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ATILIM UNIVERSITY 2022

INVESTIGATION OF THE ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES ADDED POLY (N- METHYL ANILINE)

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
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MOSTAFA KAMIL MAALA ALDARAJI

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Approval of the Graduate School of Natural and Applied Sciences, Atilim University.

Prof. Dr. Ender KESKINKILIÇ

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I certify that this thesis satisfies all the requirements as a thesis for the degree of **Master of Science in Chemical Engineering and Applied Chemistry Department, Atilim University**.

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This is to certify that we have read the thesis INVESTIGATION OF THE ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES ADDED POLY (N-METHYL ANILINE) submitted by Mostafa AlDARAJI and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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ABSTRACT

INVESTIGATION OF THE ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES ADDED POLY (N- METHYL ANILINE)

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M.S., Chemical Engineering and Applied Chemistry

Supervisor: Prof. Dr. Belgin İŞGÖR

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Humans, animals, and crops may suffer from many diseases that are caused by certain types of bacteria. These bacteria need to be deeply researched to identify the optimal means through which these can be faced. Among these types of bacteria are (*Escherichia coli*) and *(Staphylococcus aureus*). Silver is one of the most important mineral elements used in the treatment of the spread of these bacteria, as it has some chemical and physical properties that can eliminate bacteria. One of these properties whose activity has been examined is Nanotechnology. Which are particles ranging in size from 1 to 100 nanometers. It has been found that these particles can penetrate the outer membranes and enter the cell. By using so, it works to stop the production of protein and starts to kill the living cell due to the high toxicity found with silver nanoparticles. In addition, poly (n-methyl aniline) was added to the silver nanoparticles. PNMA was prepared with 0.025 M monomer solution. The size of the resulting PNMA spheres lies between 200-550 nm. The oxidative chemical polymerization and liquid impregnation methods were used to successfully prepare poly (N-methyl aniline) silver nanoparticles (PNMA-AgNPs). SEM, TEM, EDX, and ICP-OES were used to characterize the resulting particles. It has been studied the antibacterial effect of these compounds; PNMA and Ag-PNMA against the *Escherichia coli* and *Staphylococcus* *aureus* and compare the effect of these compounds with penicillin/streptomycin common antibiotic.

Keywords: poly (N-methyl aniline), silver nanoparticles, antibacterial activity, *Escherichia coli, Staphylococcus aureus*

ÖZ

POLİ (N- METİLANİLİN) EKLENMİŞ GÜMÜŞ NANOPARTİKÜLLERİN ANTİBAKTERİYEL AKTİVİTESİNİN ARAŞTIRILMASI

Aldaraji , Mostafa Kamil Maala
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Ocak 2022, 38 sayfa

İnsanlar, hayvanlar ve mahsuller, belirli bakteri türlerinin neden olduğu birçok hastalıktan muzdarip olabilir. Bu bakterilerin, karşılaşabilecekleri en uygun araçları belirlemek için derinlemesine araştırılması gerekir. Bu bakteri türleri arasında *(Escherichia coli)* ve (*Staphylococcus aureus*) bulunur. Gümüş, bakterileri yok edebilecek bazı kimyasal ve fiziksel özelliklere sahip olduğu için bu bakterilerin yayılmasının tedavisinde kullanılan en önemli mineral elementlerden biridir. Etkinliği incelenen bu özelliklerden biri Nanoteknolojidir. Bunlar 1 ila 100 nanometre arasında değişen parçacıklardır. Bu parçacıkların dış zarlara nüfuz edebildiği ve hücreye girebileceği bulunmuştur. Bunu kullanarak, protein üretimini durdurmak için çalışır ve gümüş nanopartiküller ile bulunan yüksek toksisite nedeniyle canlı hücreyi öldürmeye başlar. Ek olarak, gümüş nanopartiküllere poli (n-metil anilin) ilave edildi. PNMA 0.025 M monomer çözeltisi ile hazırlandı. Elde edilen PNMA kürelerinin boyutu 200-550 nm arasındadır. Poli (N-metil anilin) gümüş nanopartikülleri (PNMA-AGNP'LER) başarıyla hazırlamak için oksidatif kimyasal polimerizasyon ve sıvı emprenye yöntemleri kullanıldı. Elde edilen partikülleri karakterize etmek için SEM, TEM, EDX ve ICP-Oe'ler kullanıldı. Bu bileşiklerin; PNMA ve Ag-Pnma'nın *Escherichia coli ve Staphylococcus* aureus'a karşı antibakteriyel etkileri incelenmiş ve bu bileşiklerin penisilin/streptomisin ortak antibiyotiği ile etkileri karşılaştırılmıştır.

Anahtar Kelimeler: poli (N-metilanilin), gümüş nanopartiküller, antibakteriyel aktivite, *Escherichia coli, Staphylococcus aureus*

I dedicate this study to my mother and father

To the soul of my grandfather Maala Kafi

And to the soul of the brave man … to Jamal

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First and foremost, I want to express my gratitude to Allah for empowering me to trust in myself and achieve my goals.

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CHAPTER 1

INTRODUCTION

1.1 Nanotechnology

Nanotechnology is a cutting-edge field of study that enables the creation of a diverse range of materials, including nano particles with dimensions smallest than one hundred nanometers (nm). Particles larger than 100 nm in all dimensions are referred to as bulk materials. Physical properties in bulk materials are independent of size; however, different physical properties of nano particles can be affected by the shape and size of the nano particles. Researchers discovered that size affects the substance physicochemical properties, such as chemical, mechanical, electrical, and optical properties revealing the significance of Nano particles. As a result of their unique features, nano particles have attracted the curiosity of many researchers [1]. Some applications of nano particles include filtration systems, oil refineries, industrial processes, petrochemical industries, diagnostics, catalytic processes, buildings and construction supplies, and drug delivery. Condensation, attrition, chemical pre capitation, ion implantation, pyrolysis, and hydrothermal synthesis are some ways for creating nano particles [2,3].

Nanoparticles (NPs) have measured a broad group of materials that include particulate compounds with a minimum dimension of one hundred nm [4]. In general, these materials are usually 3D, 2D, 1D, or 0D [5]. (Figure 1.1).

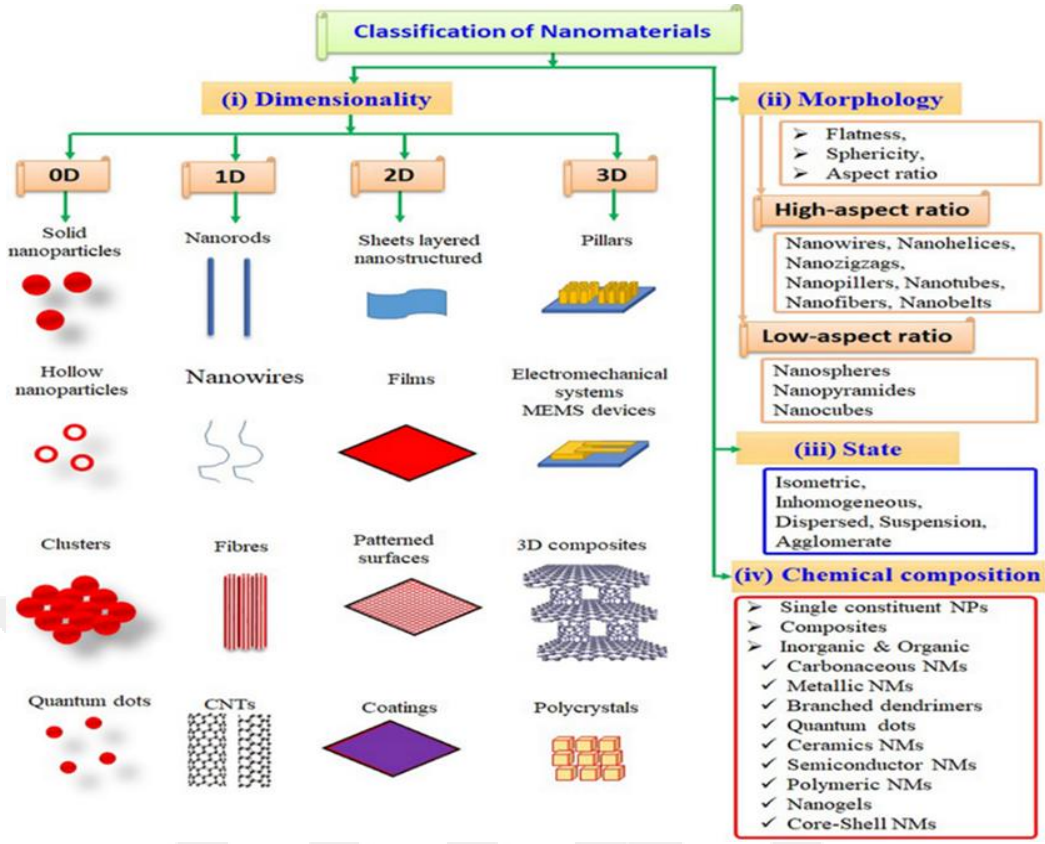


Figure 1.1. Classification of nanomaterials built on different criteria [6]

The most significant of these materials was recognized after investigators found that size could affect the chemical and physical properties of a substance e.g., the optical properties. Nano particles have special colors: A 20-nm gold (Au) have wine red color, platinum (Pt) have yellowish-gray color, silver (Ag) have black color, and palladium (Pd) has dark black color. An example of these colors is shown in Figure 1.2, in which gold nano particles were synthesized with many sizes. These nanoparticles displayed distinct colors and properties based on their shape and size, and they have the potential to be used in bioimaging applications [7]. The color of the solution variation because of the difference in nano shell thickness, (%) gold concentration and aspect ratio. The change of any of the above discussed factor affect the characteristics of absorption of the nanoparticles and then different absorption colors are observed [8] (Figure 1.2).

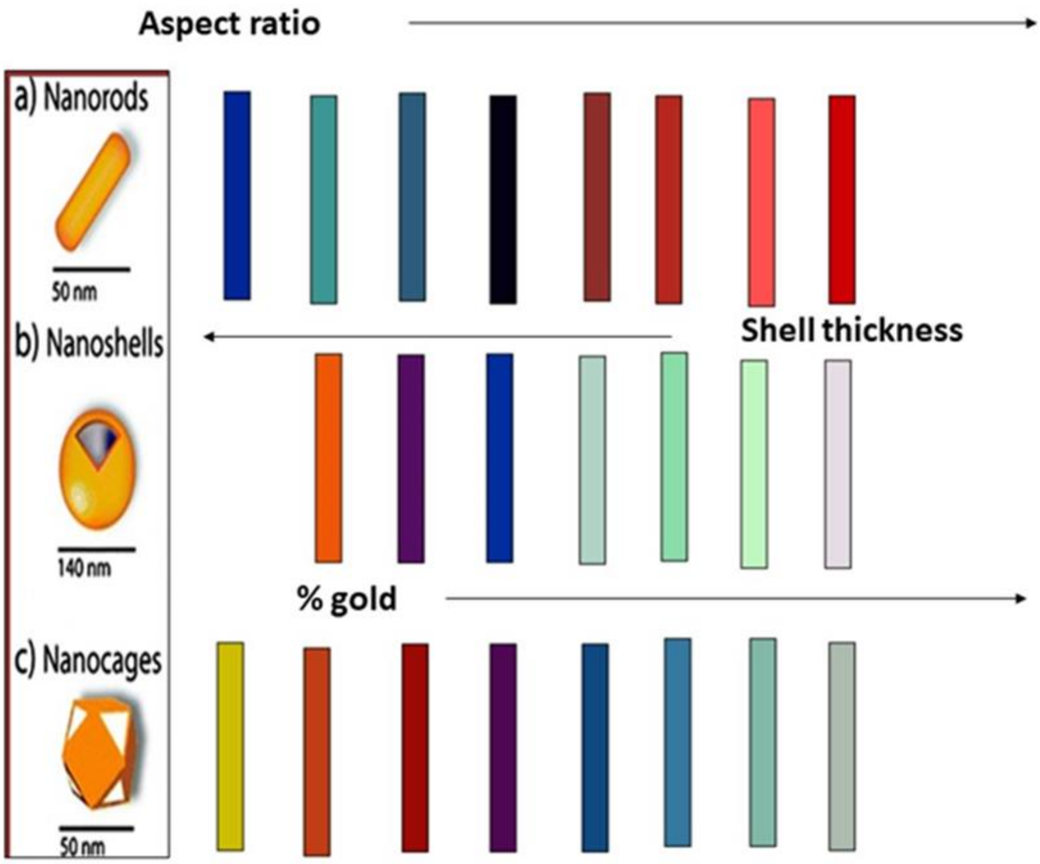


Figure 1.2 : The colour necessity of Au NPs on size and shape (a,b,c gold nano particles with difference nanometer size [6]

Because nanoparticles are more complex than simple molecules, they are consisting of three layers:

1. The top layer: which can be modified with a variety of chemicals, polymers, surfactants, and metal ions.

2. The outer layer: which is made up of chemicals that are completely different from the core in every way.

3.The core: which are the Nanoparticles' central element and frequently corresponds to the Nano particles themselves [9]. Because of their exceptional qualities, these materials have attracted the curiosity of researchers from a wide range of fields. The Nano particles are often utilized for drug delivery [10] biological and chemical sensing [11], gas sensing, greenhouse gas capturing [12] and alternative connected applications.

1.2 Properties of Nanomaterials

Nanomaterials have many important properties such as those listed below:

1) Melting point: the values of freezing point temperature are affected by the dimensions of particles for an equivalent method, for instance, the melting point for Au is 1064 -1100 C^o but when the diameter of Au particles decreased, the freezing point becomes 500-615 C^o [6,13].

2) Magnetic properties: magnetic properties have a relationship with the particle's size, magnetic properties increases when the dimensions of atoms decrease and also increase the area for the surface [6].

3) Mechanical properties: Mechanical properties of nanomaterials have been generally improved as a result of material structure perfection [14] The reduction of size to the nanometer scale introduces new technological challenges, such as particle agglomeration and matrix debonding. To completely appreciate particle interaction mechanics, atomic-scale Van der Waals attraction and electrostatic repulsion must also be considered. Because of its increased mechanical qualities, nanocomposite has aroused a lot of interest in a different automotive and general industrial applications [15].

1.3 Types of Nanoparticles

Nano particles are classified into numerous classes according to their chemical properties, morphology, and size. To exemplify chemical and physical characteristics, a number of the well-known categories of nanoparticles scale are given in Figure 1. 3 [16].

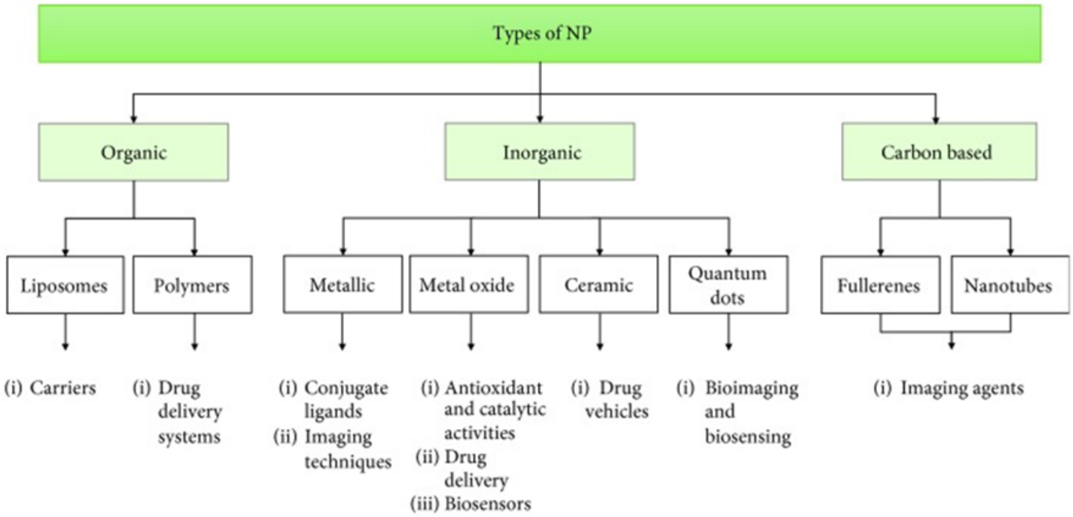


Figure 1.3: Types of nanoparticles [16]

1.4 Metallic Nanoparticles

Metal nanoparticles are the particles that only result from the metallic element precursors. These particles show well-known localized surface plasmon resonance (LSPR) characteristics. Optoelectrical characteristics are possessed by nano particles. In today's newest materials, facet, size, and form-controlled production of metal nanoparticles are critical [7], Because of their advanced optical properties, metal nanoparticles are used in several applications especially in the medicine and drug industry [17] (Figure 1.4).

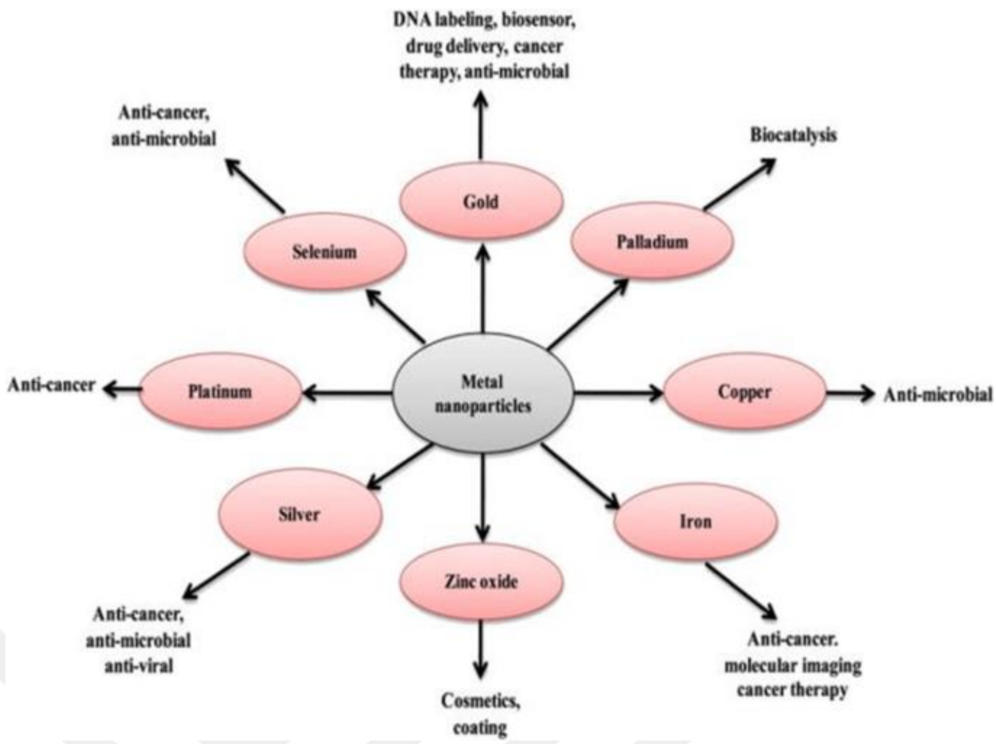


Figure 1.4: Different kinds of metal nanoparticles and their applications [18]

1.5 Synthesis of Metallic Nanoparticles

The (top-down) and (bottom-up) methodologies have both been used to produce metallic nanoparticles.

1) The top-down approach: The top-down technique works by taking a bulk piece of material and transforming it into the desired nanomaterial. Cutting, grinding, and etching are some of the advanced fabrication techniques used to work on the nanoscale. Top-down approaches can create nanomaterials with sizes ranging from 10 to 100 nanometers [19]. Despite this, top-down grading is common in nanomaterials with rough surfaces. The physical characteristics and surface chemistry of nanoparticles are influenced by their imperfect surface architectures. Because of the high surface-to-volume ratio, these imperfect surface structures harm the physical properties and surface chemistry of nanomaterials. This approach remains important when actual complex structures are selected [19,20] (Figure 1.5).

2) The Bottom-up approach: Bottom-up or self-assembly refers to the construction

of a structure atom by atom, molecule by molecule, or cluster by cluster. Colloidal dispersals, which are used to create nanoparticles, are an excellent example of this method. The sizes of the nanomaterials discovered using this method covers the entire nanoscale. This method is advantageous because it allows for the production of nanomaterials with fewer flaws and more constant chemical compositions. This is frequently attributed to the mechanisms used in nanostructure synthesis to lower the energy of the system. As a result, the produced nanomaterials are closer to thermodynamic equilibrium [19] (Figure 1. 5).

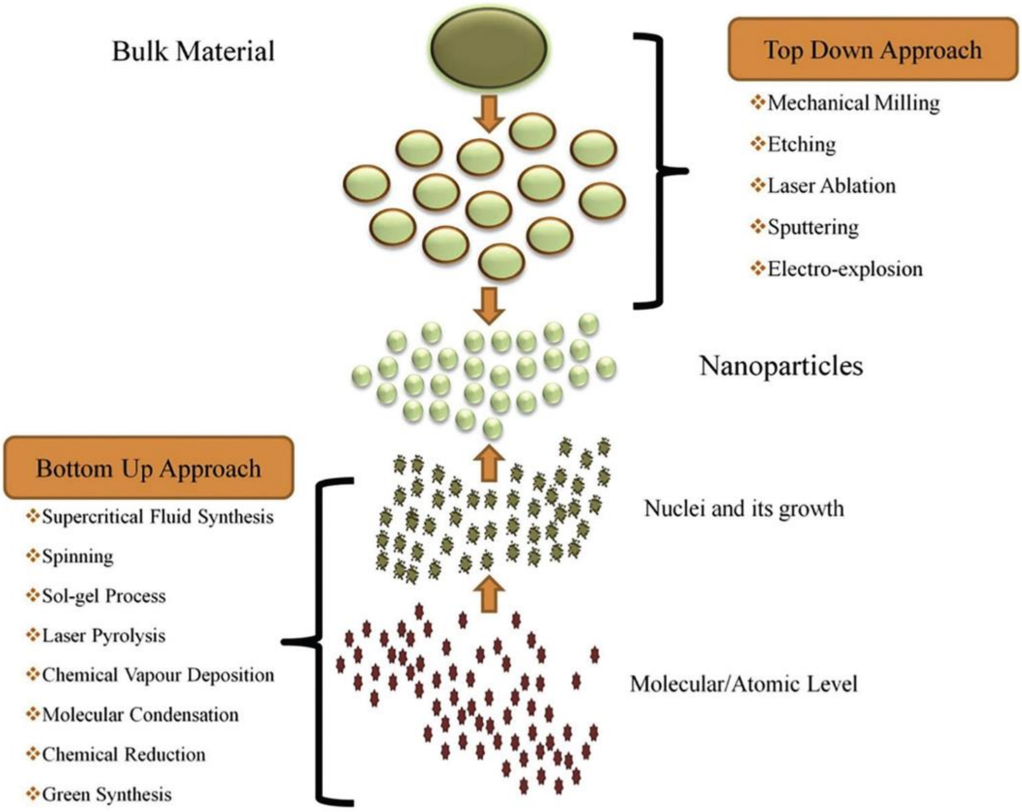


Figure 1.5: Bottom-up and Top-down approaches for synthesis of [21]

1.6 Silver Nanoparticles

Silver was used by the ancient Phoenicians, Egyptians, Romans, Greeks, and others to preserve food and water in some form or another, and this tradition remained until World War II. The Macedonians used silver plates to improve wound healing. It was the first attempt to prevent or treat surgical infections. Hippocrates prescribed silver preparations to treat ulcers and promote wound healing. Because it was mentioned in a pharmacopeia published in Rome in 69 B.C.E., silver nitrate was most likely used medically. [22,23].

In the last few years, researchers have devoted care for AgNPs to the area of intense scientific attention, because of their potential biological, optical, and electrical uses. [24,25]. Ag-NPs showed a really necessary role in nanoscience and engineering, considerably in nanomedicine. Though several noble metals area units are used for diverse functions, the AgNPs have potentially targeted the applications in cancer and medical aid [26,27]. Ag-NPs are used in the applications of drug delivery, analysis, and imaging or healing by coated stents because of their optical and physical properties. However, improvements to novel Ag-NPs containing comparable products are always required [27] (Figure 1.6).

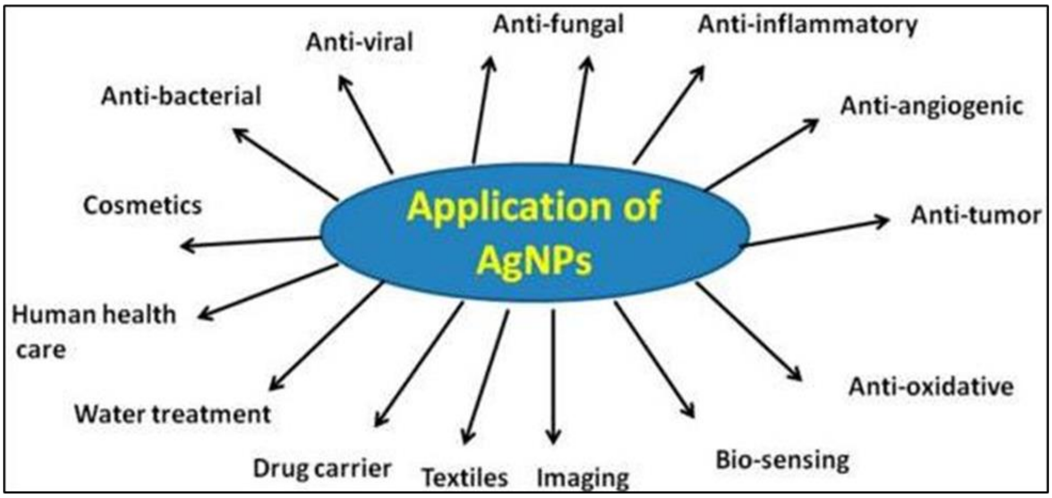


Figure 1.6: Many applications of Ag-NPs.[26]

1.6.1 Antimicrobial and Anti-inflammatory Properties of Silver Nanoparticles

Ag-NPs are efficient antibacterial agents for a wide range of gram-negative and gram-positive bacteria, as well as antibiotic-resistant strains [28]. Acinetobacter, Salmonella, Pseudomonas, and enteric and true bacteria are examples of gram-negative microorganisms. Gram-positive microorganisms include the genera Eubacteria, Bacillus, Staphylococcus, Enterococcus, and Streptococcus. Among the antibiotic-resistant microorganism strains are Enterococcus faecium, which is vancomycin-resistant and methicillin-resistant, and Staphylococcus aureus. It was recently shown that AgNPs with diameters between 5-32 nm improve the antibacterial action of different medications [29].

Small nanoparticles with a high surface area to volume ratio simply give a more effective antibacterial action, even at lower concentrations. AgNPs with triangular shapes have the most potent antimicrobial activity. AgNPs are efficient against a wide range of fungi and have faster-acting fungicides, including Aspergillus, Candida, and the species Saccharomyces. In addition, these nanoparticles are actual in contradiction of yeast isolated from bovine mastitis [30,31].

1.6.2 Antibacterial Mechanism of Silver Nanoparticles

The mechanism of the antibacterial activity of silver nanoparticles has not been completely investigated. Silver nanoparticles release silver ions regularly, which could be interpreted as a bacterial killing mechanism [32], Because of silver ions attraction to electricity and affinity for sulfur proteins, silver ions will bind to the plasma and cytoplasmic membranes [33]. The attached ions will increase cytoplasmatic membrane permeability, causing the bacterial membrane rupture [34] (Figure 1.7). After the integration of free silver ions into cells, respiratory enzymes may be inhibited, producing reactive oxygen species but interfering with adenosine triphosphate generation. [35]. The provocation of cell membrane breakdown and deoxyribonucleic acid (DNA) adjustment could be aided by reactive oxygen species. [36].

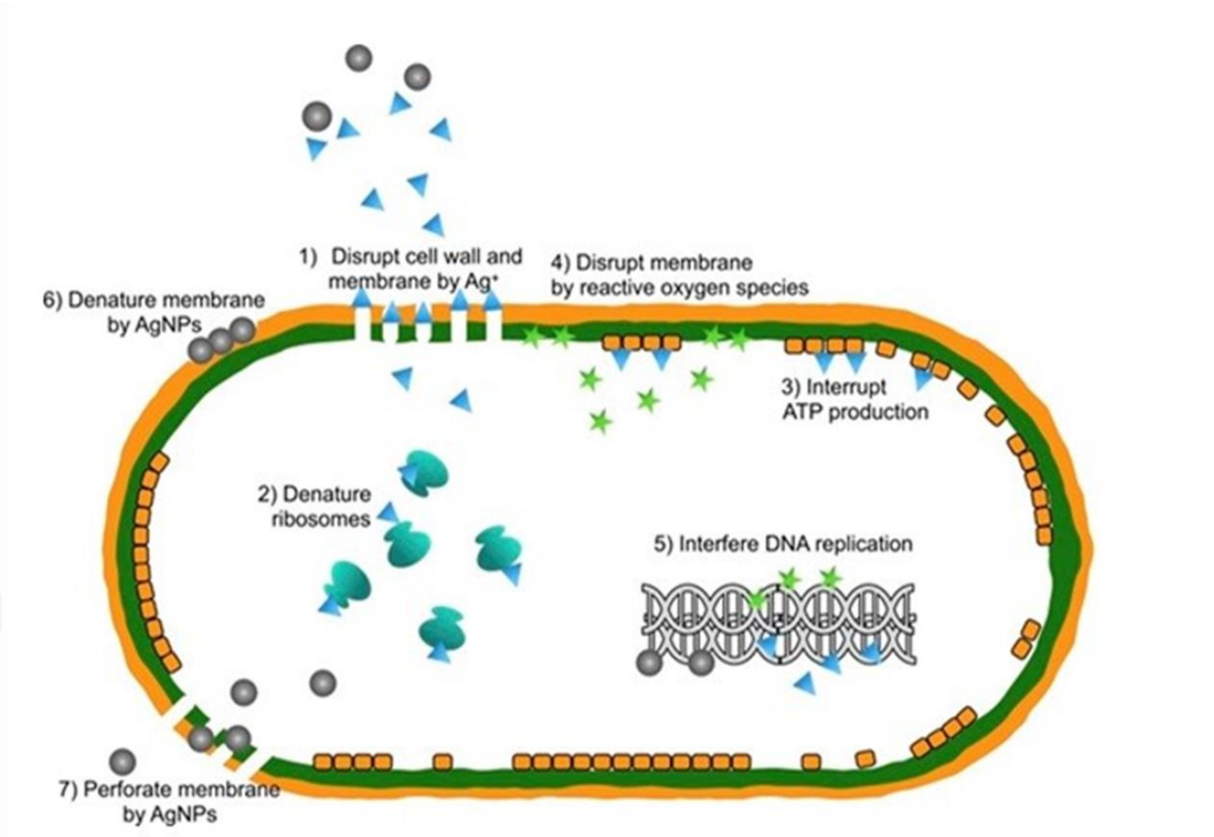


Figure1. 7: Antibacterial mechanism of Silver nanoparticles [37] . 1) Disruption of cell membrane2(denaturation of ribozomes ,3) Interruption of nucleotide (ATP)production. 4) Disruption of membrane by reactive oxygen species 6) membrane denaturation by AgNPs 7) membrane perforation by AgNPs

The interaction of silver ions with sulfur and phosphorus in DNA can create a range of issues in DNA replication, cell reproduction, and even microbe termination since sulfur and phosphorus have important mechanisms in DNA. Silver ions can stop protein synthesis by denaturing ribosomes in the cytoplasm [38].

Silver nanoparticles, in addition to being able to release silver ions, can also kill bacteria. After anchoring to the cell surface, silver nanoparticles can accumulate in pits that form on the cell wall [39].

Cell wall denaturation will be caused by the silver nanoparticles collected. Silver nanoparticles even have the flexibility to penetrate bacterial cell walls. Because of their nanoscale size, they can then affect the structure of the plasma membrane [39,40].

The denaturation of the cell membrane, cytoplasm and organelles will rupture, and even lead to cell lysis. Also, silver nanoparticles are involved in microorganism signal transduction. Bacteria signal transmission is suffering from phosphorylation of super-molecule substrates, and nanoparticles would then dephosphorylate tyrosine residues on the amide substrates. Disruption of the signal transmission will cause cell necrobiosis and termination of cell growth [41].

1.7 Properties of Bacteria Used in The Study

1) Escherichia coli (E. *coli*) bacteria are Gram-negative, rod-shaped bacteria with rounded ends that measure 2.0–6.0 µm in length and 1.1–1.5 µm in width. However, the shape of these bacteria can range from spherical (cocci) cells to elongated or filamentous rods. Escherichia coli does not produce spores and is motile due to the action of peritrichous flagella. As evidenced by acid and gas production from lactose at $37C^0$ and $44C^0$, Escherichia coli is facultatively anaerobic and produces gas from carbohydrate fermentation [24]. (E. *coli*) is a microscopic single celled organism that can survive in a variety of environments. Bacteria can be found in soil and water, as well as on living creatures such as plants, humans, and animals [1] .

2) *S.aureus*, a Gram-positive, pathogen belonging to the *Staphylococcaceae* family , is a spherical bacterium with grape like clusters having a diameter roughly 1 µm. S. aureus is a commensal bacteria that may be found asymptomatically on human body parts such as skin, skin glands, and mucous membranes, including the noses and intestines of healthy humans [44]. *S. aureus* is one of the most prevalent causes of life-threatening hospital and community-acquired infections. It may infect the bloodstream, skin and soft tissues, and the lower respiratory tract, as well as contaminated medical equipment [45,46].

1.8 Conducting Polymers

Conducting polymers (CPs) have a wide range of applications, including biological applications such as medication delivery and antimicrobial-resistant polymers. PANI (polyaniline) and polypyrene (polypyrene) are two of the most well-known. In biomedical research, polypyrimidine (Ppy), polythiophene (PTh), and polyacetylene have all been used. Because of its solubility in non-standard laboratory solvents, it may be used for a wide range of applications [47], And because they can remove microorganisms without the need of antiseptics like iodine or silver, decay of-PANI-based polymers are regarded to have the potential to be used as biocompatible scaffolds for tissue engineering and antibacterial wound dressings. Conjugated organic polymers with alternating single and double bonds along their main chains (CPs) are known as conductive polymers [48,49].

1.8.1 Polyaniline

The researchers focused on PANI because it has both nitrogen and hydrogen on both sides of a very reactive phenylene ring, making it a conducting polymer. Because of its unique properties, such as stability, ease of preparation, cost efficiency, and environmental friendliness, PANI has a wide range of applications [50]. The complex has been of interest since 1980s because of its electrical conductivity and mechanical properties. Polyaniline is one of the most extensively researched conducting polymers [51] PANI is a typical phenylene-based polymer with a chemically flexible –NH group bordered on each side by phenylene rings. The presence of this –NH-group is responsible for PANI's protonation, deprotonation, and a variety of other physicochemical characteristics. The antibacterial properties of conductive functionalized-polyaniline (f-PANI) were studied by studying their interactions with various microorganisms. Low quantities of PANI were found to be strongly limit the development of wild-type *E. coli* and *S.aureus*, as well as various antibiotic-resistant clinical pathogens[52] (Figure 1.8).

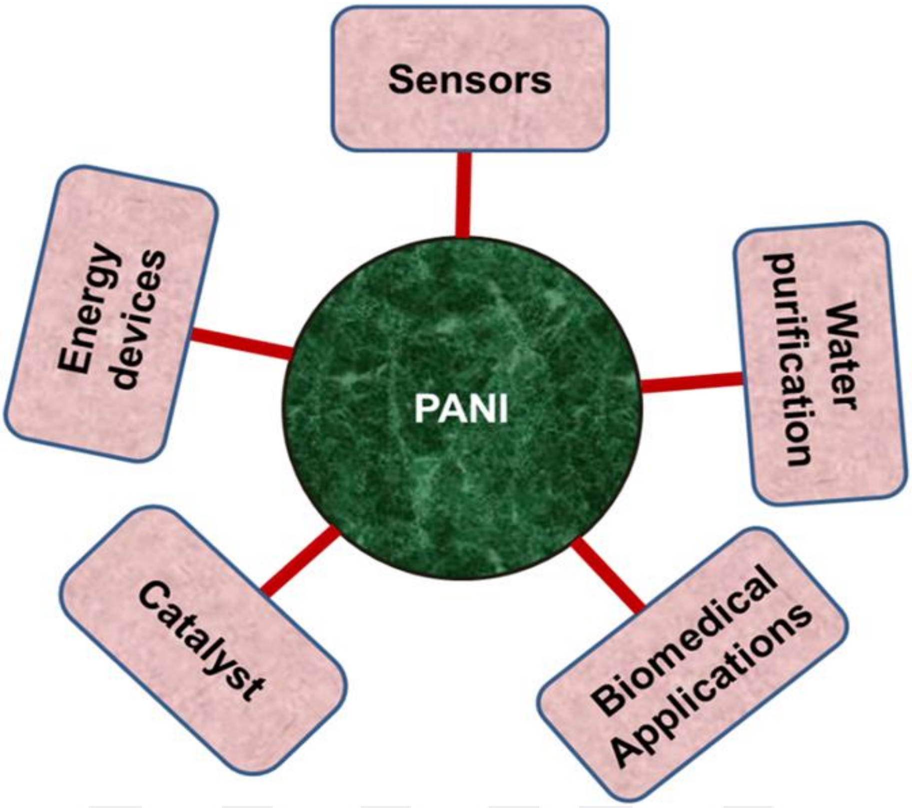


Figure1. 8 Applications of PANI-based nano- and composites materials [53]

1.8.2 Poly (N-methyl anillin)

One of the conductive polymers is poly (n-methyl anillin) PNMA$[C_6H_5NH(CH_3)]_{n.}$ Conductive polymers (CPs) are a type of conjugated organic polymer with alternating single and double bonds throughout their major [54]. PNMA is of interest because it is more soluble in organic solvents than unsubstituted polyaniline. PNMA has a methyl substituent at the nitrogen heteroatom, which increases its solubility in organic solvents [55,56].

1.9 The Aim of This Study

This study aims to synthesize and characterize silver nano particles with PNMA and test its anti-bacterial response against *E. coli* and *S. aureus*.

CHAPTER 2

EXPERIMENTAL AND METHODS

2.1 Materials

Aniline monomer (Ani), N-methylaniline monomer (NMA), Ammonium persulfate (APS) Adipic acid, Hydrochloric acid, ferric chloride, silver nitrate, sodium boron hydride, ethanol and deionized water have analytical grades and were used as received without any further treatment. The addition of silver nanoparticles was performed with silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$).

2.2 Instrumentation

For the morphological characterization of prepared particles, initially, Quanta 400 FE-SEM from FEI, Field Emission Scanning Electron Microscopy (FE-SEM) was used. For the detailed characterization to find the size and the shape of the prepared particles, JEOL JEM- 2010F transmission Emission Microscopy (TEM) was used. The instrument is an advanced field emission electron microscope with an accelerating voltage of a maximum of 200 kV. Elemental composition of the particles was performed with an energy-dispersive X-ray analyzer (EDX) coupled with SEM and TEM. SEM, TEM and EDX measurements, percent loading of silver was revealed by using ICP-OES, Elan, Perkin Elmer. Lysogeny Broth (LB) media, Chromogenic TBX agar and Baired Parker Agar were used (Laborlar Biyoteknoloji, Istanbul, Türkiye) for bacterial studies. Press (Optosense) was used to prepare NPs pellets. CO_2 incubator (New Brunswick), centrifuge (Hettich) and temperature-controlled shaker were used for bacterial growth. Antibiotic discs (Sigma) were used for growth inhibition analysis.

2.3 Preparation of PNMA Microspheres

The experimental setup used to prepare the PNMA microsphere is shown in Figure 2.2. In a beaker (A), 25ml de-ionized water containing 2.5×10^{-3} M NMA monomer and 2.5×10^{-3}M of adipic acid were placed. The mixture was allowed to stir at room temperature and 500 rpm for 15min to obtain well dispersed solution. In another beaker (B), 25ml deionized water containing 2.5×10^{-3} M of APS were placed and allowed to stir at 500 rpm at room temperature for 15min. APS solution was added drop-wise to NMA dispersion as the reaction mixture turned dark blue. The resulting reaction mixture was allowed to stir at room temperature for 1h. To stopping the polymerization reaction, 10 ml of ethanol was added to the reaction mixture. The obtained dark green product was filtered, washed with ethanol and with an excess amount of deionized water until the filtrate became colorless. The dark green product was placed in a petri dish and left to dry at room temperature overnight. The dry product was collected and stored in a labeled vessel for future use.

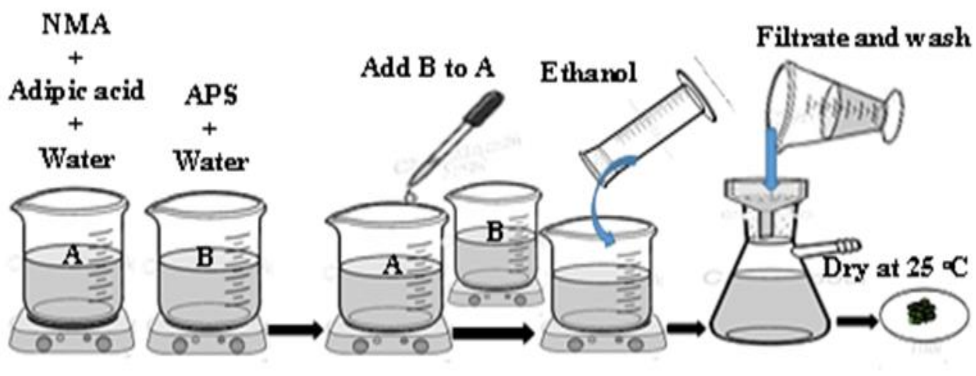


Figure 2.1 Synthesis of poly (N-methylaniline) Microspheres

2.4 Loading of Silver Ions on PNMA Microspheres

50 mg sample support, 9.5 ml deionized water and 0.5 ml metal nanoparticles 3 % for silver doping were used. The solutions were placed in labeled vessels and closed with caps and then stirred at 600 rpm, at room temperature for 5h for effective metal

loading. To avoid agglomeration of the solid support particles, each 15 min the vessels were handily shaken for a few minutes then placed back on the magnetic stirrer. Vessel contents were transferred into labeled centrifuge tubes after removing the stirrer bars using an external magnet. Centrifugation was carried out at 4000 rpm for 5 min, the clear supernatants were stored for further analysis. The remaining solid supports were washed with water and then centrifuged at 4000 rpm three times. The remaining solid supports were dried in the oven at 80 C° for 24 h. The dried solid supports were recovered prior to the reduction step.

2.5 Reduction of Silver Ions Using $NaBH_4$

13 mg solid support sample, 10 ml deionized water and 25 mg $NaBH_4$ were placed in clean labeled vessels. Upon the addition of $NaBH_4$, bubbles started to appear due to hydrogen gas generation indicating a successful reduction process. The vessels were stirred at 600 rpm at ambient atmosphere for 3h. Vessel contents were transferred into labeled centrifuge tubes. Then centrifuged at 4000 rpm for 5min and then the liquid parts were removed into the waste. In the case of samples containing MNP, it was better to use an external magnet, which easily attracts all particles to it since it allows ease of supernatant removal and avoids possible loss of solid particles. Its remaining solid supports were washed with water and centrifuged two times. The supernatants were removed and the remaining solid supports were dried up in the oven at 80 °C overnight. The dried solids were collected and stored in labeled vials for light irradiation and photocatalytic activity measurements. The loading and reduction process of catalyst supports is shown in Figure 2.2.

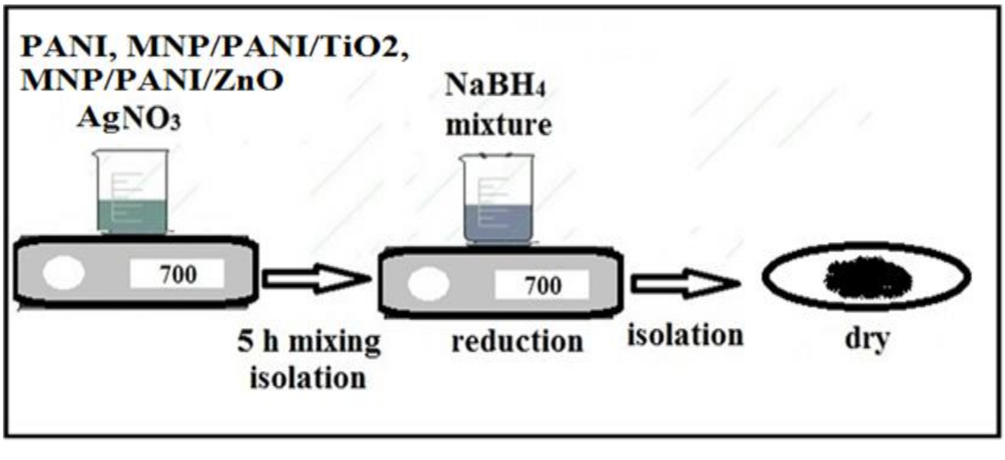


Figure 2.2. Addition of Ag NPs and reduction using $NaBH_4$

2.6 The Growing of Bacteria in LB Agar in the Laboratory.

An isolated colony was taken from a freshly streaked plate and used to inoculate sterile flasks with 5 mL of (LB) medium to start a starter culture. The flasks are incubated for 8 hours at 37 C° while shaking at 250-300 rpm. The starting culture was used to inoculate an overnight culture. The starting culture was diluted 1:500 to 1:1000 in a large flask with the required volume of medium and incubated for 12 to 16 hours at 37 °C while shaking at 250-300 rpm. OD measurements were used to calculate the concentration of this medium. The bacteria concentration was then diluted to 2.5×10^4 colonies. All of these studies were carried out in a sterile environment and under aseptic conditions.

Chromogenic TBX agar and Baired Parker Agar (Laborlar Biyoteknoloji, Istanbul, Türkiye) were used. Chromogenic TBX (Tryptone Bile X-glucuronide) agar was inoculated with E. *coli* (ATCC: 25922), while Baired Parker Agar was inoculated with S. aureus(ATCC: 29213). Both strains are standard strains for antimicrobial susceptibility testing).The discs were placed on a plate that had previously been inoculated with bacteria, and a 20 μL antibiotic (pen/strep) was applied to the discs. Nanoparticles' and antibiotics' growth inhibition zones were compared. (Figure 2.3 and 2.4).

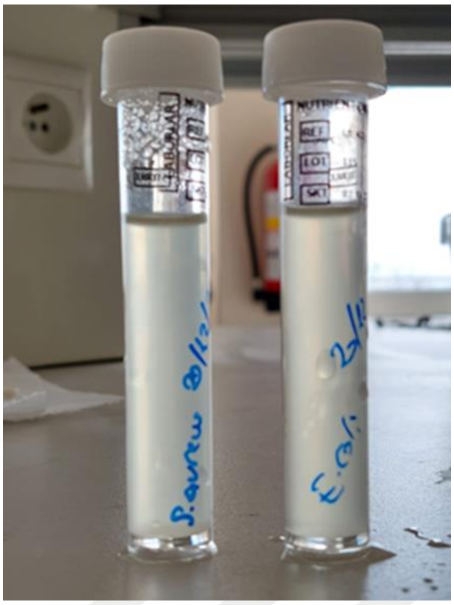


Figure 2.3 E.*coli* and S.*aureus* grown in LB Medium

Figure 2.4 Agar plates used for bacteria inocculation.

The pellets of each nanoparticle were prepared to test their antibacterial response because the NPs are insoluble in most solvents. To prepare pellets, the press shown in Figure 2.5 was used. (Optosense, Orlando Florida USA). The prepared pellets of PNMA and PNMA-AgNPs were given in Figure 2.6.

Figure 2.5 Press used to prepare pellets of nanoparticles.

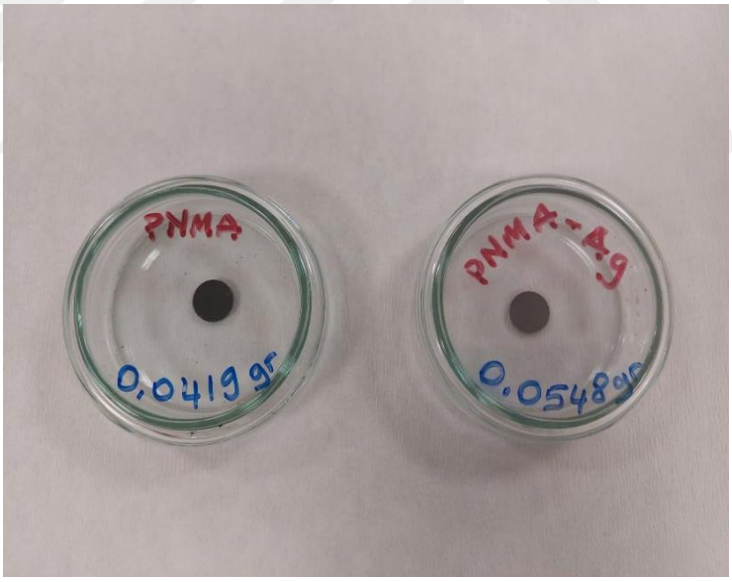


Figure 2.6 The pellets were prepared from the PNMA and PNMA-AgNPs

CHAPTER 3

RESULTS AND DISCUSSION

Polyaniline and its derivative poly(N-methylaniline) have received a great attention in newest years due to their exceptional physical, chemical properties and potential applications, particularly in photocatalysis.

The physical properties and catalytic effectiveness of photocatalyst composite structures are heavily influenced by the molecular weight and the shape of the polymers utilized. For PANI and PNMA, for example, the necessary catalyst performance for organic dye removal can be maintained by using the appropriate preparation approach during the syntheses of particles

Due to the presence of a stiff aromatic ring in their chemical structure and a stable π electronic system, PANI and PNMA polymers are generally insoluble in most common solvents and are not processable [57-60].

3.1 SEM Measurement of PNMA

The synthesis of nanoparticles PNMA were analyzed by scanning electron microscope. Figure 3.1 shows the SEM image of PNMA prepared with 0.025 M monomer solution.

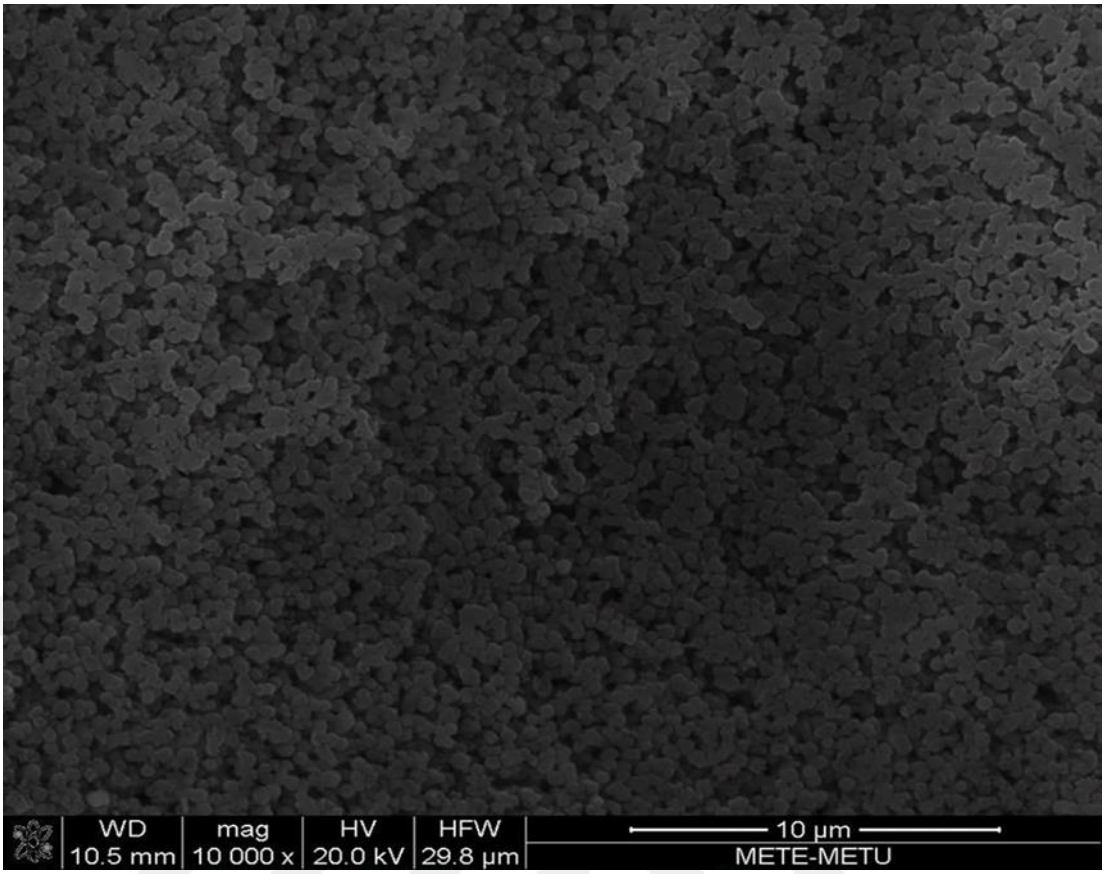
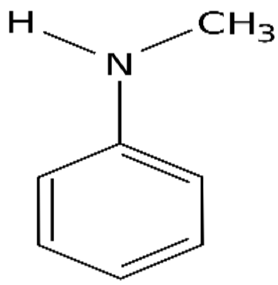


Figure 3.1: SEM image of PNMA prepared with 0.025 M monomer solution

From the SEM images it has found that the size of the resulting PNMA spheres lies between 200-550 nm.

3.2 The result of EDX Measurement of PNMA

The chemical composition of the resulting material was investigated with EDX measurement (Figure 3.2).



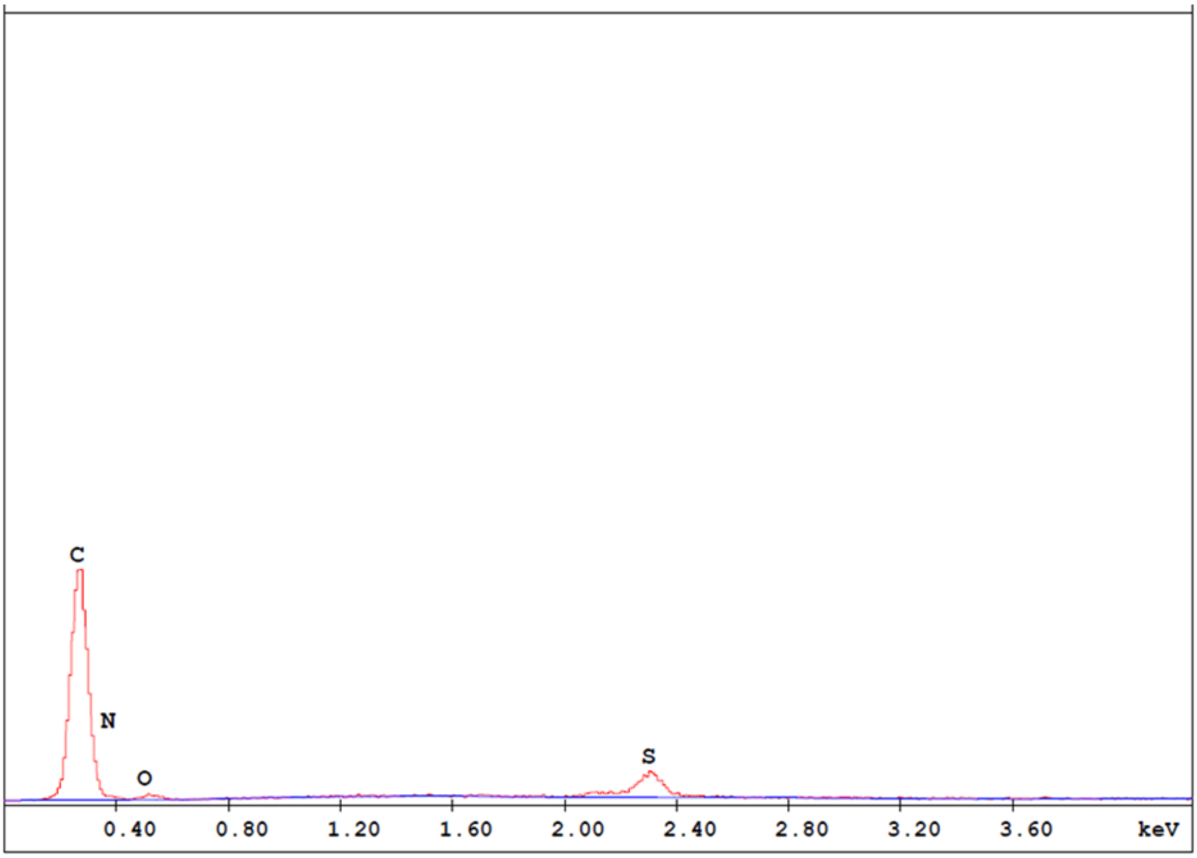


Figure 3.2: EDX pattern of PNMA spheres

The results showed that all elements found in PNMA $[C_6H_5NH(CH_3)]_n$ was absorbed in EDX pattern .

3.3 The result of TEM Measurement of silver nanoparticles added onto PNMA nanospheres

Morphological characterization of silver nanoparticles added to PNMA nanospheres was performed by TEM measurements. TEM image of resulting particles (PNMA-AgNPs) is given in Figure 3.3.

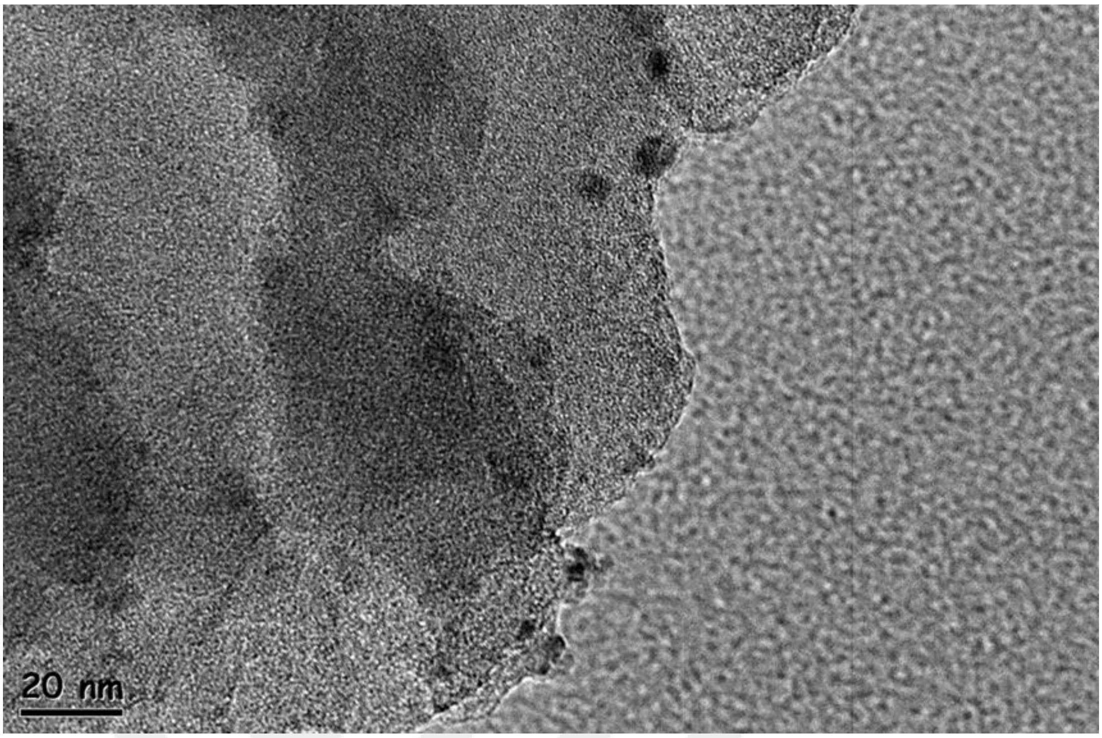


Figure 3.3: TEM image of silver nanoparticles added onto PNMA nanospheres

Silver nanoparticles were added on to PNMA nanoparticles are black spheres and the average particle size of Ag nanoparticles on the surface was measured as 3 ± 1 nm.

3.4 The result of EDX Measurement PNMA-AgNPs

In order to show the presence of silver on PNMA nanoparticles, EDX measurement was performed. EDX pattern of PNMA-AgNPs is given in Figure 3.4. As can be seen from the EDX pattern, silver nanoparticles were found in the structure besides elements found in PNMA. Where the peaks (Figure 3.4) showed all the elements in the PNMA and PNMA-Ag. The presence of silver nanoparticles on the surface of PNMA is seen by silver signal in the EDX pattern.

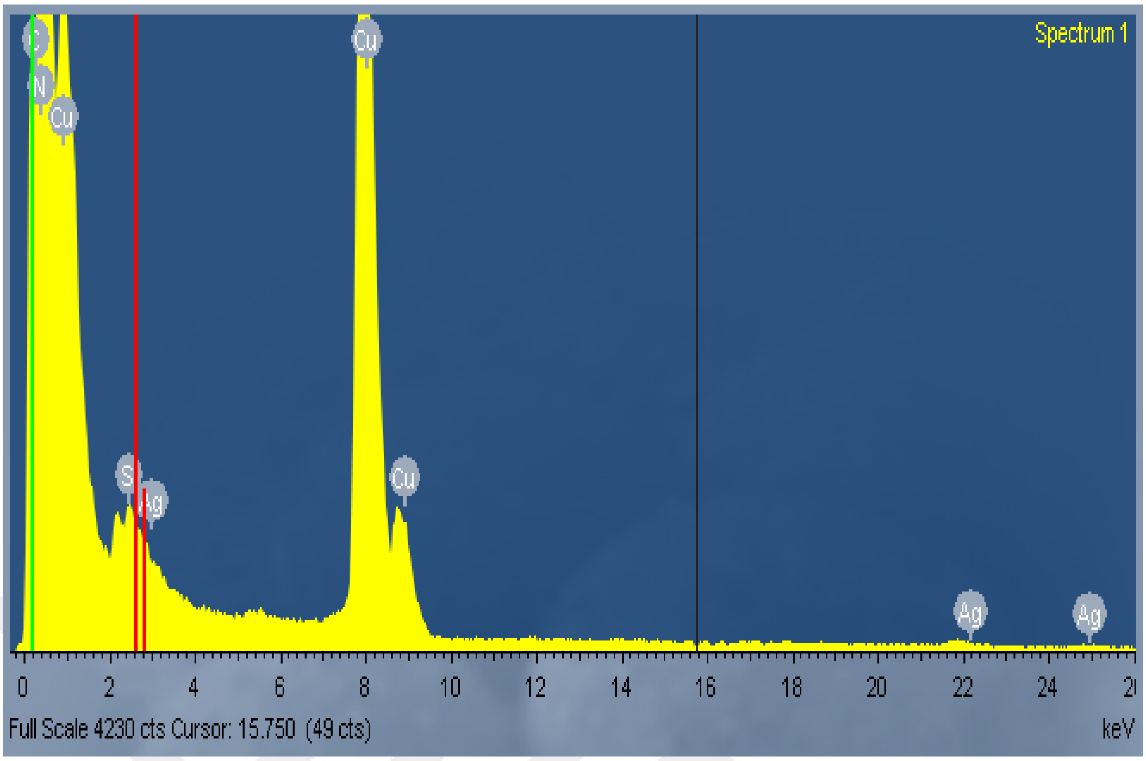


Figure 3.4: EDX pattern of PNMA-AgNPs.

The silver signal in the EDX pattern proves the attachment of silver nanoparticles to the surface of PNMA. The quantification of the total silver decorated onto PNMA nanoparticles was performed with ICP-OES measurements. According to the ICP-OES result. The total amount of silver added to the PNMA substrate was calculated as 1.97 % (w/w).

3.5 Effects of $AgNO_3$ on Bacteria

Infections of Antibiotic resistant bacteria are a serious problem because they can endanger patients. Antibiotics are antimicrobial agents produced either naturally by microorganisms or by chemically synthesized molecules to prevent or treat infections by inhibiting growth of the bacteria. They are a large and diverse group of part of Secondary metabolites of microorganism. These metabolites are complex organic molecules that are produced in the body and they aren't required for normal cell growth or reproduction. Ether for prophylactic that is use for disease prevention, or for treatment ideally all antibiotics must be accessible with low cost, chemically stable, simply to administer and nontoxic for human [62,63 and 64].

In this study, ($AgNO_3$) was used in two plates agar containing gram negative bacteria (*E. coli*) and gram positive bacteria (S.*Aureus*) as shown in (Figure 3.5 and 3.6) respectively. As seen from the figures, $AgNO_3$ did not provide any antibacterial response against both types of bacteria (without having zone around the antibiotic disc) as compared with the antibiotic used as a reference (penicillin / streptomycin) (Figure 3.5 and 3.6).

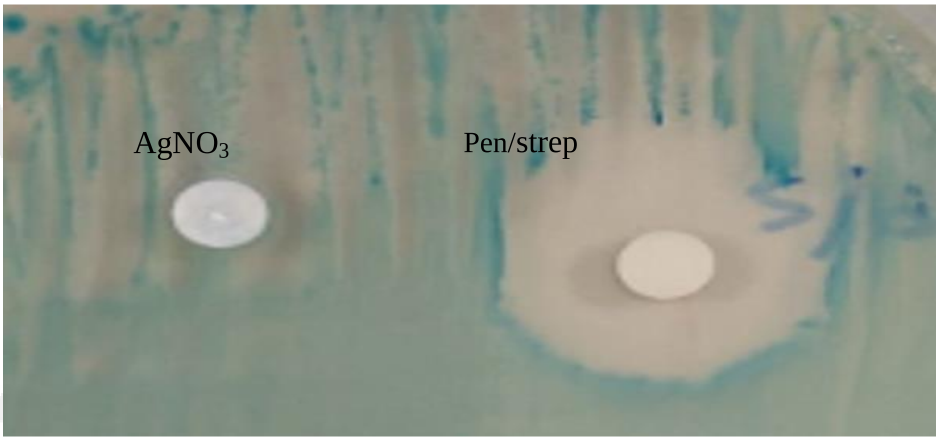


Figure 3.5 Effects of ($AgNO_3$) on E.*coli* compared to pen/strep.

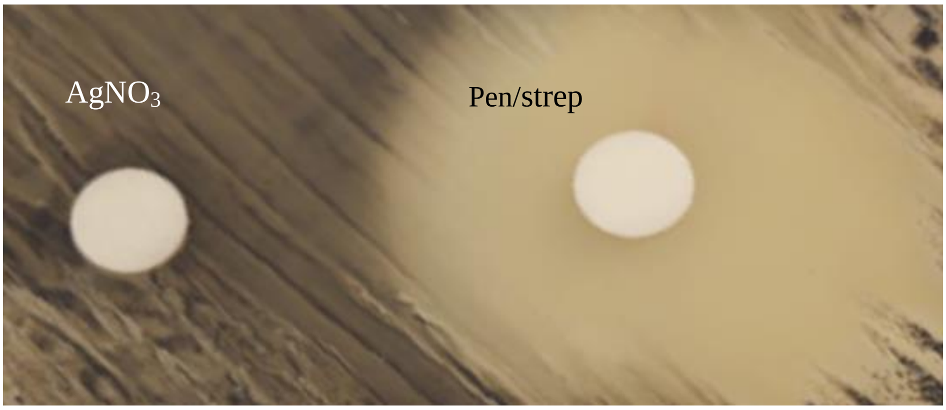


Figure 3.6 Effects of ($AgNO_3$) on S.*aureus* compared to pen/strep.

Although the silver is accepted and proven to be one important component of antibacterial agents, it is generally true for nanoscales applications. Here $AgNO_3$ is the compound test against standard types of bacteria because it's one of the ingredients used to produced PNMA.

On the other hands, as seen from the figure 3.5 and 3.6, the same antibiotic mixture has different responses with different types of bacteria, where the $AgNO_3$ did not induce any response [65-70].

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3.6 The Effect of PNMA, PNMA-Ag NPs on Bacteria

Experimental results showed that the PNMA pallet on to the plate of maculated with E.*coli* no inhibition zone was observed with respect to antibiotic control (Figure 3.7 a). In contrast, the inserted PNMA-Ag pellet showed too small and ghostly of inhibition zone as (Figure 3.7 b) as compared with the antibiotic control (Figure 3.7 c).

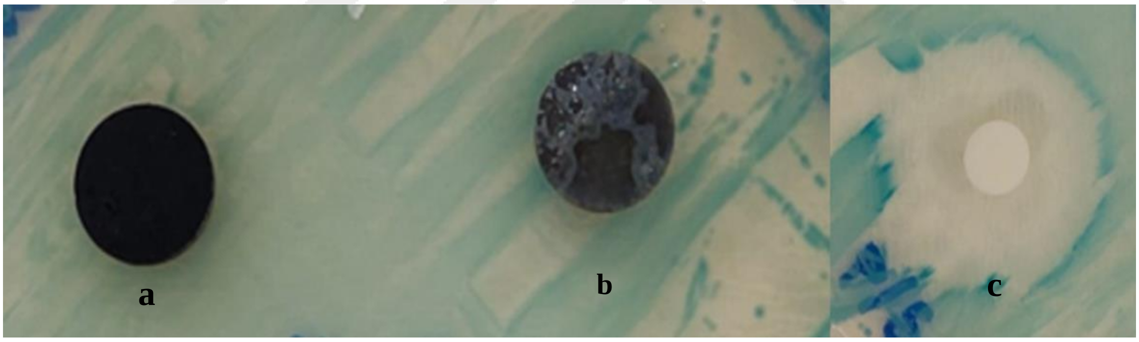

Figure 3.7 a) Effects of a) PNMA , b) Ag-PNMA c) pen/strep on E.coli

The effect of PNMA and PNMA-Ag pellets on *S.aureus* is shown in (Figure 3.8). As seen from the figure, against S.*aureus* , PNMA showed light inhibition zone (Figure 3.8 a) , compared with the antibiotic control (Figure 3.8 c). On the other hand, PNMA-Ag better and significant inhibition zone (Figure 3.8 b) compared to antibiotic control and PNMA tested.

When these two results compared it is concluded that like their response to antibiotics (Figure 3.5 and 3.6) the response of bacteria towards PNMA and PNMA-Ag was different. PNMA and PNMA-Ag was found to be more effective towards S.*aureus* than *E.coli* Due to difference in the zone length around each pellet.

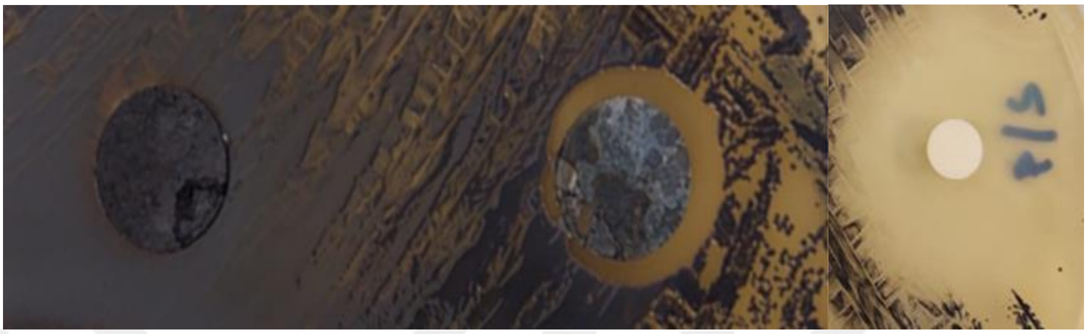

Figure 3.8 Effects of a) PNMA , b) Ag-PNMA c) pen/strep on S.aureus

CHAPTER 4

CONCLUSION

For several decays, antimicrobial agents have reactivated great attention to improve quality of health, treat and protect human from microorganism, especially from bacteria with resistance to several and well-known antibacterial agents. Shares the importance of silver nanoparticles use as antibacterial agents with the while rang of compositions. In this study 1) Silver nanoparticles and PNMA prepared. 2) $AgNO_3$ didn't show any antimicrobial response on both types of bacteria due to its low reactivity towed bacteria. 3) PNMA and PNMA-Ag both have no reasonable against E. *coli* .4) PNMA has some small effect against S.*aureus* , PNMA-Ag showed good effects on the same bacteria.

Of this information gathered it can be concluded that PNMA-Ag nanoparticles are selectivity antibacterial against gram positive bacteria. For future study, PNMA-Ag nanoparticles may be used with more other compounds to improve the solubility and antibacterial effectivity.

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