that do not relate to plasma, such as chemical vapour deposition (CVD) [7], solvothermal pathways, sol–gel methods, laser removal [8], etc. Fine-quality products might be produced if non-plasma techniques were appropriately selected and regulated for beginning materials and procedures. However, owing to the very lengthy response time, it is usually impossible to regulate the dispersion of Nanoparticles [9]. Furthermore, both Escherichia coli, gram-negative Staphylococcus aureusgram-positve bacteria, using disc diffusion bio-essay, have been quantitatively evaluated for their antibacterial properties. These two bacterial strains are very infectious in hospitals and the environment, therefore the antibacterial activity of ZnSe nanoparticles may be evaluated.

I. Procedures setup

1.1. Synthesis Colloidal Nanoparticles

The Se NPS synthesis involves the application of approximately 16 kV of high voltage and plasma is then generated between the capillary and the aqueous SeO (NO3)2 solution. The high energy and loaded ionic (Ar) electrons in the plasma assist to complete the circuit and the current (1,66 mA), the flow rate of gas (2 L/min) and start to flow in the system. Just minutes after discharge begins, the solutions begin to become burned orange. This shows the production of Se nps, as illustrated in Figure (1). The period of exposure was selected for concentration (3 min). The coloured solution was then described using a measuring device.

Fig. 1. Colored solution of Se NPs.

1.2. Synthesis ZnSe Colloidal Nanoparticles

After synthesizing Se NPs as a core, we introduced a 99.95 percent purity strip of zinc metal to the Se NPs solution. After exposure to plasma, we moved it to achieve homogeneity where a change of colour was noticed after a minute, as shown in Figure (2), which shows that The Se NPs were rapidly combined with the zinc particles produced by a cold plasma ablation at a concentration near the selenium nanoparticles concentration of 0.04 mM for the obtaining of the nanoparticle. A colour shift in the mixture was noticed after 3 minutes, indicating the production of ZnSe C.S NPs.

Fig. 2. Colored solution of ZnSe NPs.

1.3. Bacterial isolates activation and preparation

The bacterial isolates utilised were derived from the Nanotechnology Laboratory / Biotechnology Department / Baghdad University; they were Gram-positive bacteria (Staphylococcus aureus), and gram-negative bacteria (Escherichia coli). Bacterial isolates were streaked and cultured at 37°C for 24 hours on the heart infusion agar. A single colony was then taken out of a medium plate and inoculated into 5 mL of brain heart infusion broth, and then incubated at 37°C overnight.

1.4. Culture Media Preparation

All media mentioned below have been prepared by the manufacturing firm and sterilised at 121°C for 15 minutes below 15 Psi by autoclaving.

1.5. Bacterial Growth Method Inhibition

The diffusion technique of the tablet was utilised to determine the growth inhibition of the studied microorganisms. Bacterial suspension cells were distributed into the prepared agar medium in the two types of bacteria used in study. Then tablets with the size of the antibiotic pill were made and soaked with the prepared nanopart solution for a few minutes.The plates were then maintained in the incubator for 24 hours at a temperature of 37^0C. On the second day the results were measured by using a ruler to measure the inhibition area around each pill.

II. Result and Discussion

2.1. Structural Properties

2.1.1. X-Ray Diffraction Investigation

The produced ZnSe Nanoparticles are displayed in the figure as X-ray diffraction patterns of non-thermal plasma (3). The XRD models of ZnSe, prepared by non-thermal plasma, have shown major peaks at diffraction angles (27.22, 29.25, 38.02, 45.37) and (35.28) of Zn (111), (101), (110) and (002) corresponding planes.This result agrees well with that presented in references [10]. In general, ZnSe forms in both hexagonal wurtzite structure and cubic zinc blend structure, structure which were matches well the standard peaks (JCPDS NO. 00-005-0522). From the x-ray patterns, the broadening of the diffraction peaks of the nanoparticles is obvious, the crystallite size (D) calculated by using Scherrer formula (1)[4]:

$$D = \frac{0.9\,\lambda}{\beta \cos\theta}, \qquad (1)$$

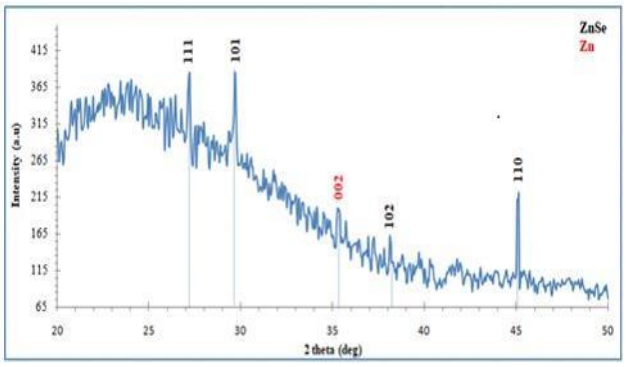

Fig. 3. XRD diffraction spectrum of ZnSe NPs.

The peak widths of a strong diffraction plane were calculated which they found value of D shows that the size lies within the Nanoparticles range which the average crystalline size of ZnSe NTP is (51.28)nm and listed in the table (1).

Table 1. Structural properties resulting from XRD of ZnSe NPs prepared by physicalmethod.

Sample	FWHM (Deg.)	2 theta (Deg.)	C.S (nm)	Avg. C.S ZnSe	hkl
ZnSe NTP	0.15	27.2243	54.62	51.28	**(111)**
	0.19	29.257	43.31		**(101)**
	0.12	36.2964	69.83		**(102)**
	0.14	37.9659	60.14		**(110)**
	0.3	43.2313	28.54		**(002) Zn**

2.2. Morphological Properties

2.2.1. Atomic Force Microscopy Analysis (AFM)

Three dimensional (3D) AFM profiles are used to provide materials with information on the morphology of the surface. Figures (4) demonstrate the ZnSe NPs preparation surface morphology by non-thermal plasma. Where it was noted that the average granular material diameter prepared in the green method is less than the granular diameter of the prepared substance without the extract, meaning that the extract produces a particle capping and thus the material cannot be aggregated together and thus keeps the particulate size intact. Table (2) provides physical technique AFM parameters for ZnSe NPs.

2.2.2. Transmission Electron Microscopy (TEM)

The ZnSe produced nanoparticles were used to analyse the size, shape and morphology of the transmission electron microscopy (TEM), as shown in figure (5). These findings demonstrate that the particles have spherical shape and are evenly distributed (mono dispersed) without major agglomerations.

2.3. Optical Properties

2.3.1. Optical Energy Band Gap (E_g)

The band gap energy can be obtained in nanomaterial's from maximum absorption. According to the theory of quantum confinement, the electrons in the conductivity band, and the holes in the valence band are spatially confined to the potential barrier of the surface. Due to confinement of both electrons and holes, the lowest optical transmission of energy from the valence to the conduction band increases the energy, effectively increasing the band gap (E_g) [11]. A shoulder or peak spectrum corresponds to the fundamental absorption edges of samples; the optical energy band gap for ZnSe Nanoparticles has been calculated using absorption edge.The bulk ZnSe exhibit a narrow band gap of about 2.7 eV, while in this study the showed a very large energy gap as show in table (3) increasing the band gap energies

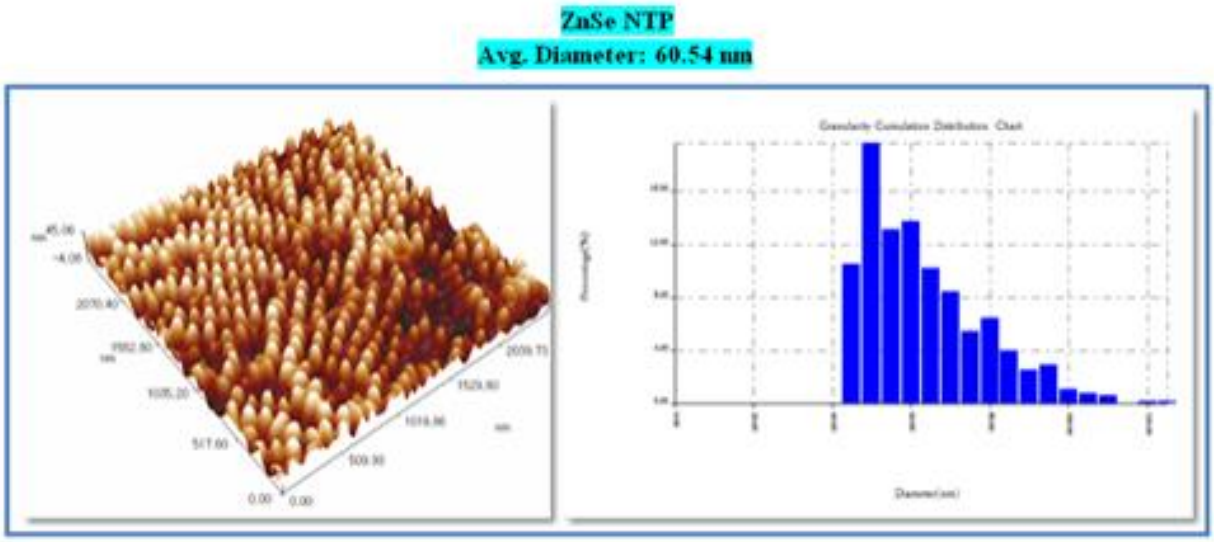

Fig. 4. 3D AFMof ZnSe NPs.

Table 2

Shows the AFM parameters for ZnSe NPs by physical method.

Sample	Avg. Diameter(nm)	Root Mean Sq. (nm)	Ave. Roughness (nm)
ZnSe NTP	60.54	5.87	6.50

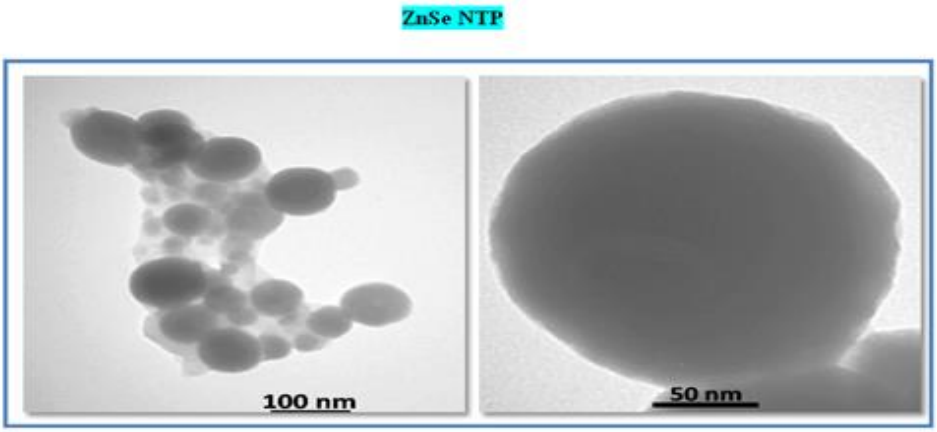

Fig. 5. TEM imageof ZnSe NPs.

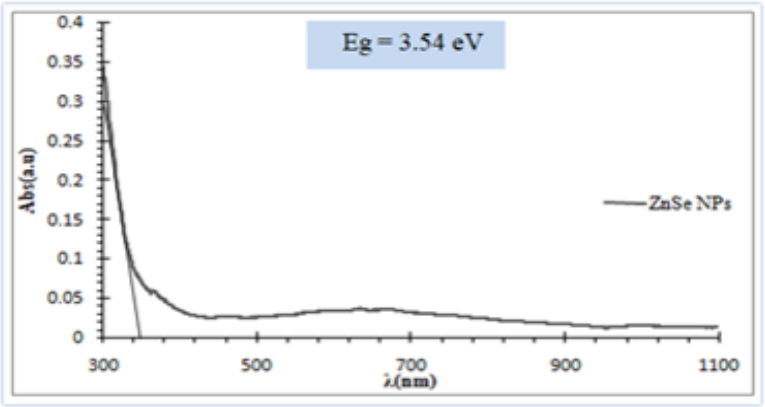

Fig. 6. UV-VIS Absorbance spectrumof ZnSe NPs.

Table 3

Band gaps from UV-Vis absorption spectra of the samples.

Manufacturing method	Absorption wave length(nm)	Band gap (eV)
ZnSe NPs physical method	350	3.54

of ZnSe nanostructure could be an indication of the quantum confinement effect due to decreasing size of structure. These results have good agreement with the results obtained from XRD, AFM, and TEM measurements. Figure (6) show the UV-VIS absorption spectra of the ZnSe NPs has been recorded, to measure their band - gap.

2.4. The inhibitory effect of ZnSe NPs prepared physical and green synthesis against bacteria by Agar diffusion method

In this study, the experimental of the effect of ZnSe NPs against different human pathogens, which include two isolates of bacteria negative gram (Escherichia coli) and positive gram (Staphylococcus aureus) were used in this experiment. Selected pathogenic bacteria were achieved in triplicates and the obtained results were statistically analyzed. The means and standard deviation (means ± SD) were calculated and reported for all treatments and compared with control.

The antibacterial effect of ZnSe against pathogens that infect the human body is measured on the basis of the area of inhibition compared to the control, which did not show any inhibition on bacteria compared to Nanoparticles, for which the inhibition was very clear when adding concentrations (100,50, 25, and 12.5)% The reason for this is because of the size and area of the Nanoparticles. As the lowest inhibition of all *E.* coli species was at a concentration of 12.5%, which is 9 mm for ZnSe NPs. As for *S.* aureus bacteria, the lowest inhibition diameter was recorded at the same concentration, which is 8mm with respect to ZnSe NPs, as shown in the table (4), Figures (7A) and (7C) and (8).

The inhibition of nanoparticle bacteria is explained by several hypotheses as the properties of bacterial killing are similar to the nanoparticles in terms of their dependence on size, stability and concentration added to the growing medium which provides more time to interact nanoparticles with bacteria as the surface-to-volume ratio in nanoparticles increases by one million [12]. However, it is essential that the nanostructions have the capacity to cross or pierce such membranes and adequately stabilise them in order to decide the development of bacteria, by influencing the regular operation of the cells [13]. Notwithstanding the various methods used by researchers to inhibit the growth of bacteria from the nanoparticles, their principle is that these particles have a positive charge that can be associated with a negative charge on the bacterial cell surface, resulting in particle collections on the cell membrane surface and therefore changes in physique and chemical properties [14]. And that the positive ions of nanoparticles used in the trial can be released from these particles within the cell which can be

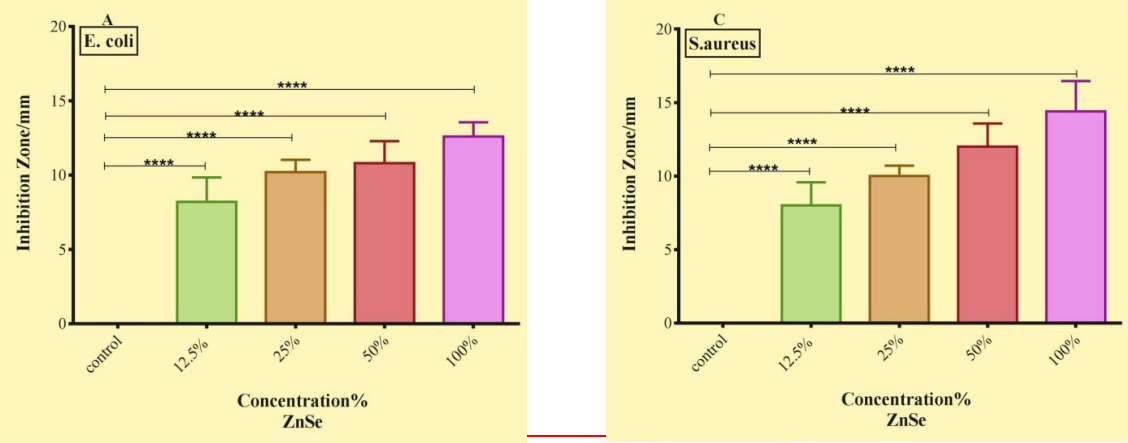

Fig. 7. Effect of ZnSe NPs prepared in four different concentration (100, 50, 25, and 12.5) % against some clinical isolates of bacteria and yeasts, A: *E. coli* for ZnSe prepared by physical method, C: *S. aureus* for ZnSe prepared by physical method, Theeffect was measured by the inhibition zone formed around wells filed by ZnSeNPs, statistical analysis reported as the means values followed by the letters aresignificantly p≤0.0001, Errors bars represent SD of Triplicate experiments.

Table 4

Inhibition zone of the ZnSe NPs activity towards selected pathogenic bacteria with concentration (100, 50, 25 and 12.5) % prepared by non-thermal plasma.

Type bacterial	**Organisms**	**sample**	**Inhibition zone / mm**			
			100%	**50%**	**25%**	**12.5%**
Gram - Negative	E. coli	ZnSe	12.6 ± 0.4301	10.8 ± 0.6633	10.2 ± 0.3742	8.2 ± 0.7348
Gram - Positive	S. aureus	ZnSe	14.4 ± 0.9274	12 ± 0.7071	10 ± 0.3162	8 ± 0.7071
Control	-	ZnSe	0 ± 0	0 ± 0	0 ± 0	0 ± 0

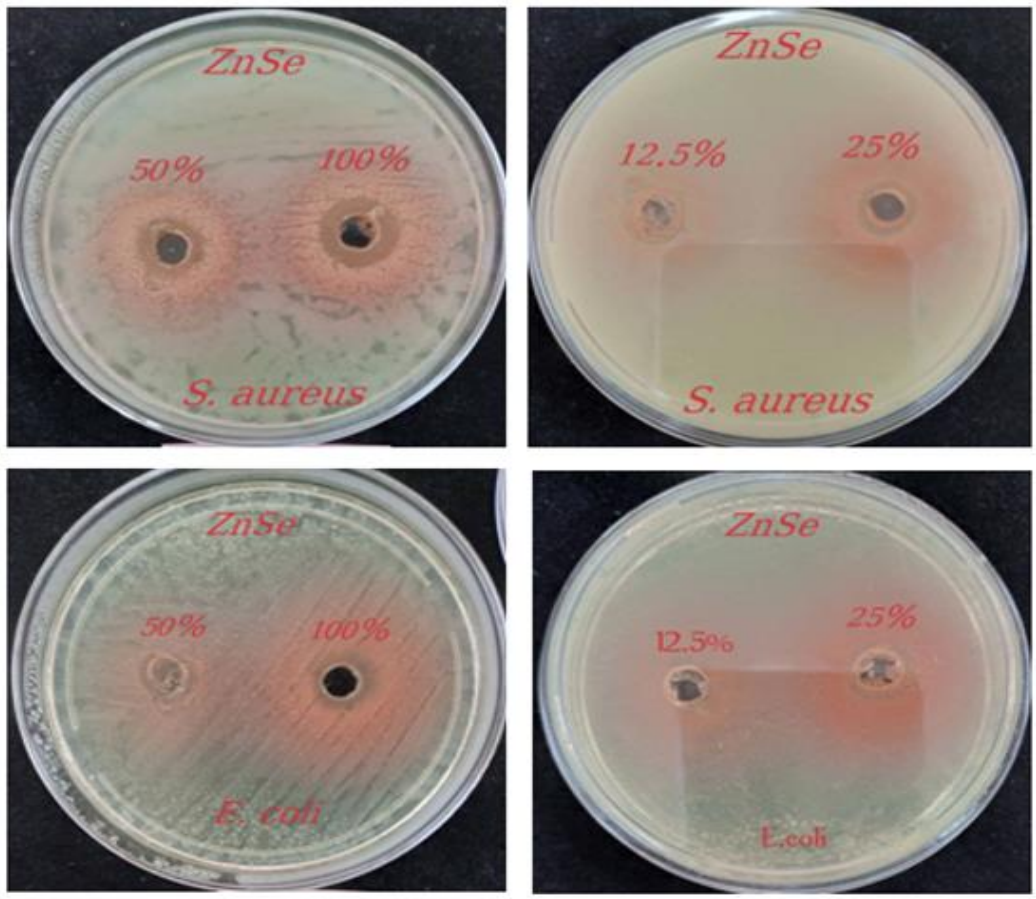

Fig .8. Antibacterial activityof ZnSe NPs.

linked to the bacterial ribosome to stop the synthesis of proteins or prevent the propensity of the genetic material in bacteria by attaching them to the genetic material and thereby destroy the DNA, which leads to the death of bacteria [15,16].

Conclusion

Different semi-conducting nanoparticles demonstrate the high-end achievements of nano-biotechnology as powerful antibacterial agent against bacteria. The current research shows nanosized ZnSe particles' antibacterial effect characteristics produced using the cold plasma technique. ZnSe nanoparticles were shown to display strong antibacterial action against gram-negative and gram-positive bacteria. By looking at the surface morphology and concentration impact, the findings show that the percentage of the inhibition of microorganism rises with the increase in the loading size of nanoparticles and the reduction in nanopart size. The present validation is thus helpful to establish that nanoparticles are a potential antibacterial substance.

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Синтез наночастинок селеніду цинку методом холодної плазми для інгібування бактерій

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Наночастинки селеніду цинку (НЧ) виготовлено рідинною технологією із використанням холодної плазми під атмосферним тиском з часом експозиції 3 хв і швидкістю потоку газу 3 л. на хвилину. Структурні та морфологічні характеристики плівок досліджували за допомогою рентгенівського дифрактометра, атомно-силової мікроскопії (АСМ) та скануючої електронної мікроскопії (FE-SEM). Крім того, розраховано такий параметр, як розмір кристала. Результати показали, що рентгенограми демонструють структуру полікристалів із переважною орієнтацією (111). Техніка SEM показує, що представлені наночастинки є сферичними. Сферичні частинки плівки, що підтверджено АСМ-зображеннями, розподілені рівномірно. Антибактеріальна дифузійна властивість цих наночастинок досліджувалася на грамнегативних бактеріях Escherichia coli та грампозитивних бактеріях Staphylococcus aureos, що показало хороший контроль над цими ними. Максимальний рівень інгібування в coli утворюють бактерії із середнім діаметром зони інгібування з staphelоscous aureous, що означає тенденцію до зростання зі збільшенням/зменшенням об'єму навантаження NC. Таким чином, ці наноматеріали, які можна отримати простим і економічно ефективним способом, можуть бути придатними як нові типи бактерицидних матеріалів.

Ключові слова: селенід цинку, наночастинки, холодна плазма, Escherichia coli, staphelоscous aureous.