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ATILIM UNIVERSITY 2022

PREPARATION OF TITANIUM DIOXIDE NANOPARTICLES ADDED PANI
AND THEIR ANTIBACTERIAL RESPONSE ANALYSIS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
ATILIM UNIVERSITY

BY
MAHA ALTMARI

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMICAL ENGINEERING

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

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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, Atilim University.**

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ABSTRACT

PREPARATION OF TITANIUM DIOXIDE NANOPARTICLES ADDED PANI AND THEIR ANTIBACTERIAL RESPONSE ANALYSIS

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MS. Chemical Engineering and Applied Chemistry

Supervisor: Prof. Dr. Belgin İşgör

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Because of an increase the bacterial attack in different sectors of our life, it become necessary to search about a new antibacterial material. The aim of this research is to produce an anti-bacterial and environmentally friendly material used as a surface coating to reduce diseases caused by bacterial infection. In this work, we prepared polyaniline (PANI) by using the chemical polymerization of aniline with ammonium persulfate (APS), after that we used commercially available titanium dioxide nanopowder for preparation (PANI-TiO₂) nanocomposite material by chemical polymerization. The morphologies, structures, and elemental composition of the nanocomposite material are characterized by using TEM, SEM and EDX. After that, study the antibacterial activity of TiO₂, PANI and (PANI-TiO₂) nanocomposite was investigated by using *E. coli* and *S. aureus*. The results of this study show that no antibacterial effect of TiO₂ and PANINPs for each type of bacteria, but the antibacterial effect for (PANI- TiO₂) was very weak on *S. aureus* and very strong on *E. coli*.

Keywords: Polyaniline, TiO₂, Antibacterial activity

ÖZ

TİTANYUM DİOKSİT NANOPARTİKÜLLERİ EKLENMİŞ POLİANİLİN HAZIRLANMASI VE ANTİBAKTERİYEL YANIT ANALİZİ

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Yaşamımızın farklı sektörlerinde bakteriyel saldırının artması nedeniyle yeni bir antibakteriyel malzeme arayışı gerekli hale gelmiştir. Bu araştırmanın amacı, bakteriyel enfeksiyonun neden olduğu hastalıkları azaltmak için yüzey kaplaması olarak kullanılan anti bakteriyel ve çevre dostu bir malzeme üretmektir. Bu çalışmada, anilinin amonyum persülfat (APS) ile kimyasal polimerizasyonunu kullanarak polianilin (PANI) hazırladık, ardından kimyasal polimerizasyon ile hazırlanan (PANI-TiO₂) nanokompozit malzeme için piyasada bulunan titanyum dioksit nanotozunu kullanıldı. Nanokompozit malzemenin morfolojileri ve elementel bileşimi TEM, SEM, EDX ve ICP-OES kullanılarak karakterize edildi. Daha sonra *E. coli* ve *S. aureus* kullanarak TiO₂, PANI ve (PANI-TiO₂) nanokompozitinin antibakteriyel aktivitesi incelendi. Bu çalışmanın sonucunda, her bakteri türü için TiO₂ ve PANI NP'lerinin antibakteriyel etkisinin olmadığını, ancak (PANI-TiO₂) antibakteriyel etkisinin *S. aureus* üzerinde çok zayıf ve *E. coli* üzerinde çok güçlü olduğu gösterildi.

AnahtarKelimeler: Polianilin, TiO₂, Antibakteriyel aktivite

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CHAPTER 1

INTRODUCTION

Nanotechnology is the field that deals with materials in nanometer scale that has a great application in many sectors aimed to improving the human life. The development of nanotechnology led to increase the production of nanoparticles (NPs) materials that eventually lead to rapid development in their applications in various areas of life [1]. NPs have ability to cellular interactions in the biological environments and destroy their structure and functions. So, for that, the toxicity property of NPs is a very complicated issue [2]. Because of an increase the bacterial attack in different sectors of our life, such as food, textiles, medicines, water disinfection and food packaging it is become necessary to search about anew antibacterial material. Some of bacteria have a high resistance to some antibiotics and the toxicity of some organic antimicrobial to the human body that lead to increase the development of inorganic antimicrobial substances. Among these compounds, metal and metal oxide compounds because of their broad-spectrum antibacterial activities. In addition to the nanoscale materials have a very important properties because of their high surface area-to-volume ratio and have a different activity from their bulk materials [3]. In the final years, metal oxide nanoparticles, such as zinc oxide (ZnO_2), manganese oxide (MgO), titanium dioxide (TiO_2), and press oxide (Fe_2O_3), have been used in biological applications because of their unique physical and chemical properties.

Titanium is the ninth most common element in the Earth's crust [4], could be found in rocks, plants, natural water and creatures. TiO_2 is a semiconductor transition metal oxide material and has special properties, such as easy control, low cost, non-toxicity, excellent surface morphology, photocatalytic action and have high resistance to chemical corrosion [5], it is a very important for several industrial applications such as solar cells, electronics, chemical sensors and antibacterial agents [6]. Many studies have reported that

TiO₂ NPs is one of the most important metal oxide nanoparticles because of their photocatalytic and antibacterial action [7]. Antibacterial action of nanoparticles is affected by many factors such as their morphology, size, chemical composition, source, and nanostructure [8] and the antibacterial action of TiO₂ NPs is depended on the photocatalytic action of TiO₂, which depends on its structural, morphological and textural properties [9]. There are two shapes for titanium dioxide amorphous and crystalline structure and the crystalline structure have three crystalline polymorphous: anatase, rutile, and brookite, the crystal structures and the shape of TiO₂ NPs are a critical point that affects their physicochemical properties and antibacterial properties [10]. British mineralogist William Gregor discovered titanium in 1791 during analysis black magnetic sands in England. After several years, TiO₂ is separated from the minerals by a German chemist M.H. Klaproth and he named the new metal titanium. In 1825 a Swedish chemist J.J. Berzelius separated the crude titanium after that, an American chemist M.A. Hunter produced the pure titanium in 1910. But the primary commercial generation of titanium dioxide did not appear until the 1920 [11]. The production of titanium metal was increased between the years 1950 - 2008 because titanium was entered in the aircraft industry. Titanium dioxide has been widely used in coating industries such as wood and metal products [12] and also entered in the pharmaceutical and cosmetic industries including hair treatments, lotions, sunscreens, lipsticks, and protect baby cream because very small amount of titanium dioxide can add a new property to the product. Titanium dioxide is used as coloring in the food industries because it's low toxicity [13]. Titanium dioxide nanoparticles have a widely use in medical sector in dental restoration, orthopedic implants and medical devices [14] also uses in wastewater treatment [15] and cancer treatment by destroying the cancer cells [16].

Polyaniline is the most important conductive polymer due to many advantages including, low cost of monomers (aniline), easy preparation, low toxicity, environmental stability, biocompatibility good conductivity [17] and have ability to make composites with different materials [18]. Polyaniline have great applications in different sectors such as in solar cells, sensor elements, anticorrosion materials [19] and antibacterial material [20].

The first product of aniline was synthesized by thermo lysis of indigo plant in 1826 by German chemist Otto Unverdorben. At that time, this product was called "Krystallin" meaning crystallization. Fdtzsche could produce aniline by colorless oily material from indigo in 1840, this production was used in the coloring industry in 1856. In the early 20th century, the polyaniline synthesis has begun. Dr. Green and Dr. Willstatter from Germany used different oxidants and reaction conditions to obtain polyaniline [21]. Dr. Green could synthesize organic aniline with different degrees of oxidation by using sodium chlorate and hydrogen peroxide as oxidants [22]. In 1968 Dr. Surville synthesized and proposed polyaniline structure [23].

Organic/inorganic nanocomposites show many synergistic properties, PANI/TiO₂ nanocomposites are formed by the electron interaction between PANI and TiO₂ [24]. PANI-TiO₂ nanocomposite has been used in various research fields because of their high performance in different applications such as antibacterial [25], antifungal [26] and degradation of pollutants [25].

In this study, PANI was prepared using the chemical polymerization of aniline with ammonium persulfate (APS) after that, commercially available titanium dioxide nanopowder was used for preparation PANI-TiO₂ nanocomposite material to test its antibacterial potential by using *E. coli* and *S. aureus*.

1.1 What are Nanoparticles?

Nanoparticles (NPs) are defined as particles less than 100 nm in at least one dimension and because of its small size have anew characteristic. Nanoparticles have attracted a great attention for their properties of higher surface area to volume ratios. Nanotechnology is considered as one of the most important developments in different industries that deal with materials at the atomic or molecular level [27]. Nanomaterials are considered as one of the important materials that contribute to the development of many devices in various fields such as the physics [28], biology [29], biomedicine [30] and pharmacy [31].

1.2 Types of Nanoparticles

Nanoparticles are classified into four types, metal-based materials, carbon-based materials, dendrimers, and composites [32]. The metal-based materials include gold, silver, and titanium oxides NPs include zinc oxide (ZnO_2) and titanium dioxide (TiO_2). The carbon-based materials consist mostly of carbon, in the form of balls or tubes and because of its strong and light material they are used in the coatings. Dendrimers are nano polymers consist of branched units and the surface contains many chain ends, which can be designed for catalyzing the chemical functions. Composites are nanomaterials that combines two types of NPs, this type is used in the auto parts and packaging industry [28].

1.3 The Antibacterial Activity of Nanoparticles

The antibacterial activity of nanoparticles is very complicated. Nanoparticle have ability to destroy the integrity of bacterial membrane through the electrostatic interaction between nanoparticles and bacteria and generating reactive oxygen species [33]. The cell wall of bacteria plays a very important role to protect the cell from mechanical damage and provide shape, rigidity and strength [34]. There are two types of the cell wall depending on their structure and composition: Gram-positive (+) and Gram negative (-). Gram positive has a thick layer of peptidoglycan and no outer lipid membrane but Gram negative has a thin layer of peptidoglycan and have an outer lipid membrane[35]. The advantages of nanoparticles as antibacterial agents are their nanostructure design that makes it difficult for bacteria to protect themselves against these type of materials[36]. The antibacterial activity of nanoparticles depends on several factors such as the composition of the cell wall, contact surface, species of bacteria, concentration of nanoparticles and the properties of nanoparticles.

1.4 Titanium Dioxide Nanoparticles (TiO₂)

Titanium dioxide also called ultrafine titanium dioxide or nanocrystalline is a white inorganic compound having the chemical formula TiO₂ with diameter less than 100 nm. Titanium dioxide (TiO₂) is safe, inert, semiconductor material that exhibits photo reactivity in the presence of light and has been used in many applications for many years [5]. With the development of nanotechnologies, TiO₂ nanoparticles are being synthesized and used, with many new and useful properties.

1.4.1 The General Properties of TiO₂ Nanoparticles

Titanium dioxide has many special characteristics that make it interesting to use in different applications such as:

TiO₂ is a solid white material in particle form with odorless powder, the molecular weight of TiO₂ is 79.9 g/mol, the density of TiO₂ is 4.26 g/cm³ at 25°C, the melting point is 1,843°C, the boiling point is 2,972°C, TiO₂ is an insulator material, TiO₂ is a nontoxic material, TiO₂ is inexpensive material, high dielectric constant, chemical stability and TiO₂ is a semiconductor [37].

1.4.2 The Antibacterial Applications of TiO₂ Nanoparticles

The intensity and type of damage on the bacterial cells depends on the physical and chemical properties of the TiO₂ nanoparticles [38]. TiO₂ nanoparticles have photocatalytic activity and generates reactive oxygen species so for that it is used in antibacterial applications such as:

1.4.2.1 Medical Application

In the clinic after surgery, the infection of surgical site, medical devices and orthopedic implant are the most complications and to be controlled, the biomaterial surfaces must be adjusted to resist bacterial colonization and coating the orthopedic implant and medical devices by TiO_2 reduces the bacterial attack [14]. TiO_2 nanoparticles have been used to inhibit bacterial growth because of their photocatalytic property [39]. TiO_2 nanoparticles play an important role in dentistry, they are used in dental implant to reduce bacterial infections[40].

1.4.2.2 Environmental Application

Water is an essential requirement in all industrial processes and commercial activities that produce wastewater contains various pollutants depending on the type of process. And now there has been much interest in the photocatalytic activity of TiO_2 nanoparticles to remove these organic compounds. TiO_2 as a photocatalyst has been widely used in wastewater treatment because the operation is simple, environmentally friendly and its lower cost [41]. In addition, the TiO_2 catalyst is non-toxic and stable in water for a period of time. The efficiency of the decomposition process increases with increase in concentration, light intensity and time of operation [42].

1.4.2.3 Food Safety Application

In the food safety application TiO_2 nanoparticles are used as antibacterial coating due to its photocatalytic activity [43]. TiO_2 nanoparticles have a good antibacterial effect so, it is used to coat the surface of steel and glass in which food is found and when the intensity of UV light is increased photocatalytic activity is increased too, when reduces the bacterial growth [40]. That is why the method was considered as the best way to improve food safety.

1.4.3 Antibacterial Effect of TiO₂ Nanoparticles

Several investigations have been focused for studying TiO₂ nanoparticles for treatment bacterial infections. Initially, TiO₂ nanoparticles have the ability to inactivate the bacterial cells because bacterial cells and TiO₂ nanoparticles have the opposite charge and close contact by the electrostatic attraction between bacterial cells and TiO₂ nanoparticles ruptures the cell membrane[44].TiO₂ nanoparticles are used for inhibition growth for both Gram-negative and Gram-positive bacteria due to its photocatalytic performance by using UV light irradiation[45].The toxicity of TiO₂ NPs to bacteria are related with the particle size. Many of the studies have shown that the toxicity increases with decreased particle size [46]. When TiO₂ nanoparticles are activated by UV light, that lead to generating holes (h⁺) and electrons in the valence band and the conduction band. The holes and electrons can generate ROS, which have strong oxidizing property towards bacteria by damaging the cell wall and membranes [47].

1.4.4 Photocatalytic Mechanism of TiO₂Nanoparticles

The photocatalytic properties of titanium dioxide have been well known for 90 years and use for disinfection of surfaces, air and water, because the photocatalytic properties have a high activity for killing a wide range of Gram-negative and Gram-positive bacteria [46]. The mechanism of photocatalysis starts whenTiO₂take in the photon with sufficient energy by UV light promoting the electrons transport from the valence band (e_{vb}⁻) to theconduction band (e_{cb}⁻) and leaving the hole with positively charged in the valence band (h_{vb}⁺). In this case the electrons are ready to migrate to the conduction band. The holes can be filled by another migrate electrons from other molecules. This process may be repeated, in sometimes the electrons and holes recombine again in this case the reaction will be non-productive, but when they reach to the surface and react with absorbed water on the surface and oxygen in the environment to give reactive oxygen species (ROS) including the superoxide anion (O₂⁻), the hydroxyl anion (OH⁻), hydroxyl radical (. OH) and hydrogen peroxide (H₂O₂) [46,48]. (Figure1.1)

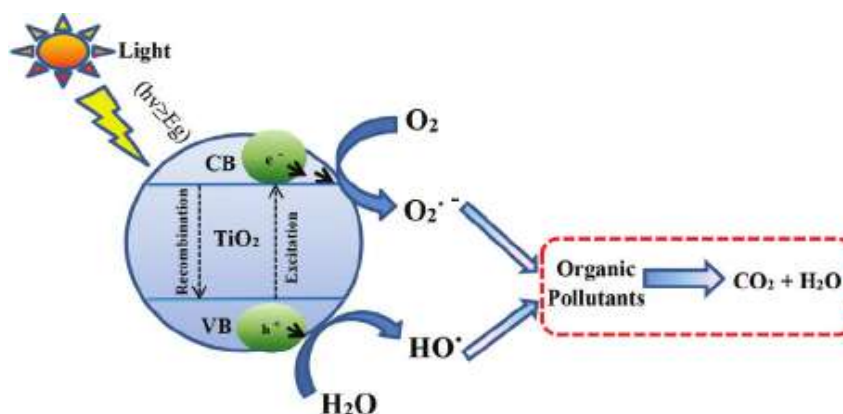


Figure 1.1: Photocatalytic mechanism of TiO₂ nanoparticles

1.4.5 Toxicity Mechanism of TiO₂ Nanoparticles to Bacteria

The mechanism of TiO₂ NP toxicity to bacteria can be summarized in three points: (1) production electron-hole pairs by UV light and generate ROS from TiO₂ NPs, (2) attachment between TiO₂ NP and the bacterial cells by electrostatic force that lead to damage the cell wall and peroxidation the lipid of the cell membrane, (3) damage to the cell membrane and cytoplasm flow out then, TiO₂ NP connect with biological macromolecules and intracellular organelles [46]. Many of studies have shown that ROS types are associated with inhibition of bacteria, they include hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻), hydroxyl anion (OH⁻), and hydroxyl radical (. OH) [49]. WHEN ROS is generated by TiO₂ NPs, it is adsorbed on the surface of bacteria by electrostatic force [50] causing lipid peroxidation in the cell membrane [51], increases the permeability of cell membrane [52]. The level of lipid peroxidation in the cell membrane is considered as a very important indicator of oxidative stress, when ROS level increases lipid peroxidation increases [53]. ROS can directly destroy all components of the cell membrane, damage the integrity and increase the permeability of bacterial membrane and then cross through the bacterial membrane [54]. The concentration of (. OH) have a very important direct relationship with bacterial mortality which means that when the concentration of (. OH) increases bacterial mortality increases too. Because it plays an essential role in the growth inhibition of the bacteria [55]. When the cell wall and

membrane are damaged, the cytoplasm flow-out, TiO₂ NPs can enter inside the bacterial cell and oxidizes the organelles such as DNA and mitochondria and denatures protein [56]. In the cell, the first to be oxidized are proteins after the lipid membranes and DNA in the nucleus [57]. (Figure 1.2)

The antibacterial activity of TiO₂ NPs is affected by the intensity of illumination because in the dark condition the level of ROS is very low compared with the light condition [54]. There is another factor which is affected on the TiO₂ NP toxicity such as microbial cell types, NP properties and environmental factors [47].

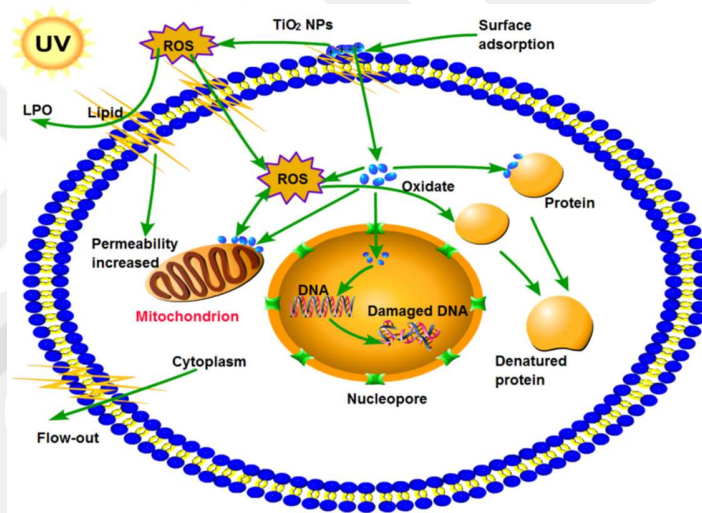


Figure 1.2: Toxicity mechanism of TiO₂ nanoparticles to bacteria

1.5 Polyaniline (PANI)

The conductive polymers have received a great attention in the past 20 years because of their good optical, electrical and redox properties [58], which allows it to be used in many applications such as memory devices [59], energy devices [60] and sensor devices [61], biomedical applications and in chemical reactions [62]. Among the others Polyaniline (PANI) is one of the most conducting polymers due to having many advantages include, low cost of monomers (aniline), easy preparation, low toxicity, environmental stability, good conductivity and having ability to make composites with different materials [18].

Dr. Mac Diarmid was the first to suggest the structural formula for polyaniline in 1984 and this model was widely accepted and called emeraldine [63]. (Figure 1.3).

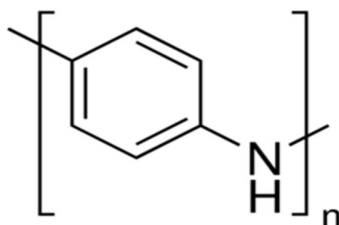


Figure 1.3: The polyaniline formula structure

Polyaniline have different structure depending on the condition of reaction [64] but emeraldine is the most important form which is prepared by chemical oxidation under acidic conditions and have a great electrical conductivity [65].

1.5.1 The Antibacterial Mechanism of Polyaniline (PANI)

Many of polymers are environmentally friendly and antibacterial agents. They can inactivate the bacterial growth by destroying the cell membrane. The antibacterial activity of polymers can be affected by the physical properties of the polymer chain, such as the type of monomer and the length of chain [66]. The structure of the bacterial cell wall consisting of peptidoglycans provides the mechanical support to the bacteria. There is a specific lipid which is responsible for synthesis of the peptidoglycan layers [67] and this lipid could be targeted by many of the polymers that prevent the cell wall synthesis and lead to kill the bacteria [68]. Polymers have ability to destroy bacteria by the electrostatic attraction to the cell membrane. Many of Polymers generally contain positively charged while bacterial membranes are negatively charged and by the electrostatic force between positive and negative charges, polymers are attracted to the bacterial membrane, and penetrate into the phospholipid bilayer that led to broken and lysis the membrane [69]. The Gram-negative bacteria have outer lipid membrane so they have higher resistance than Gram positive against the antibacterial activity of the polymers. When destroy the

cell membrane, the polymers can enter inside the bacterial cell and damage DNA these that will affect the synthesis and replication of bacteria, also damage the mitochondria and the ribosome responsible for protein synthesis. PANI are antibacterial agents and active against a wide range of bacteria, including Gram positive and Gram-negative bacteria [70] because of that, PANI are used as a coating material on the surfaces of medical devices for controlling the bacterial infection and food safety applications [71]. THE electrostatic force between polyaniline and the bacterial cell membrane which has negative charge increases the production of H_2O_2 [72].

When oxidation by H_2O_2 occur, it causes inactivation of metabolic enzymes and the respiratory enzymes and their function will be lost within minutes [73] and then iron atoms will be oxidized within the enzymes and iron releases from enzymes and the level of free iron increases [74]. The increase free iron level increases the production of Hydroxyl radicals ($\cdot OH$) and increases the oxidative stress. Hydroxyl radicals are powerful oxidants that can damage biomolecules including proteins, lipids and DNA [75].

In the mechanism of PANI of its antibacterial activity, PANI generates H_2O_2 which causes DNA double strand to break and then bacteria cannot protect themselves from oxidative stress and oxidation of biomolecules [76]. The repair systems detect and repair this damage in DNA, but if the repair systems are inactive, the damage will not be repaired. Increases hydroxyl radicals ($\cdot OH$) results in increase in the rate of biomolecule damage, which then causes the bacterial cells death [71]. H_2O_2 also causes oxidation of amino acid in the proteins, causes protein backbone degradation which leads to bacterial cells death again [77].

1.6 The Antibacterial Effect of TiO_2 -PANI Nanocomposite

TiO_2 -PANI nanocomposite used in various research fields because of their high performance in different applications such as antibacterial [25], antifungal [26] and degradation of pollutants [25]. The mechanism of TiO_2 /PANI nanocomposite for killing bacteria is shown in figure 1.4, in which ($\cdot OH$) penetrates through cell wall that increases

the cell permeability due to oxidative degradation of lipid in the cell membrane and oxidation of cellular biomolecules [66]. The thickness and the structure of cell walls play very important role in the death rate of bacteria. On the other hand, DNA cannot replicate to protect themselves from an oxidative stress by (H_2O_2) generated from PANI and finally the bacteria cannot be viable in other words bacteria are successfully killed by the use of TiO_2 / PANI nanocomposite [25]

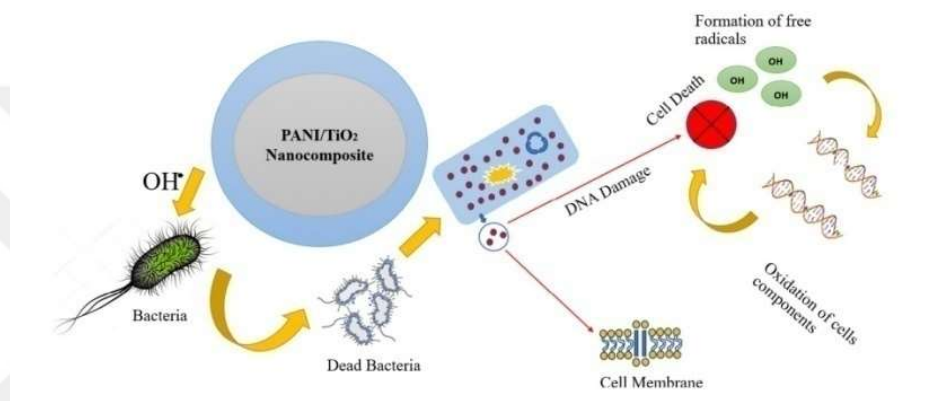


Figure 1.4: The mechanism of TiO_2 /PANI nanocomposite as antibacterial agent

CHAPTER 2

MATERIAL AND METHODS

2.1 Chemicals and Reagents

In this study all chemical materials were purchased in analytical grade and used in the experiments without any processing. Using the MilliQ water purification system to distill the water that is used in experiments. In this study all experiments were performed at room temperature and under the same conditions. The chemical synthesis of PANI conducting polymer by using aniline monomer, ammonium persulfate (APS) and hydrochloric acid purchased from Sigma-Aldrich. By using commercially available titanium dioxide nanopowder, (TiO₂, P25-Degussa-Evonik) to prepare PANI-TiO₂.

2.2 Instrumentation

2.2.1 Field Emission Scanning Electron Microscope (FE-SEM)

In this study was used Quanta 400F Field Emission Scanning Electron Microscope (FE-SEM, METU Central Laboratory) was used to figure out the components of prepared nanocomposite materials and their structure and morphology

2.2.2 Transmission Electron Microscopy (TEM)

2100 F, 200 kV Transmission Electron Microscopy (TEM, METU Central Laboratory) TEM was used to figure out the components of prepared nanocomposite materials and their structure and morphology

2.2.3 Energy Dispersive X-ray Spectrometer (EDX)

2100 F, 200 kV TEM and EDX coupled to Quanta 400F Field Emission Scanning Electron Microscope (FE-SEM) were used to figure out the elemental compositions of nanocomposite prepared materials.

2.3 Preparation of Polyaniline (PANI)

By using an oxidative polymerization technique and ammonium persulfate (APS) [100] to carry out the chemical polymerization of aniline shown in figure 2.1

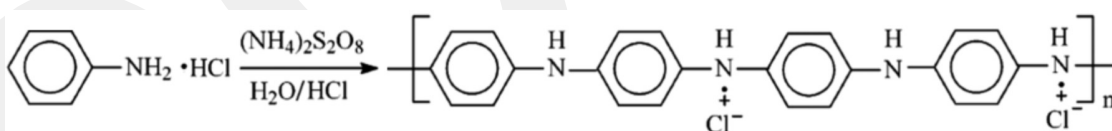


Figure 2.1: Oxidative polymerization of aniline hydrochloride with presence ammonium persulfate

In beaker (A) was added 245 μL of aniline monomer and 3.75 mL 1 M HCl then allowed to stir at 500 rpm for 15 min. In another beaker (B) was added 0.615 g of ammonium persulfate (APS) to dissolve in 3.5 mL 1 M HCl and also allowed to mix at 500 rpm for 15 min at room temperature. After that was added some of drops from solution (B) to solution (A) until the color of the reaction mixture change to the dark blue. After that, the generating solution was mixed for 1h. Then, filtered the product and washed by water

until obtaining a colorless filtrate to ensure remove all unreacted chemicals and impurities. After that, the product was put in a petri dish to dry at room temperature. Finally, the product was collected and stored in a vial (figure 2.2).

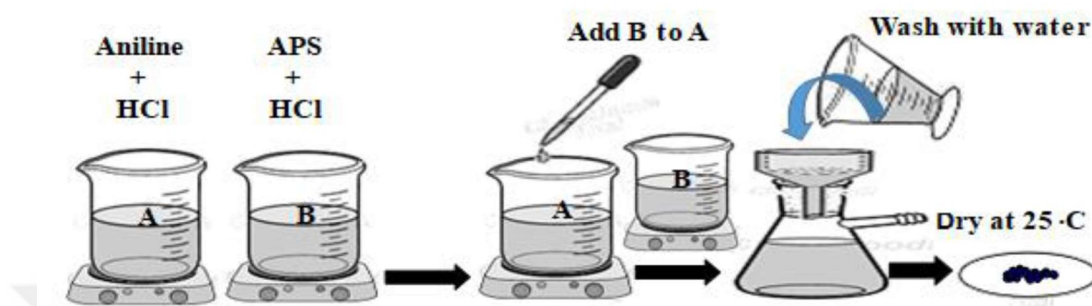


Figure 2.2: Preparation of PANI by using the oxidative polymerization method.

2.4 TiO₂ Nanoparticles Added PANI (PANI-TiO₂)

The addition of TiO₂ nanoparticles into PANI was carried out by applying the same procedure of preparation PANI. In beaker (A) was added 123 μ L of aniline monomer and 1.9 mL 1 M HCl. After that 0.060 g TiO₂ was added to the solution(A). In another beaker (B) was added 0.308 g of ammonium persulfate (APS) to dissolve in 1.8 mL 1 M HCl. At room temperature both beakers were mixed for 30 min with a magnetic mixer. After that was added some of drops from solution (B) to solution (A) until the color of the reaction mixture change to the dark blue. Then, the product was mixed for 1h at room temperature. After that, filtered the product and washed by de-ionized water until the filtrate become without color to ensure remove all unreacted chemicals and impurities. The product was dry at room temperature then was collected and stored in a vial.

2.5 Antibacterial Applications of NPS

After preparation PANI and (PANI-TiO₂) NP_s and because of these NP_s are insoluble in many solvents, we were prepared the pellets from PANI and (PANI-TiO₂) NP_s by using the press (Optosense, Orlando Florida USA) (Figure 2.3.)

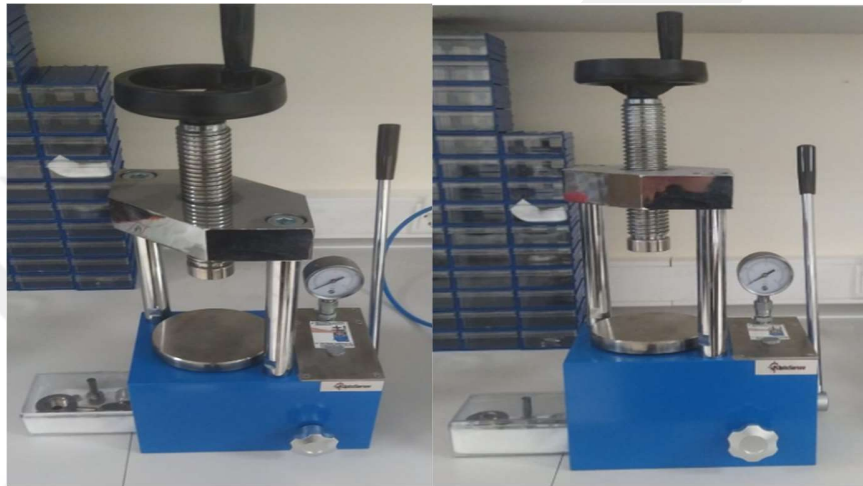


Figure 2.3: Press used to prepare pellets of nanoparticles.

These pellets were exposed to ultraviolet light for 60 minutes before the bacterial test. Some of colonies were collected from plate freshly inoculated by the starter culture in sterile flasks with 3 to 5 mL of Lysogeny Broth (LB) medium. The flasks were incubated at 37°C for 8 hours with shaking at 250-300 rpm. By using a large flask the starter culture was diluted 1:500 to 1:1000 and incubate at 37°C for 12 to 16 hours with shaking at 250 - 300 rpm. By OD measurement the concentration of this medium was calculated. After that the concentration of bacteria was diluted to 2.5×10^4 colonies (Figure 2.4). All these studies were done under aseptic conditions and by using sterile medium. In the laboratory, *S.aureus* (ATCC29213) and *E. coli*(ATCC25922) strains are used which are the standard strains for antimicrobial susceptibility testing and were grown in (LB) medium. *E.coli* were inoculated on the Chromogenic TBX agar and *S. aureus* were inoculated on the Baired Parker agar. These types of agar were ready to use and commercially available (Laborlar Biyoteknoloji, İstanbul, Türkiye). Additionally,

Penicilline / Streptomycine was used as reference antibiotic. The test of antibacterial response of Pen/Strep reference and TiO₂ were tested by using antibiotic discs.



Figure 2.4: a) Agar plates used for bacteria inoculation. b) *E. coli* and *S. aureus* grown in LB medium.

In this study, 20 uL all of each of TiO₂ (0.01 g/ml) and antibiotics Penicilline /Streptomycin (Biological Industries, USA) were added on to the antibiotic discs and the discs were placed on to the agar plate which contains *S. aureus* and *E. coli* to test their antibacterial effects.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Characterization of PANI

In this study, TEM and SEM measurements were used to figure out the morphology of the prepared materials. Energy-dispersive X-ray (EDX) spectroscopy coupled to TEM and SEM was used to figure out the chemical composition of the prepared materials. The production amount of polymer (PANI) and composite materials (PANI-TiO₂) by applying the chemical oxidation polymerization method and morphological characterization of PANI was performed by using SEM. The result is given in Figure 3.1

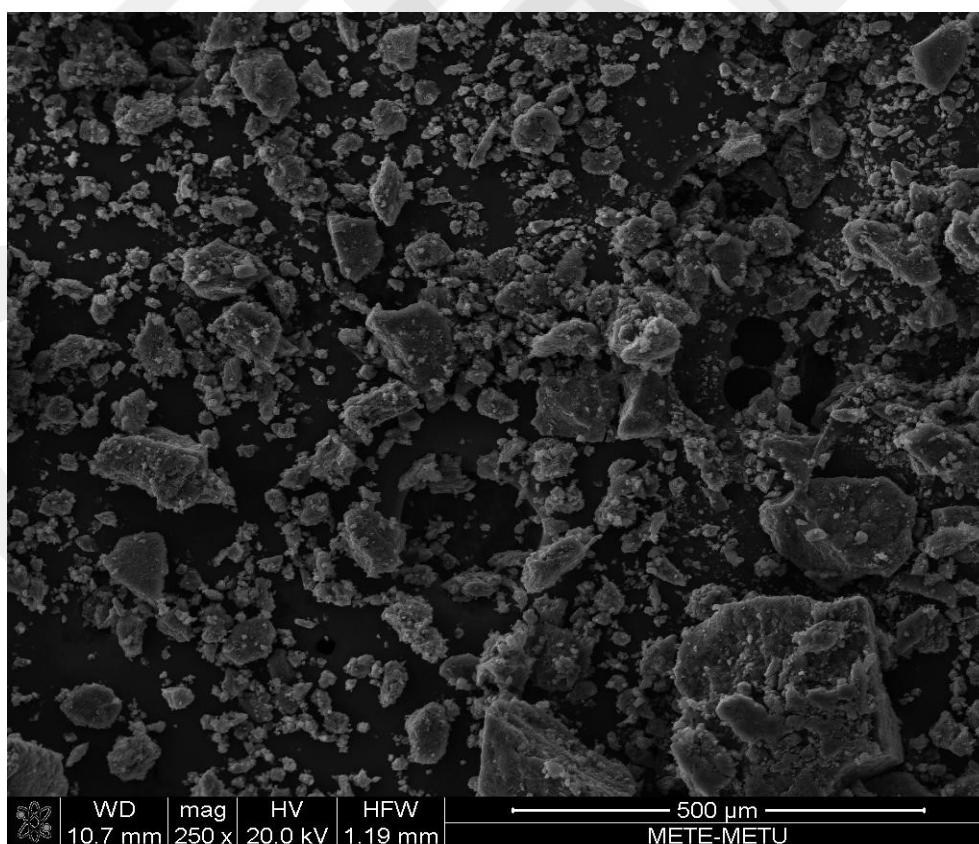


Figure 3.1: SEM image of PANI

From the SEM image, it has shown that the PANI particles have non-uniform distribution and their size were measured as micron-size.

3.2 Characterization of TiO₂ Nanoparticles

TiO₂ nanoparticles commercially known as Degussa P25 are used for the preparation of PANI-TiO₂ nanocomposite material. The TEM image of TiO₂ is given in Figure 3.2

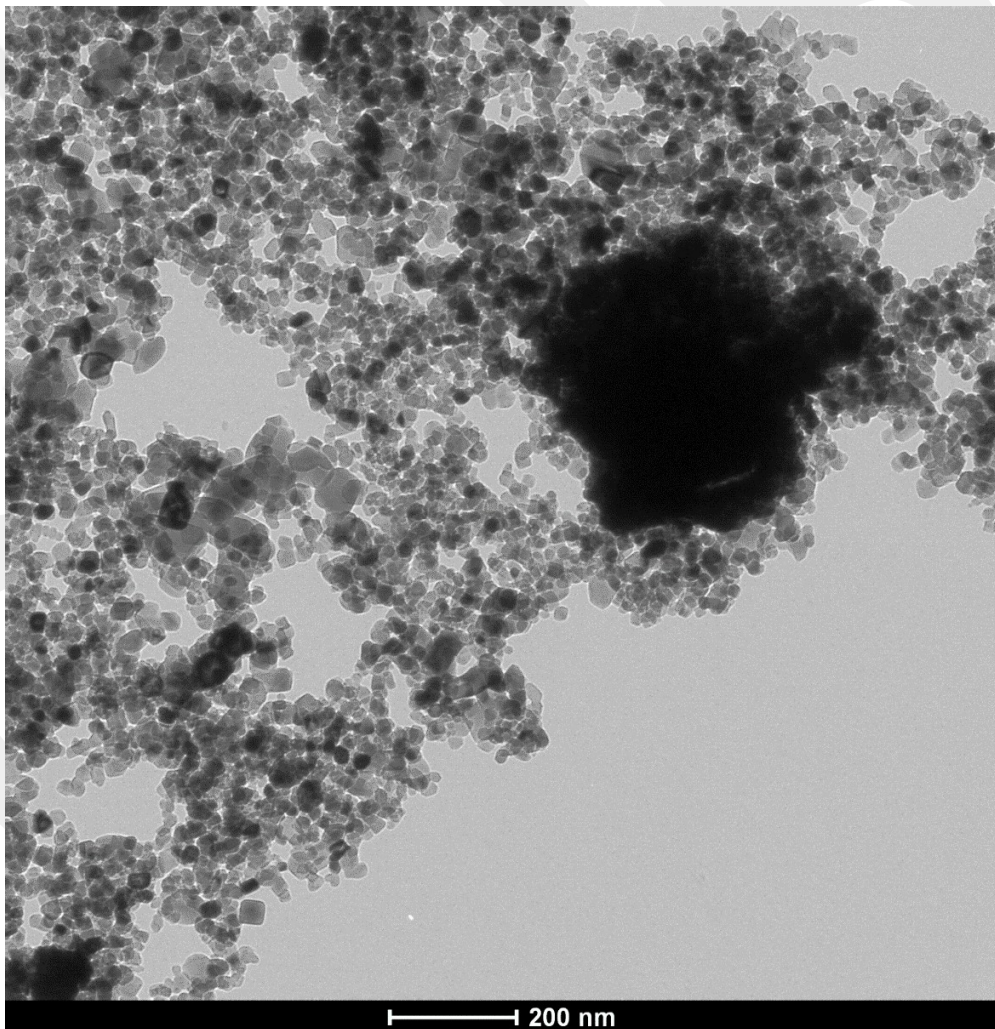


Figure 3.2: TEM image of TiO₂ nanoparticles (Degussa P25).

From the TEM image the particle size of TiO₂ nanoparticles was measured as 25±5 nm in size by using almost 50 particles which were selected randomly.

3.3 Characterization of PANI-TiO₂ Composite

The results by using SEM, and EDX measurements to characterize PANI-TiO₂ nanocomposite are given in Figure 3.3.

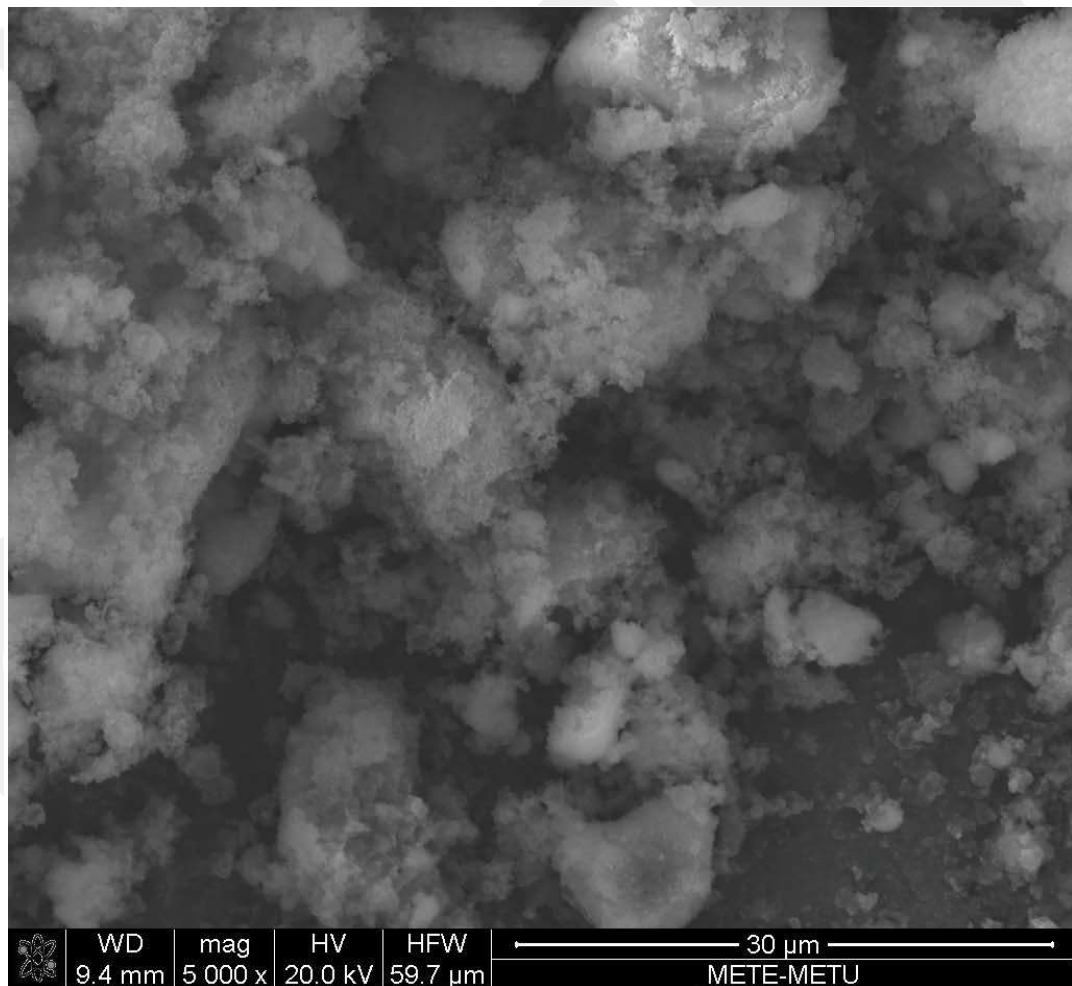


Figure 3.3: SEM image of PANI-TiO₂ composite material

From the image the presence of TiO₂ nanoparticles can be recognized easily when the SEM image is compared with the image of PANI. The particle has pluffy appearance much like clouds than non-uniform distribution of PANI (Figure 3.1).

The chemical composition of the resulting PANI-TiO₂ composite material was analyzed by EDX measurement (Figure 3.4).

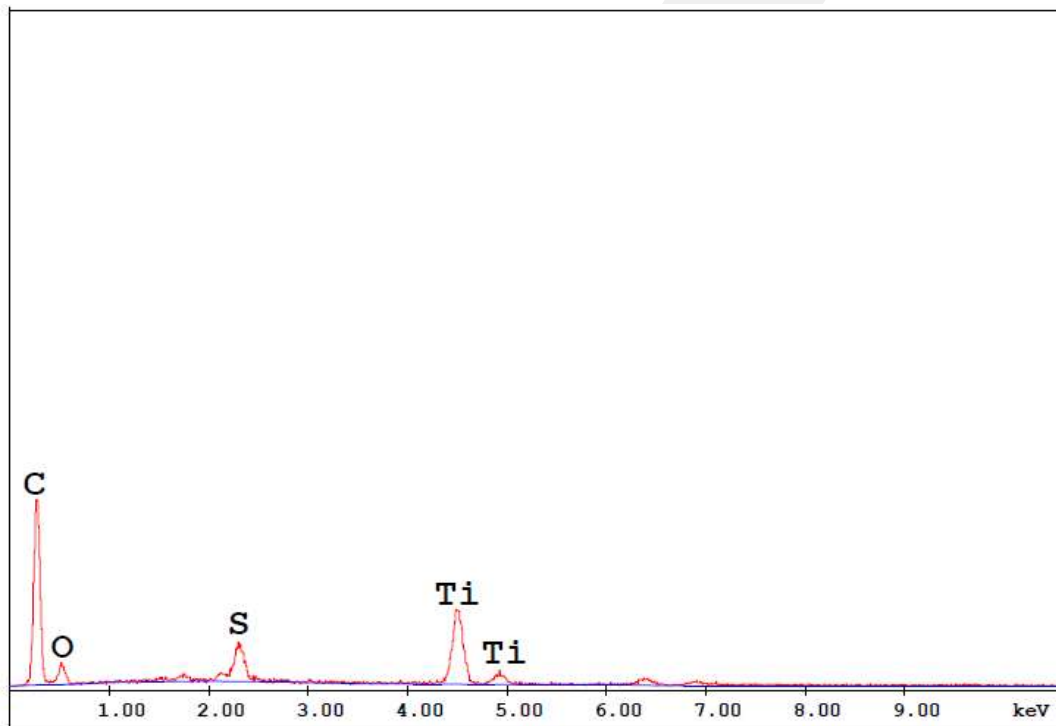


Figure 3.4: EDX pattern of PANI-TiO₂

The result showed that the PANI-TiO₂ composite material contains all the elements found in the composition.

3.4 Antibacterial Applications:

To test the anti-bacterial response of PANI-TiO₂ composite material first the pellets of the material was prepared. The antibacterial response of PANI was used as control and the Pen/ strep was used as reference. Since the PANI and PANI-TiO₂ composite materials were insoluble in many solvents that can be used for biological samples treatments, it was concluded that the pellets of the materials must be prepared. The pellets of PANI and PANI-TiO₂ NPs were prepared by using the press given in figure 2.3. The weights of the pellets of PANI and PANI-TiO₂ were measured as 0.0743 gr and 0.0989 gr respectively (Figure 3.5).



Figure 3.5: The pellets from PANI and (PANI-TiO₂) NPs.

The results of antibacterial effect of TiO₂ compared to the reference antibiotics Penicillin /Streptomycin for *E. coli* and *S. aureus* were shown in figure 3.6.

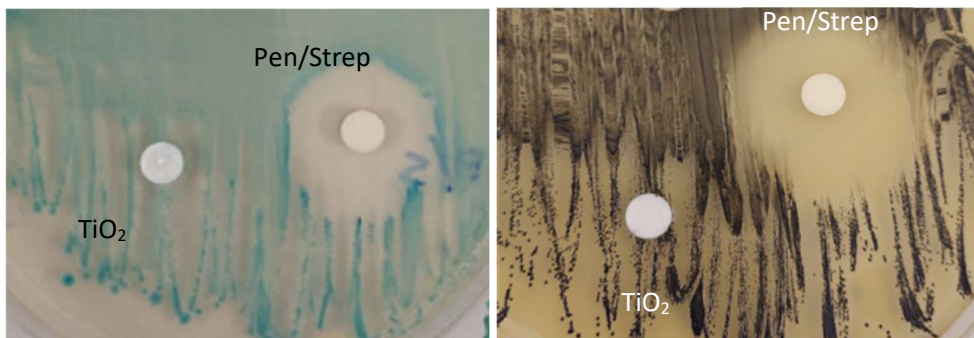


Figure 3.6: The effect of TiO_2 and antibiotics Penicilline /Streptomycin for *E. coli* (a) and *S. aureus* (b).

The antibacterial response was analyzed by the appearance of growth inhibition zone around antibiotic discs. As it is seen from the picture above the TiO_2 solution did not show any antibacterial response towards each of the bacteria; *E. coli* and *S. aureus*.

The pellets PANI and PANI- TiO_2 composite material were used to analyze their antibacterial response towards *S.aureus* (Figure 3.7.a)).The result showed that PANI has not growth inhibition effect on the bacteria by comparing the zone around the sample and Pen/strep reference. Since a very slight zone appeared around the PANI- TiO_2 pellet, it was concluded that PANI- TiO_2 composite material showe slight growth inhibition effect on *S.aureus*. (Figure 3.7.b)).

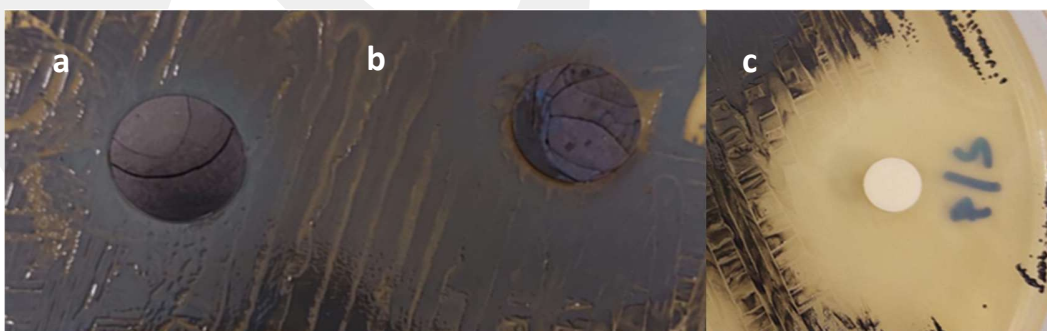


Figure 3.7: The effect of a) PANI b) PANI- TiO_2 c) Pen/strep on *S.aureus*

The antibacterial response of PANI and PANI-TiO₂ pellets on *E. coli* were analyzed and results showed that, PANI has no any antibacterial effect towards the bacteria due to not clear zone appeared around the pellet (Figure 3.8-a)). Since a very clear growth inhibition zone appeared around the pellet of PANI- TiO₂, the composite material was found to be very effective towards *E. coli* as antibacterial agent (Figure 3.8-b)). All these results were concluded by comparing the growth inhibition zone result of pen/strep (Figure 3.7 and 3.8-c)). From the antibacterial studies of PANI and PANI-TiO₂ pellets towards *S. aureus* and *E. coli*, it was concluded that PANI and TiO₂ did not show antibacterial effect on both type of bacteria but PANI-TiO₂ was found to be more effective against *E. coli* than *S. aureus* due the comparison of growth inhibition zone produced by two bacteria towards PANI-TiO₂ (Figure 3.7. and 3.8-b)).

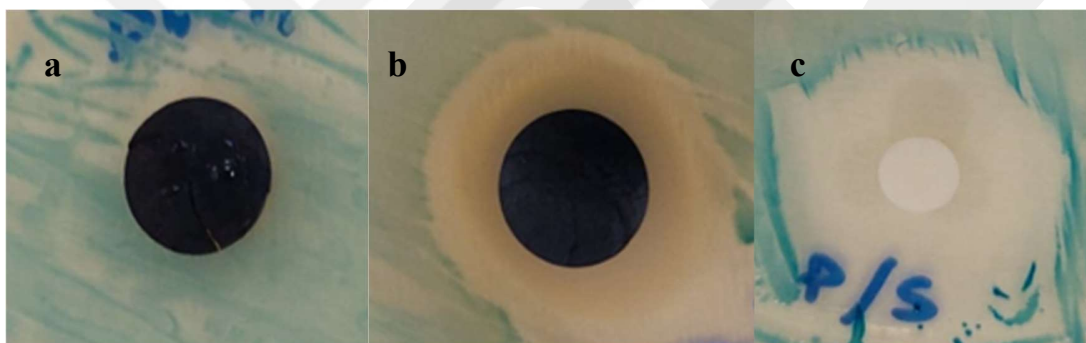


Figure 3.8: The effect of a) PANI b) PANI-TiO₂ c) Pen/strep on *E. coli*.

In the previous studies it has shown that UV irradiated TiO₂ showed antibacterial response on *E. coli*. Under UV light and dark condition, the bacteria would be contacted with TiO₂ nanoparticles because of the electrostatic interaction between them. When bacteria were covered by TiO₂ nanoparticles and the plates were put only in the dark most of the bacteria were killed after 60 minutes. It has been explained that the direct contact between TiO₂ nanoparticles and bacteria causes to destroy the bacterial membrane and kill them all. When the bacteria were exposed to UV light, together with TiO₂ nanoparticle incubation 99.9% of the bacteria were killed after 30 minutes. While only bacteria were exposed to UV light no changes in bacteria colony forming capacity were seen during two hours period of time. The results of this study showed that the direct contact between TiO₂

nanoparticles and bacteria without UV light is not sufficient to inactivate the bacteria. But with the presence of UV light, TiO₂ nanoparticles have ability to generate ROS, which has increased their antibacterial activity [39].

Another previous study explained that the antibacterial activity of TiO₂ nanoparticles by using *E. coli* with different concentration of TiO₂ nanoparticles ranging from 5 to 100 mg/ml. The results showed that, the number of bacteria decreases with an increasing concentration of TiO₂ nanoparticles through 14–16 hours. It has shown that for the concentration 50 and 100 mg/ml of TiO₂ nanoparticles no bacteria were survived. [39].

In another study in which the antibacterial activity of polyaniline and that of pure polyvinyl alcohol, (PVA) and polyaniline- PANI /polyvinyl alcohol PANI-PVA were compared. In the study *E. coli* and *S. aureus* were used as bacterial source under dark and visible light conditions. The study showed that after 24 hours, pure PVA did not show the antibacterial effect both under dark and visible light conditions both on the *E. coli* and *S. aureus*. When polyaniline PANI /polyvinyl alcohol PVA was used against *E. coli* and *S. aureus* the killing rate of the compound was found as 100% both in dark and visible light conditions due to the electrostatic force between PANI and the bacteria [20].

Bo Wen and coworkers in their study in 2019 showed the difference in the number of colonies for *E. coli* by using different coatings like acrylic coating, TiO₂ coating and PANI-TiO₂ composite coating, through 30 min sunlight illumination. It was found that the number of *E. coli* colonies on the plates were 130 for acrylic coating, 127 for TiO₂ coating and 38 for PANI-TiO₂ composite coating. From the result the antibacterial activity of TiO₂ coating was found to be 2.31% and the antibacterial activity of PANI-TiO₂ coating was 70.70% [43].

In another study, in which the antibacterial activity of polyaniline-TiO₂ was shown by using five bacteria (*Staphylococcus aureus*, *Bacillus megateriu*, *Bacillus subtilis*, *Escherichia coli* and *Bacillus cereus*) studied under visible light condition. In this study one step-interfacial polymerization was used to synthesise polyaniline-TiO₂ nanocomposites by using aniline monomer, tetrabutyl titanate, ammonium persulfate and HCl. The antibacterial test was performed by using different molar ratio of aniline to

tetrabutyl titanate. Among those, PANI/TiO₂ nanocomposites with 3:2 molar ratio showed the best antibacterial property on *S. aureus* and *E. coli* [24].

In our study the chemical polymerization method has been used to prepare PANI and PANI-TiO₂ composite because this method is the most common method in most of the literature review, because it is simple, not take much time and this method does not change the nanostructure of TiO₂ that was shown in the SEM image of PANI-TiO₂. In the composite material the presence of TiO₂ nanoparticles can be recognized easily when compared with the SEM image of PANI. For antibacterial analysis, pellets of PANI and PANI-TiO₂ NP_s were prepared. Because these NP_s are insoluble in many solvents like acidic or organic since these solvents have ability to kill bacteria, they will affect the antibacterial test results. In this study the pellets of PANI and PANI-TiO₂ were used for the first time as their advantages of keeping all particles together and easily used them several times.

When this study was compared with the previous studies especially on the basis of antibacterial study part, the previous studies were mainly depending on either coating the agar first then inoculating bacterial colonies or distributing the bacterial colonies on the agar first and then applying PANI-TiO₂ composite on the bacteria.

In this study the bacteria were inoculated on the agar plate and then the pellets were applied on to it. Before putting on the agar palte the pellets were exposed to UV light for 60 min.

CONCLUSION

In this study, was used commercially available titanium dioxide nanopowder, and we prepared polyaniline (PANI) by using the chemical polymerization of aniline with ammonium persulfate (APS), after that, preparation of PANI-TiO₂ nanocomposite material by polymerization were done. The antibacterial response of PANI and PANI-TiO₂ by using *E. coli* and *S. aureus* were completed. We concluded from the results of this study that TiO₂ nanoparticles have antibacterial activity because when used TiO₂ molecules and PANI don't have antibacterial activity on each types of bacteria but PANI-TiO₂ have antibacterial activity on *E. Coli* and *S. aureus* because in this composite we used TiO₂ NPs. PANI-TiO₂ was found to be more effective against *E. coli* than *S. aureus* because *E. coli* gram negative has a thin layer of peptidoglycan and *S. aureus* gram positive has a thick layer of peptidoglycan so, *S. aureus* has higher resistance than *E. coli*. From this study PANI- TiO₂ NPs can be a good coating material for its anti-bacterial activity to reduce diseases caused by bacterial infection.

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