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Evaluation of the antibacterial activity of PVA-stabilized nanoparticles against MDR bacteria

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Finally to all our friends and colleagues, we present our thanks.

Content

Abstract

In the present work selenium nanoparticles have been synthesized by chemical reduction method by using ascorbic acid as reducing agent, polyvinyl alcohol has been used as stabilizing agent by capping the formed nanoparticles and preventing their aggregation, the nano size, shape, charge and structure of the synthesized nanoparticles were confirmed by TEM, DLS, Zeta potential, and FT-IR analysis. Then, the effect of the PVA Se NPs on two MDR bacterial strains (Staphylococcus aureus and Klebsiella pneumonia) was estimated in comparison with Levofloxacin as standard antibiotic. The obtained results reveled that PVA Se NPs can be considered as promising antibacterial agent to tackle the problem of drug resistance.

1.1 Introduction

Antibiotic resistance, also known as antimicrobial resistance (AMR), is a growing global concern. It refers to the ability of bacteria, viruses, and other microorganisms to evolve and develop resistance to the drugs that were once effective in treating them. This means that the medications that were once considered "wonder medicines" are becoming less effective in fighting infections .Resistant is indeed a major threat to humanity, causing a significant number of deaths worldwide each year. It is estimated that AMR is responsible for around 700,000 deaths annually. If this issue is not effectively addressed, it is predicted that millions of deaths could occur by 2050. This highlights the urgent need for action to combat AMR and preserve the effectiveness of antibiotics for future generation.1

1.2 Reasons behind antibiotic resistance

Microbial (natural) reasons AMR is primarily caused by alterations within the bacteria, and may occur in a variety of ways:

1. **Genetic mutation** changes in the target site of the antibiotic, or the efflux of the antibiotic from the bacterial cell.
2. **Genetic material transfer** allows bacteria to acquire antibiotic resistance genes from other bacteria, even those of different species or genus. This contributes to the spread of antibiotic resistance.
3. **Selective pressure** plays a crucial role in driving the evolution and spread of resistance in microbial populations. As a result, they are able to reproduce and pass on their resistance genes to future generations, leading to the rapid spread of resistance within the microbial population.

4. **Inaccurate diagnosis and Inappropriate prescription of antibiotics** the reliance on unreliable or inaccurate knowledge, overprescribing, and patient pressure can all contribute to the development and spread of antimicrobial resistance. It is crucial for healthcare professionals to stay updated on the latest guidelines and evidence-based practices to ensure the appropriate use of antibiotics and minimize the risk of resistance.
5. **Self-medication** the most significant concern with SMA is the development of drug resistance in microbes. Antibiotic resistance occurs when bacteria evolve and become resistant to the drugs used to treat them. This can happen when antibiotics are used inappropriately, such as in cases of viral infections or when the wrong antibiotic is used. SMA contributes to the overuse and misuse of antibiotics, which accelerates the development of drug-resistant bacteria, making infections harder to treat and increasing the risk of complications.
6. **Poor hospital environment**, hospitals are particularly vulnerable to the spread of AMR due to the high concentration of patients with compromised immune systems, the frequent use of antibiotics, and the close proximity of individuals in healthcare settings. The presence of bacteria on clothing, surfaces, and in the air can contribute to the transmission of AMR. 2-12

1.3 Mechanism of action of antibiotics

Bacteria use various mechanisms of resistance 1, some are ‘intrinsic’, whereby the cell can use genes it already possesses to survive antibiotic exposure, and some are ‘acquired’, whereby gain of new genetic material provides new capacities that mediate survival.

Antibacterial activity is usually classified as one of five mechanisms: interfering with bacterial cell wall synthesis, inhibition of bacterial protein biosynthesis, inhibition of

bacterial nucleic acid synthesis, inhibition of metabolic pathways, and inhibition of bacterial membrane function.

1. **Antibiotics inhibiting cell wall synthesis** Bacterial cell walls are made of cross-linked Peptidoglycan. Different types of antibiotics target different steps in the cell wall synthesis process. For example, beta-lactam antibiotics like penicillin and cephalosporins inhibit an enzyme called transpeptidase, which is responsible for cross-linking the building blocks of the cell wall. Without these cross-links, the cell wall becomes weak and unable to maintain its integrity, leading to cell lysis. Other classes of antibiotics, such as glycopeptides (e.g., vancomycin) and lipopeptides (e.g., daptomycin), act by interfering with the assembly of the cell wall components or by disrupting the cell membrane, which leads to cell death.
2. **Antibiotics inhibiting protein synthesis**. The 70S ribosome of bacteria (based on the protein sedimentation rates, expressed as "Svedberg" units) constitutes of 30S and 50S subunits.
3. **Inhibition of bacterial nucleic acid synthesis** Most bacterial species maintain two distinct type II topoisomerases that assist with deoxyribonucleic acid (DNA) replication, DNA gyrase, and topoisomerase IV. DNA gyrase is responsible for reducing torsional stress ahead of replicating forks by breaking double-strand DNA and introducing negative supercoil.
4. **inhibition of metabolic pathways**.

   sulfonamides: compete with PABA to inhibit dihydropteroate synthetase and the genesis of bacterial dihydrofolic acid.

   Trimethoprim is a potent inhibitor of bacterial dihydrofolate reductase . Inhibition of this enzyme prevents the formation of the metabolically active

form of folic acid, tetrahydrofolic acid, and thus, interferes with normal bacterial cell functions. 13

Table 1.1: Major classes of antibiotics, primary targets and mechanisms of resistance

Antibiotic class (examples)	Mechanism of action	Mechanism of resistance
Aminoglycosides (gentamicin, streptomycin, kanamycin)	Interact with the 30S ribosomal subunit of 16S rRNA causing misreading and/or truncated proteins and cell death[75]; positively charged, attach to outer membrane causing pores to increase accumulation[169]	Aminoglycoside-modifying enzymes, for example, acetyltransferases, phosphotransferases and nucleotidyltransferases[131]; 16S ribosomal methylases[170]; mutations in the 16S rRNA gene[171]; decreased influx and/or increased efflux[172]
β-Lactams (penicillins, cephalosporins, cephamycins, carbapenems, monobactams)	Target peptidoglycan crosslinking by inhibiting penicillin-binding proteins, which crosslink the peptide chain in the cell wall[107], leading to lysis of the cell[75]	Production of β-lactamases[173]; modification of penicillin-binding proteins[174]; reduced permeability and increased efflux[174]
Cationic peptides (colistin)	Bind to lipid A in lipopolysaccharide[175]; permeabilizing the outer membrane causing cell death[175]	Modification or removal of lipid A[176,177]
Glycopeptides (vancomycin)	Inhibit crosslinking and therefore synthesis of peptidoglycan by binding to D-alanyl-D-alanine in the peptide chain[178]	Intrinsic resistance in Gram-negative cells by impermeable outer membrane[178]; in Gram-positive cells, enzymes can modify and hydrolyse peptidoglycan precursors[179]; intermediate susceptibility phenotype conferred by mutations leading to thickened membrane and low permeability[180]
Lincosamides (clindamycin)	Target the translation of proteins, specifically 23S rRNA of the 50S ribosomal subunit, causing truncated peptide chains[181]	Methyltransferases that modify 23S rRNA[182]; expression of proteins that inactivate lincosamides and efflux[183]
Lipopeptides (daptomycin)	Insert in the cell membrane and cause depolarization, reducing the ability to create ATP and cell death[184]	Thickening of and increasing the positive charge in the cell wall[185]; reducing the depolarization of membranes induced by lipopeptides[185]
Macrolides (azithromycin, erythromycin)	Inhibit the translation of proteins by targeting 23S rRNA of the 50S ribosomal subunit, causing truncated peptide chains[186]	rRNA methyltransferases, which methylate 23S rRNA[187]; mutations in the ribosome[188]; efflux[188]; macrolide phosphotransferases and esterases[135]; ribosomal protection by ATP-binding cassette F (ABC-F) proteins[189]
Oxazolidinones (linezolid)	Limit translation by binding to 23S rRNA of the 50S subunit and preventing the formation of a functional 70S subunit[190]	Modifications of 23S rRNA, for example, by methyltransferases[191]; protection of the ribosome via ABC-F proteins[191]
Phenicols (chloramphenicol)	Inhibit translation by binding to the A site of the 50S ribosomal subunit, inhibiting protein synthesis[192]	Mutations within 23S rRNA of the 50S ribosomal subunit[192]; enzymatic inactivation via acetyltransferases and efflux[192]
Pyrimidines (trimethoprim)	Affect C1 metabolism and folate synthesis by inhibiting dihydrofolate reductase, blocking production of tetrahydrofolate[193]	The modification or acquisition of novel dihydrofolate reductase genes and efflux of trimethoprim[194]
Quinolones and fluoroquinolones (ciprofloxacin)	Inhibit DNA replication by DNA gyrase and topoisomerase IV, which are involved in DNA supercoiling, strand cutting and ligating[195]	Mutations in DNA gyrase or topoisomerase IV[195]; the efflux of quinolones or proteins that protect DNA gyrase and topoisomerase IV[195]
Rifamycins (rifampicin)	Inhibit transcription, specifically DNA-dependent RNA synthesis, by binding to RNA polymerase[196]	Mutations in the drug target *rpoB*[196]; enzymatic ribosylation or inactivation of rifampicin[144]
Streptogramins (dalfopristin)	Target protein translation by binding to 23S rRNA of the 50S ribosomal subunit at the peptidyl-transferase domain causing truncated peptides[197]	Mutations in 23S rRNA[192]; modification of streptogramins by acetyltransferases[192]; efflux out of the cell[192]
Sulfonamides (sulfamethizole)	Stop dihydrofolate acid synthesis by inhibiting dihydropteroate synthase and arresting cell growth[198]	Mutations in the dihydropteroate synthase gene and *sul1/2* genes, which encode distinct dihydropteroate synthases that are less susceptible to sulfonamides[198]
Tetracyclines (tigecycline, tetracycline)	Inhibit translation by binding to 16S rRNA of the 30S ribosomal subunit, preventing tRNA binding to 30S at the A site[199]	Efflux[199]; protein-mediated ribosome protection[199]; ribosomal mutations[199]; enzymatic inactivation of the drug[199]

1.4 Mechanisms of antibiotic resistance

There are several mechanisms by which bacteria can develop resistance to antibiotics 14-21. These include:

1.Mutation: Bacteria can acquire resistance through spontaneous mutations in their DNA.These mutations can alter the target site of the antibiotic, making it less effective. For example, a mutation in the DNA gyrase gene can lead to resistance to fluoroquinolone antibiotics. Parallel selection of mutations within fusA1 and ptsPin response to tobramycin in Acinetobacter baumannii grown in diferent conditions demonstrated the primary importance of these mutations in resistance.33-44

2. Efflux pumps: Bacteria can develop resistance by pumping out the antibiotic before it can reach its target site. Efflux pumps are membrane proteins that actively transport antibiotics out of the bacterial cell. This mechanism is particularly common in Gram-negative bacteria. Tetracycline resistance is a textbook example of effluxmediated resistance, the efflux pumps are capable of recognizing and binding to tetracycline molecules, and then actively pumping them out of the cell before they can exert their antibacterial effect. This reduces the intracellular concentration of tetracycline, making it less effective against the bacteria Resistance to macrolides is another clinically relevant phenotypeinduced by the efflux mechanism. Resistance to macrolides is another clinically relevant phenotypeinduced by the efflux mechanism. The mef genes, which extrude themacrolide class of antibiotics, encode the most well-characterized efflux pumps (e.g., erythromycin). MacB, an ABC family membe ,acts as a tripartite pump (MacAB-TolC) for extruding macrolide drugs.53-59

3. Altered target site: Bacteria can develop resistance by altering the target site of the antibiotic. This can involve mutations in the target protein or the overexpression of the target protein, making it less susceptible to the antibiotic. The addition of moieties to the drug target can prevent antibiotic access and thus protect the target.

This is a well-known Mechanism of resistance to macrolides, whereby the 16S rRNA target can be methylated by ribosomal methyltransferases, thereby preventing binding of macrolides.45-52

4. Inactivation of antibiotics: Another important example of the inactivation of antibiotics is exemplified by tetracycline-inactivating enzymes that catalyse the oxidation of tetracyclines. The best known is the Tet(X) family, whichHas been characterized in many different classes of bacteria and can move horizontally on transposable elements, conferring high-level tetracycline resistance.22-32

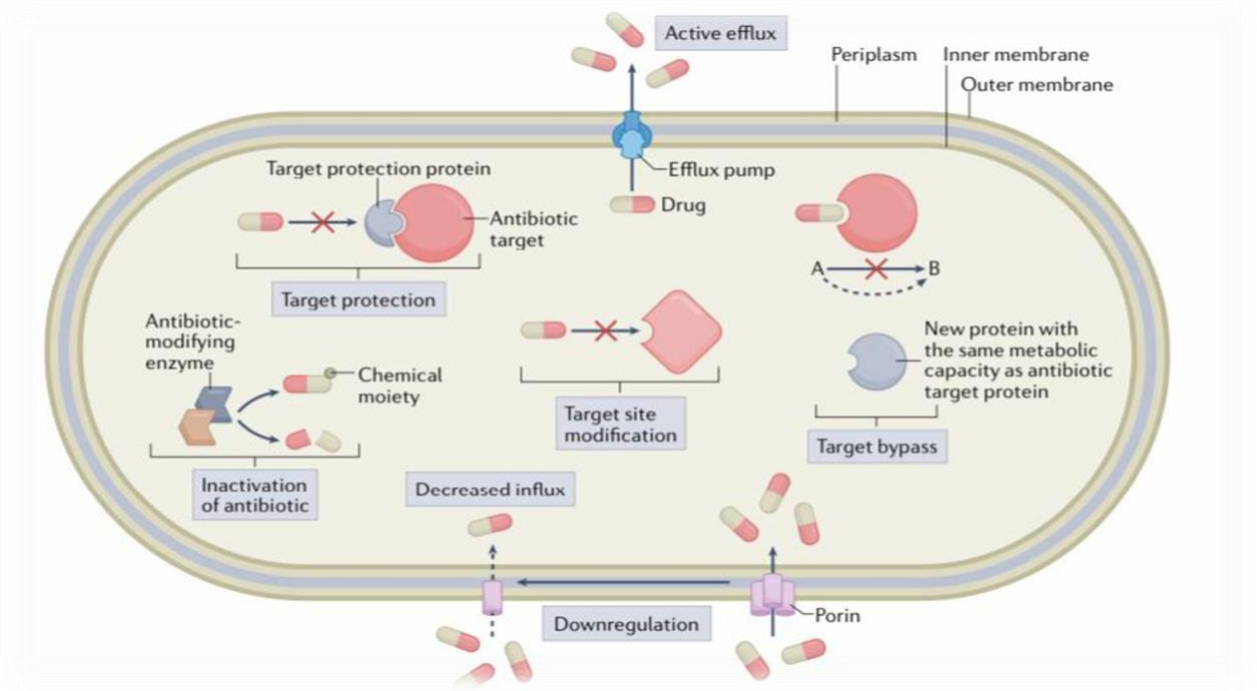


Figure 1.1: The molecular mechanisms of antibiotic resistance.

1.5 Selenium nanoparticles

Nanoparticles have gained significant interest in various fields due to their unique properties and potential applications in areas such as biomedicine, materials science, and environmental remediation. In biomedicine, selenium nanoparticles have been studied for their antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. They have shown promise in combating oxidative stress, reducing inflammation, fighting infections, and inhibiting the growth of cancer cells. These nanoparticles can be used in drug delivery systems, diagnostic tools, and therapeutic agents.

Selenium nanoparticles are synthesized by:

- **Physical method**
- **Chemical method**
- **Biological method**
- **Microbial method**

However, compared to other conventional physical and chemical methods, microbial and plant-mediated synthesis of biogenic selenium nanoparticles (Bio-SeNPs) with various bioactive substances have extensive biological applications.

Bio-SeNPs (Biosynthesized Selenium Nanoparticles) exhibit greater effectiveness against gram-negative bacteria, likely because of the thinner peptidoglycan layer in these bacteria, allowing the selenium nanoparticles to penetrate more easily and disrupt the cell membrane.

Some of the key properties of selenium nanoparticles include:

1. Antioxidant Activity: Selenium nanoparticles exhibit strong antioxidant properties, helping to scavenge free radicals and reduce oxidative stress in cells and tissues.

2. Antimicrobial Activity: These nanoparticles have shown antimicrobial properties, making them effective against a wide range of microorganisms, including bacteria, fungi, and viruses.

3. Anti-inflammatory Effects: Selenium nanoparticles can help reduce inflammation by modulating immune responses and cytokine production.

4. Anticancer Properties: Studies have shown that selenium nanoparticles may inhibit the growth of cancer cells and induce apoptosis, making them potential candidates for cancer therapy.

1.6 *Staphylococcus aureus*

Is gram+, well recognised as a significant pathogen in both human and animal medicine. It can cause a wide range of conditions in humans and animals, from mild skin infections to life-threatening bacteraemia. More than 80% of S.aureus strains produce penicillinases and thus b-lactam antibiotics such as meticillin, which are resistant to penicillinases were widely used to treat S. aureus infections.

S. aureus is a ubiquitous microorganism that is able to colonise the anterior nares and other skin districts of healthy individuals. It has been estimated that ∼50% of adults are either persistent or intermittent S.aureus carriers . This microorganism can become a versatile pathogen causing a broad spectrum of infections thanks to a large arsenal of virulence factors. S. aureus infections range from common skin infections, such as furunculosis and impetigo, to severe deep-seated infections.

S.aureus ranks first or second among bacterial pathogens causing bloodstream infections according to different studies , and is the leading cause of nosocomial pneumonia .In addition, S.aureus causes infections of surgical wounds and prosthetic implants.

1.7 *Klebsiella pneumonia*

Is a common type of bacteria found in your intestines. They are normally harmless. But Klebsiella pneumoniae can be dangerous if they get into other parts of your body, especially if you're already sick. They can turn into "superbugs" that are very hard to fight with antibiotics. The germs can give you pneumonia, infect your or blood, and cause other serious problems.

Klebsiella pneumoniae causes a wide range of infections, including pneumonias, urinary tract infections, bacteremias, and liver abscesses. Historically, K. pneumoniae has caused serious infection primarily in immunocompromised individuals, but the recent emergence and spread of hypervirulent strains have broadened the number of people susceptible to infections to include those who are healthy and immunosufficient. Furthermore, K. pneumoniae strains have become increasingly resistant to antibiotics, rendering infection by these strains very challenging to treat.

Four factors, capsule, lipopolysaccharide, fimbriae, and siderophores, have been well studied and are important for virulence in at least one infection model.

1.8 Techniques used for characterization of nanomaterial

1.8.1 Transmission Electron Microscopy (TEM) is extensively used for imaging and characterizing nanoparticles at the nanoscale. TEM allows researchers to visualize the size, shape, and distribution of nanoparticles with high resolution. The detailed imaging capabilities of TEM contribute to a better understanding of the properties and behavior of nanoparticles, facilitating advancements in nanotechnology and related applications. In a Transmission Electron Microscope (TEM), a beam of electrons is accelerated and focused onto a thin specimen. As electrons pass through the specimen, they interact, and their transmission is influenced by the specimen's density and

thickness. Electromagnetic lenses then focus the transmitted electrons to form an image on a fluorescent screen or a digital detector.

1.8.2 Dynamic Light Scattering (DLS) is commonly employed for characterizing the size distribution of nanoparticles in solution. DLS can determine the hydrodynamic size of particles, which includes both the core particle size and the surrounding solvent layers. This technique is particularly useful for nanoparticles in the submicron and nanometer range, providing insights into their size distribution, polydispersity, and overall stability in a liquid environment.

The principle of Dynamic Light Scattering (DLS) is based on the analysis of the temporal fluctuations in the intensity of scattered light from particles undergoing Brownian motion in a liquid medium. These fluctuations are related to the size of the particles, and by applying mathematical models, DLS extracts information about the distribution of particle sizes in the sample.

1.8.3 Zeta potential is crucial in nanoparticle research as it affects the stability and behavior of nanoparticles in colloidal systems. Nanoparticles with high zeta potential tend to repel each other, reducing aggregation and enhancing stability, which is essential in applications like drug delivery and nanotechnology.

The principle behind zeta potential is based on the electro kinetic behavior of particles in a liquid medium. It measures the potential difference between the dispersion medium and the stationary layer of fluid attached to the particle's surface. This potential provides insights into the stability and interactions of colloidal systems, particularly important in understanding and manipulating nanoparticles for various applications.

1.8.4 FTIR spectroscopy

It is a vibrational spectroscopic technique that can be used to optically probe the molecular changes associated with diseased tissues. This method is relatively simple, reproducible, non-destructive to the tissue and only small amounts of material (micrograms to nano grams) with a minimum sample preparation are required, thus it can employed to find more conservative ways of analysis to measure characteristics within tumor tissue and cells that would allow accurate and precise assignment of the functional groups, bonding types, and molecular conformations.

1.9 Aims of the work:

The aim of this work is to develop a new nanomaterial with potential anti-bacterial activity against gram positive and gram negative MDR species. The study involves the below steps:

1- Synthesis of polyvinyl alcohol stabilized nanoparticles (PVA Se NPs)

2- Characterization of the synthesized PVA Se NPs

3- Determination of the effect of PVA Se NPs on the growth of two bacterial species (*Staphylococcus aureus and Klebsiella pneumonia*)

2. Experimental

2.1 Synthesis of PVA-stabilized selenium nanoparticles.

Selenium nanoparticles stabilized capped with polyvinyl alcohol were prepared by the reduction of sodium selenite by sing ascorbic acid as the reducing agent [27], and in the presence of PVA as a stabilizer in a one-pot reaction. To the solution of PVA (0.1% in acetic acid, 1.0%) ascorbic acid solution (15.054 g, 0.08548 mol in 100 ml distilled water) was added, then a solution of sodium selenite (1.314 g, 0.0076 moles in 100 ml distilled water) was added gradually with continuous stirring. The volume was then completed to 1000 ml with distilled water, and the mixture was left to stir for 30 minutes at room temperature. A change in the color of the solution from colorless to orange indicated the formation of Se-PVA NPs. The concentration of the resulting solution is 600 ppm based on the concentration of selenium metal.

2.2 Biological activity

2.2.1 The materials and methods

The instruments and the materials used in the experiments

- Refrigerator
- Incubator
- Laminar air flow hood
- Autoclave
- Sensitive electronic balanced

- petri dishes
- Tubes, beakers, flask
- Loop
- Nutrient broth
- Muller Hinton broth

2.2.2 Preparation the cultural media

The variety selective and differential media were prepared for isolating and cultivating the gram positive and gram negative tested.

2.2.3 Preparation Nutrient broth

Nutrient Broth is a liquid medium used for the cultivation of a wide variety of organisms from clinical specimens and other materials. Upon to the synthesized company, suspend 13 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.

2.2.4 Preparation Muller Hinton broth

Mueller Hinton Broth is used for determining minimal inhibitory concentrations (MICs).It is low in sulfonamide, trimethoprim, and tetracycline inhibitors and yields satisfactory growth of most non-fastidious pathogens. Beef infusion and Casein provide nitrogenous compounds, vitamins, carbon, sulphur and amino acids in Mueller Hinton media. Starch is added to absorb any toxic metabolites produced. For testing streptococci, supplementation with 5% defibrinated sheep or horse blood is recommended. The media is prepared by dissolving 21 g in 1 liter of distilled water. Sterilize by autoclaving at 121°C for 15 minutes.

2.2.5 Evaluation the antibacterial efficacy of PVA –selenium nanoparticles (PVA-SeNPs)

The antibacterial impact of PVA –selenium nanoparticles (PVA-SeNPs) and Levofloxacin was estimated separately against two pure cultures of bacterial strains (*Staphylococcus aureus and Klebsiella pneumonia*) that isolated from clinical samples characterized as MDR by vitek technique. The 96-well plate technique is the most accurate procedure to detect minimum inhibitory concentration and minimum bactericidal concentration of Se NPs stabilized by PVA. At first, two -fold dilution of Ch-SeNPs , and Levofloxacin (LEV) in 50µl was done in the well of 96- micro-titer plate separately to obtain the following concentrations: (400, 200, 100, 50, 25.12.5, 6.2, 3.1, 1.5, 0.7 µg/L) for PVA-SeNPs and (1600, 800, 400, 200, 100, 50, 25, 12.5, 6.2, 3.1 µg/L) for LEV. The volumes 50µl of bacterial growth at 0.5×10^5 cells/ml were transferred to the all walls of microtiter plate except the walls of negative control. Finally, the inoculated micotiter plates were incubated at 37˚C for 24 hours. After incubation, the MIC was detected as the first well not showing bacterial growth, and then 10 µl from the first clear well noticed after the wells having bacterial growth were cultured on nutrient agar and kept at 37˚C for 24 h to determine the MBC values that reduce 99% of bacterial growth.

3.Results and Discussion:

3.1. Synthesis and characterization of Se-PVA NPs

Selenium nanoparticles stabilized by polyvinyl alcohol were prepared by chemical reduction method; the reduction of sodium selenite was performed by using ascorbic acid in the presence of PVA in the acidic aqueous reaction mixture. The first indicator for the formation of the Se-PVA NPs the visible colour change from colourless to orange.

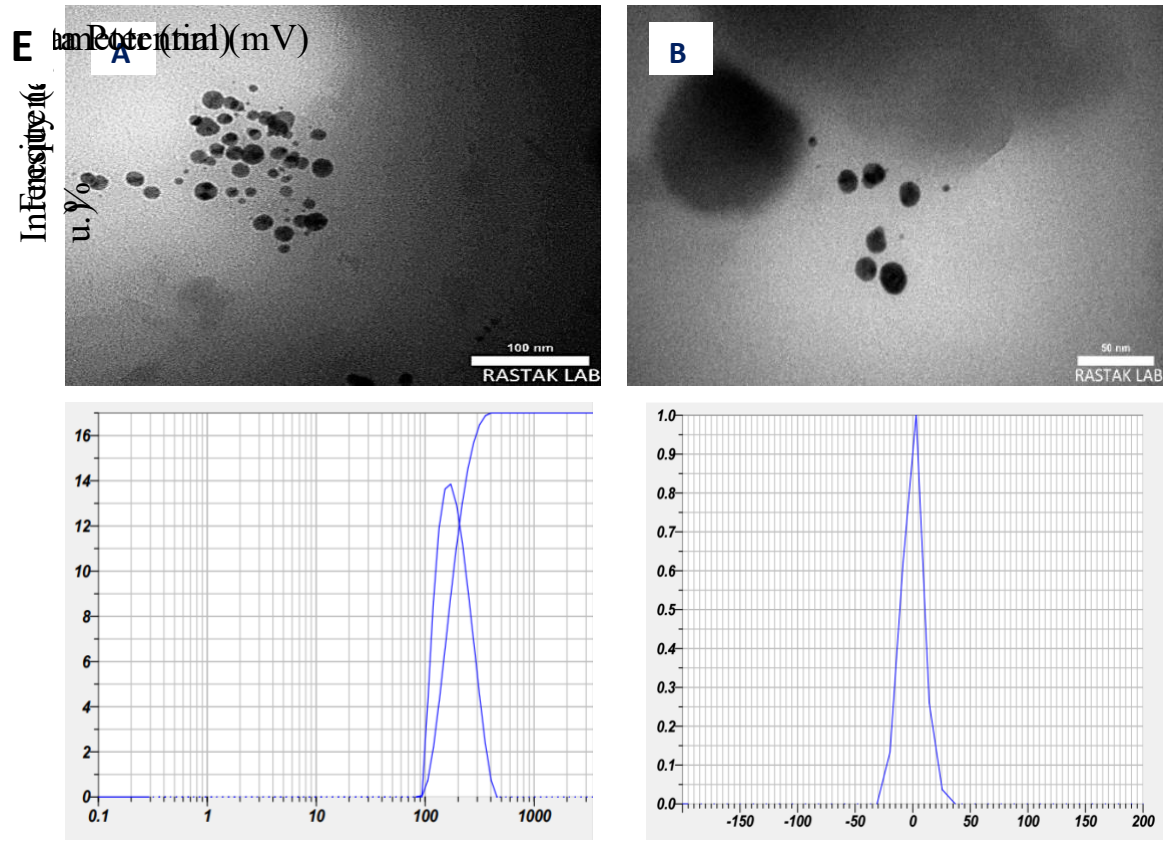
Transmission electron microscopy was used to investigate the size and the shape of the Se-PVA NPs, the obtained micrographs are shown in Figure 3.1 (A and B). The results showed that the shape of the selenium nanoparticles was spherical, and the diameter of the particles was in the range of 20-92 nm. The average diameter of PVA-SeNPs is 58.14 ±23 nm and this result is within the limits of the nanoscale.

Dynamic light scattering analysis (DLS) was also performed to get more information about the average size of selenium nanoparticles, Figure 3.1 (C). An average size of 164.3 nm was observed, this result came in agreement with previous studies; the DLS results show size values greater than the size that TEM provided. The rationale is that TEM is more sensitive to particles carrying electrons, whereas DLS analysis yields the particles' hydrodynamic

radius. Moreover, a variety of parameters, including sample concentration and preparation, influence the outcomes of DLS analysis.

The zeta potential of Se-PVA NPs was measured, Figure 3.1 (D). It was found to be -0.4 mv. In terms of zeta potential analysis, such results refer to the lack of stability (±30 mV is the value which refer to the significant stability). However, in our case, stable nanoparticles were synthesized with a relatively long shelf life, where no precipitate was observed. On the other hand, in a test experiment conducted in the absence of PVA, a solid precipitate was formed quickly, indicating that the formed nanoparticles were immediately aggregated (unstable). All of that confirms that the stability of the synthesized Se-PVA NPs comes from steric stabilization rather than electrostatic stabilization.

The FT-IR analysis, Figure 3.1 (E), confirmed the presence of the PVA polymer on the surface of the Se NPs nanoparticles, where a wide absorption band was observed at 3426 cm-1, which is due to the stretching vibration of the OH group, while the absorptions at 2850 and 2930 cm-1 are due to the stretching vibration. Aliphatic C-H, absorption band at 1637cm-1 attributed to C=O in ascorbic acid. A band at 720 cm^{-1} could be attributed to the Se-Se metal surface stretching vibrations.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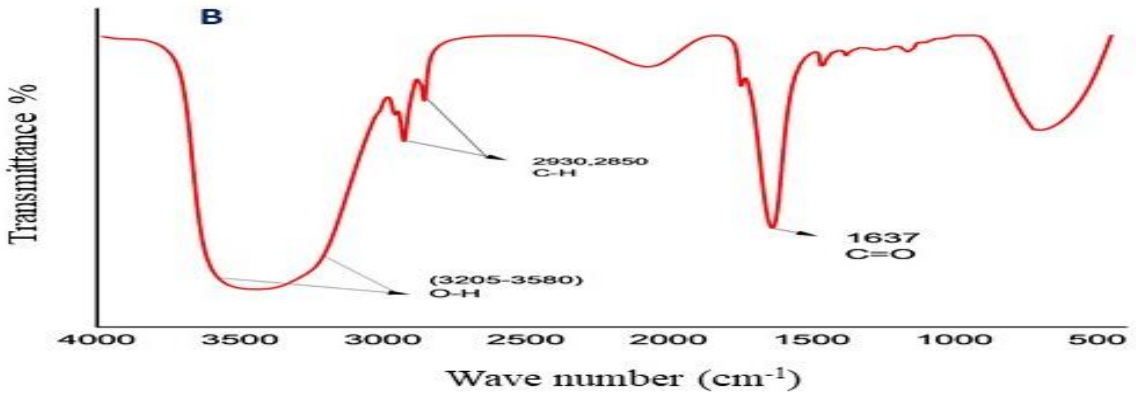
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O-H
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500
Wave number (cm-1)

Figure 3.1: Characterization of PVA-SeNPs with different techniques: TEM micrograph (A, B), DLS (C), Zeta potential (D), and FT-IR (E)

3.2 Antibacterial activity of PVA Se NPs

This study was mostly focused on an evaluation of the MIC and MBC values of PVA-SeNps and LEV upon pathogenic bacteria including: *Staphylococcus aureus and Klebsiella pneumonia*. The obtained results as exhibited in Figures (3.2, and 3.3).

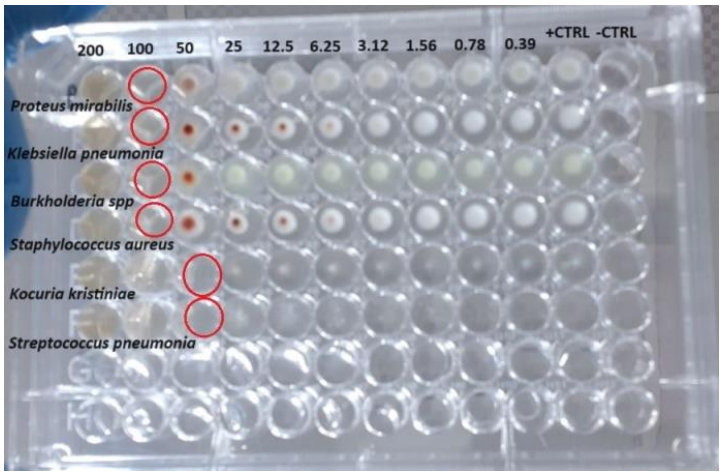

Figure 3.2: Minimum inhibition concentration of selenium nanoparticles coated by Chitosan against different types of bacterial strains

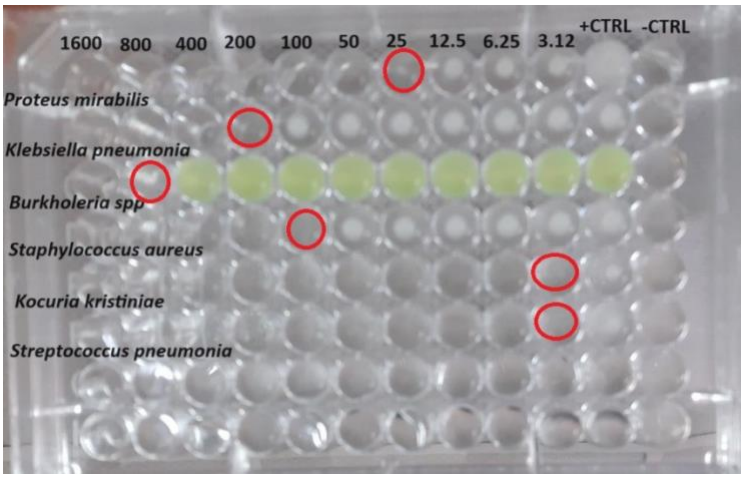

Figure 3.3: Minimum inhibition concentration of levofloxacin (LEV) against different types of bacterial strains

The minimum inhibition concentration (MIC) of Ch-SeNps for the tested bacteria was found between 100.0±0 µg/mL, for *Staphylococcus aureus* and *Klebsiella pneumonia* while for the value of Levofloxacin the MIC24 values were found to be 133. 3±57 µg/mL and 200±0 µg/mL for *Staphylococcus aureus* and *Klebsiella pneumonia* respectively. Table 3.1 shows the obtained results.

Table 3.1: Minimum inhibition concentration (MIC24) of chitosan-selenium nanoparticles, and Levofloxacin against different bacterial strains.

Bacterial species	MIC_{24}	
	PVA-SeNPs	LEV
Staphylococcus aureus (MRSA)	100±0	133.3±57
Klebsiella pneumoniae	100±0	200±0

The minimum inhibition concentration (MBC_{24}) of Ch-SeNps for the tested bacteria was found between 166. 7±57 µg/mL, and 200±0 µg/mL for *Staphylococcus aureus* and *Klebsiella pneumonia* respectively while for the value of Levofloxacin the MIC24 values were found to be 166. 7±57 µg/mL and 266. 7±115 µg/mL for *Staphylococcus aureus* and *Klebsiella pneumonia* respectively. Table 3.1 shows the obtained results.

Table 3.2: Minimum bactericidal concentration (MBC_{24}) of chitosan-selenium nanoparticles, and Levofloxacin against different bacterial strains.

Bacterial species	MBC_{24}	
	PVA-SeNPs	**LEV**
Staphylococcus aureus (MRSA)	166. 7±57	166. 7±57
Klebsiella pneumoniae	200±0	266. 7±115

Conclusion

An important to understand the molecular mechanisms of resistance is to use this information to design novel strategies that interfere with or inhibit the resistance mechanism. So-called antibiotic resistance breakers are compounds that can disrupt or inhibit a specific mechanism of antibiotic resistance to restore the clinical efficacy of a specific antibiotic. This is an attractive strategy because it could be used to potentiate the use of existing antibiotics.

Selenium nanoparticles stabilized by chitosan were synthesized and characterized by TEM, FT-IR, DLS and zeta potential analysis. The antibacterial activity of synthesized nanoparticles was evaluated against *Proteus mirabilis* and *Streptococcus pneumonia* as MDR bacteria, in comparison with Levofloxacin, the synthesized nanoparticles exhibited a good level of activity.

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