

T.R.
GEBZE TECHNICAL UNIVERSITY
INSTITUTE OF BIOTECHNOLOGY

**SYNTHESIS OF ZnO NANOPARTICLES VIA SOL-GEL
METHOD FOR ITS ANTIBACTERIAL PROPERTIES**

BÜŞRA EREN
A THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF SCIENCE
INSTITUTE OF BIOTECHNOLOGY

GEBZE
2022

T.R.
GEBZE TECHNICAL UNIVERSITY
INSTITUTE OF BIOTECHNOLOGY

SYNTHESIS OF ZnO NANOPARTICLES
VIA SOL-GEL METHOD FOR ITS
ANTIBACTERIAL PROPERTIES

BÜŞRA EREN

A THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF SCIENCE
INSTITUTE OF BIOTECHNOLOGY

THESIS SUPERVISOR
ASST. PROF. ZEHRA BANU BAHSI ORAL

GEBZE
2022

T.C.
GEBZE TEKNİK ÜNİVERSİTESİ
BİYOTEKNOLOJİ ENSTİTÜSÜ

ANTİBAKTERİYEL ÖZELLİKLERİ İÇİN
SOL-JEL YÖNTEMİ İLE ZnO
NANOPARTİKÜLLERİNİN SENTEZİ

BÜŞRA EREN
YÜKSEK LİSANS TEZİ
BİYOTEKNOLOJİ ENSTİTÜSÜ

DANIŞMANI
DR. ÖĞR. ÜYESİ ZEHRA BANU BAHSİ ORAL

GEBZE

2022

GTÜ Biyoteknoloji Enstitüsü Yönetim Kurulu’nun 08.06.2022 tarih ve 2022/13 sayılı kararıyla oluşturulan jüri tarafından 16/06/2022 tarihinde tez savunma sınavı yapılan Büşra EREN’ in tez çalışması Biyoteknoloji Anabilim Dalında YÜKSEK LİSANS tezi olarak kabul edilmiştir.

JÜRİ

ÜYE

(TEZ DANIŞMANI) : Dr. Öğr. Üyesi Zehra Banu BAHŞİ ORAL

ÜYE

: Prof. Dr. Işıl AKSAN KURNAZ

ÜYE

: Doç. Dr. Mustafa Burak TELLİ

ONAY

Gebze Teknik Üniversitesi Biyoteknoloji Enstitüsü Yönetim Kurulu’nun
...../...../..... tarih ve/..... sayılı kararı.

İMZA/MÜHÜR

SUMMARY

Zinc oxide nanoparticles (ZnO NPs) have a promising and remarkable potential for many areas of technology due to their unique properties.

In this study, ZnO NPs were prepared by the sol-gel method to demonstrate the influence of the particle size of ZnO NPs on their optical properties and also antibacterial activity of ZnO NPs. 0.2M ZnO sol solution was prepared to produce NPs. The pH was adjusted to 12 with a 1.0M NaOH solution. Two different ZnO NPs were obtained by washing 3 times and 6 times and calcination temperature of 800°C and 450°C, respectively.

The microstructural characterization of the ZnO NPs was determined by X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). The optical properties were determined using the UV-Vis-NIR spectrophotometer. In XRD analysis, both samples have a hexagonal wurtzite ZnO structure (JCPDS No. 00-036-1451) and the average NP sizes were calculated at 52.65 nm at 800°C, with 3 washes and 25.11 nm at 450°C with 6 washes from XRD data. The results showed that the crystallite size of the particles was decrease when the calcination temperature was lowerred and the washing time was increased. It was observed that the optical properties of the ZnO NPs were significantly affected by the size of the NPs. The antibacterial activity of ZnO-800°C and ZnO-450°C NPs was investigated against three different bacterial strains using two different. Promising results were obtained with ZnO-450°C NPs exposed to UV irradiation on Gram-positive bacteria *S. aureus*.

Key Words: ZnO NPs, sol-gel, UV-VIS-NIR spectrophotometer, antibacterial activity.

ÖZET

Çinko oksit nanoparçacıkları (ZnO NP'ler), benzersiz özellikleri nedeniyle teknolojinin birçok alanı için umut verici ve dikkate değer bir potansiyele sahiptir.

Bu çalışmada, ZnO NP'lerin partikül boyutunun optik özellikleri ve ayrıca ZnO NP'lerin antibakteriyel aktivitesi üzerindeki etkisini göstermek için sol-jel yöntemiyle ZnO NP'ler hazırlanmıştır. NP'leri üretmek için 0.2M ZnO sol çözeltisi hazırlanmıştır. 1.0M NaOH solüsyonu ile pH 12'ye ayarlanmıştır. Sırasıyla 800°C ve 450°C kalsinasyon sıcaklığı ile 3 kez ve 6 kez yıkama yapılarak iki farklı ZnO NP elde edilmiştir.

ZnO NP'lerin mikroyapısal karakterizasyonu, X-ışını kırınımı (XRD), taramalı elektron mikroskobu (SEM) ve enerji dağılımlı X-ışını spektroskopisi (EDS) ile belirlenmiştir. Optik özellikler, UV-Vis-NIR spektrofotometre kullanılarak belirlenmiştir. XRD analizinde, her iki numune de altıgen wurtzite ZnO yapısına sahiptir (JCPDS No. 00-036-1451) ve ortalama NP boyutları, XRD verilerinden 800°C'de 52.65 nm'de 3 yıkama ile ve 450°C'de 25.11 nm'de 6 yıkama ile hesaplanmıştır. Sonuçlar, kalsinasyon sıcaklığı düşürüldüğünde ve yıkama süresi artırıldığında partiküllerin kristalit boyutunun azaldığını göstermiştir. ZnO NP'lerin optik özelliklerinin, NP'lerin boyutundan önemli ölçüde etkilendiği gözlenmiştir. ZnO-800°C ve ZnO-450°C NP'lerinin antibakteriyel aktivitesi, üç farklı bakteri suşuna karşı iki farklı yöntem kullanılarak araştırılmıştır. Gram pozitif bakteri *S. aureus* üzerinde UV ışınlarına maruz bırakılan ZnO-450°C NP'ler ile umut verici sonuçlar elde edilmiştir.

Anahtar Kelimeler: ZnO NP'ler, sol-jel, UV-VIS-NIR spektrofotometre, antibakteriyel aktivite.

ACKNOWLEDGEMENTS

First of all, I would like to thank with a great sincerity to Asst. Prof. Zehra Banu BAHSI ORAL for her supervision, guidance and advice during the period of my research.

I would like to thank Assoc. Prof. Dr. Mge İŞLETEN HOŞOĞLU for opening her laboratory and for her kindness and also Cansu YAY her help and support on antibacterial activity.

I would like to thank my dear laboratory colleagues Gamze SEKİCEK, Seda KOL, Onur Alp AKSAN and Mehmet SEZER, who always shared their help and advice with me in my experimental studies.

I would like to thank Ahmet NAZIM ve Adem ŞEN for the ZnO nanoparticle characterization.

Above all, I thank all members of my family, especially my mother Oya EREN, my father Mustafa EREN and my dear brother Ömer EREN, who have never spared their support, love and interest throughout my life.

TABLE OF CONTENTS

	<u>Page</u>
SUMMARY	v
ACKNOWLEDGEMENTS	vii
TABLE of CONTENTS	viii
LIST OF ABBREVIATIONS AND ACRONYMS	x
LIST OF FIGURES	xiii
LIST OF TABLES	xiv
1. INTRODUCTION	1
1.1. Nanotechnology and Biotechnology	1
1.2. Classes of Nanomaterials	3
1.3. Zinc Oxide (ZnO) Nanoparticles	6
1.4. Applications of ZnO Nanoparticles	9
1.4.1. Agricultural Applications	10
1.4.2. Cosmetic Applications	10
1.4.3. Biomedical Applications	11
1.5. Fabrication Techniques of ZnO Nanoparticles (NPs)	15
1.5.1. Physical Fabrication	16
1.5.2. Biological Fabrication	17
1.5.3. Chemical Fabrication	18
1.6. Sol-Gel Method	19
1.6.1. Mechanism of Sol-Gel Fabrication	21
1.6.2. Mechanism of Hydrolysis and Condensation	22
1.6.3. Mechanism of Gelation	23
1.6.4. Mechanism of Aging, Drying	23
1.6.5. Mechanism of Calcination	23
2. MATERIALS AND METHODS	25
2.1. Schematic Representation of the ZnO Nanoparticles Synthesised by the Sol-gel Method	25

2.2.	Precursors	25
2.3.	Synthesis of ZnO Nanoparticles	26
2.4.	Characterization Techniques	29
2.4.1.	X-ray Diffraction (XRD)	29
2.4.2.	Scanning Electron Microscope (SEM)	29
2.4.3.	Energy Dispersive X-Ray Spectroscopy (EDS)	29
2.4.4.	UV-VIS-NIR Spectrophotometer	30
2.5.	Antibacterial Activity of ZnO NPs	30
2.5.1.	Preparation of Bacterial Culture and Stock Solutions	30
2.5.2.	Assessment of Antibacterial Activity of ZnO NPs by Agar Well Diffusion Method and Agar Disc Diffusion Method	30
3.	RESULTS	33
3.1.	Microstructural and Optical Properties of ZnO NPs Produced by Sol-Gel Method	33
3.1.1.	XRD Analysis of ZnO NPs	33
3.1.2.	SEM Analysis of ZnO NPs	36
3.1.3.	Energy Dispersive X-Ray Spectroscopy (EDS) Analysis of ZnO NPs	37
3.1.4.	UV-VIS-NIR Spectrophotometer Analysis of ZnO NPs	37
3.2.	Antimicrobial Activity of ZnO NPs	39
4.	DISCUSSION AND CONCLUSION	46
	REFERENCES	49
	BIOGRAPHY	55

LIST OF ABBREVIATIONS AND ACRONYMS

Abbreviations

<u>and Acronyms</u>	<u>Explanations</u>
°C	: Celcius
m	: Meter
nm	: Nanometer
λ	: Wavelength
M	: Molarity
μ L	: Microliter
g	: Gram
mL	: Milliliter
mm	: Millimeter
eV	: Electronvolt
pH	: Power of Hydrogen
0D	: Zero-dimensional
1D	: One-dimensional
2D	: Two-dimensional
3D	: Three-dimensional
NMs	: Nanomaterials
NPs	: Nanoparticles
AD	: Atopic dermatitis
ALD	: Atomic layer deposition
Ag	: Silver
Ag ₂ O	: Silver oxide
Ag-NPs	: Silver Nanoparticles
Au	: Gold
Au-NPs	: Gold Nanoparticles
<i>B. cereus</i>	: Bacillus cereus
CaO	: Calcium oxide
CB	: Conduction band
CH ₃ OH	: Methanol

Cu	: Copper
CuO	: Copper oxide
CVD	: Chemical vapor deposition
DNA	: Deoxyribonucleic Acid
DM	: Diabetes mellitus
<i>E. coli</i>	: Escherichia coli
EDS	: Energy dispersive X-Ray spectroscopy
FWHM	: Full width at half maximum
h	: Hour
H ₂ O ₂	: Hydrogen peroxide
H ₂ O	: Deionised water
MBE	: Molecular beam epitaxy
min	: Minute
MgO	: Magnesium oxide
MONPs	: Metal oxide nanoparticles
NaOH	: Sodium hydroxide
OH	: Hydroxyl
PLD	: Pulsed laser deposition
PVD	: Physical vapor deposition
ROS	: Reactive oxygen species
<i>S. aureus</i>	: Staphylococcus aureus
SEM	: Scanning electron microscopy
Si	: Silicon
SP	: Spray pyrolysis
SPR	: Surface plasmon resonance
Ti	: Titanium
TiO ₂	: Titanium dioxide
US FDA	: The United States Food and Drug Administration
UV	: Ultraviolet
UV-VIS-NIR	: Ultraviolet-Visible (UV Vis) spectrophotometer
VB	: Valence band
VLS	: Vapor-solid-liquid deposition
ZnO	: Zinc oxide

ZnO NPs : Zinc oxide nanoparticles

$\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$: Zinc acetate dihydrate

QDs : Quantum dots

XRD : X-ray diffraction



LIST OF FIGURES

<u>Figure No:</u>	<u>Page</u>
1.1: Comparison of size ranges with nano-sized scales.	2
1.2: Classification of nanomaterials according to different criteria.	4
1.3: Crystallographic structures of ZnO nanocrystals: hexagonal wurtzite (A) and cubic zincblende (B).	7
1.4: Universal consumption of ZnO.	9
1.5: Schematic illustration of antibacterial activity of ZnO NPs.	14
1.6: The fabrication techniques of ZnO nanoparticles.	16
2.1: A schematic view of ZnO nanoparticle syntheses via sol-gel.	25
2.2: Flowchart of ZnO NPs (ZnO-800°C).	27
2.3: Flowchart of ZnO NPs (ZnO-450°C).	28
3.1: XRD pattern of ZnO-800°C NPs.	34
3.2: XRD pattern of ZnO-450°C NPs.	34
3.3: SEM images of ZnO NPs synthesized by sol-gel method: a) ZnO-800°C b) ZnO-450°C.	36
3.4: EDS spectra for ZnO NPs a) ZnO-800°C b) ZnO-450°C.	37
3.5: Reflectance spectra of ZnO NPs calcined at 800°C and 450°C.	38
3.6: Band gap energy of ZnO NPs a) ZnO-800°C b) ZnO-450°C.	39
3.7: Comparison of bacterial activity of ZnO-800°C and ZnO-450°C on a) B. cereus b) E. coli c) S. aureus in the 1st experiment.	40
3.8: Comparison of bacterial activity of ZnO-800°C and ZnO-450°C on a) B. cereus b) E. coli c) S. aureus in the 2nd experiment.	41
3.9: Comparison of bacterial activity of ZnO-800°C and ZnO-450°C on B. Cereus in the 3rd experiment.	43
3.10: Comparison of bacterial activity of ZnO-800°C and ZnO-450°C on E.coli in the 3rd experiment.	44
3.11: Comparison of bacterial activity of ZnO-800°C and ZnO-450°C on S.aureus in the 3rd experiment.	45

LIST OF TABLES

<u>Table No:</u>	<u>Page</u>
1.1: Physical properties of ZnO nanoparticles.	8
2.1: Chemical formula of substances for NPs.	26
3.1: Average crystallization size of the ZnO-800°C and ZnO-450°C.	35

1. INTRODUCTION

1.1. Nanotechnology and Biotechnology

Nanotechnology, which has emerged in the 21st century and is of great importance, is used in the design, construction, research and use of systems with nanoscale [1]. Definitions of nanotechnology have diversified and evolved over time. Although its original definition is "technology at the nanoscale," it can also be referred to as "atomically precise technology" or "technology with atomic precision" [2]. Nanotechnology is a science that works with materials at the nanoscale (1-100 nm) [1], [2]. The nano size is on the scale of 1 billionth of a meter (i.e. 10^{-9} m = 1 nm) [3]. Nanotechnology can be used to manipulate structures at the atomic and molecular level [3]. Thanks to nanotechnology, the theory of nanoscience can be transformed into effective applications with new features such as biocompatibility, bioconjugation and ease of use, as well as a large surface-to-volume ratio, uniform particle size, adjustable optical, magnetic and electronic properties, improving the quality of life [1], [3]. Nanotechnology plays an important role in innovative methods of manufacturing new products, formulating new materials and chemicals, and reducing material and energy consumption and environmental impact [2]. In addition, it has become an important point in diagnosis, distribution of medicines, sunscreens, antimicrobials, disinfectants, elimination of waste products (zero waste) and more environmentally friendly energy production [4]. In this context, this technology offers the possibility to create a more sustainable solution to overcome the problems [2]. Nanotechnology has the potential to combine almost all branches of science, such as physics, chemistry, engineering, electronics, computer science, materials science, biology, and medicine, and produce remarkable results [1], [2], [5].

Nanotechnology is the most widespread technology of the 21st century and the result of years of human research into "nano" dimensions [1], [2]. The Greek term "nano", meaning "dwarf", refers to 1/1000 of a micron [2]. Figure 1.1 shows the size ranges for micro- and nano-scale objects [2].

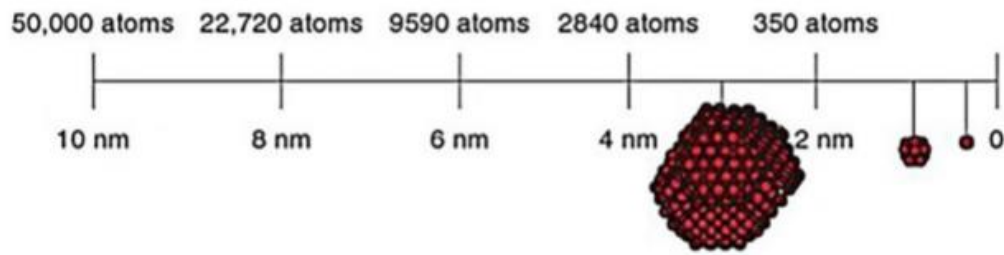


Figure 1. 1: Comparison of size ranges with nano-sized scales.

In 1959, Richard Feynman laid the foundation for nanoscale research and development, and perhaps even smaller, by using the phrase "There's plenty of room at the bottom" in a speech [6]. In the 1960s, Richard Feynman was awarded the Nobel Prize for this new window of science [7]. In 1974, fifteen years after Feynman introduced the concept of nanotechnology, Prof. Norio Taniguchi defined nanotechnology as "basically consisting of the processing of separation, consolidation and deformation of materials by an atom or a molecule." [1], [3], [7].

Biotechnology is another scientific research discipline that offers the possibility of understanding complex biological systems and using the information gained for industrial production [1]. Applications in biotechnology are closely related to the physical, chemical and pharmacological properties of particles, such as chemical composition, crystalline structure, surface structure, adsorption properties and solubility, and low self-toxicity [8]. Nanobiotechnological developments in biotechnology will have a great impact in the fields of science and technology [9]. Nanobiotechnology is a branch that uses nano-level principles and techniques that combine biotechnology and nanotechnology to integrate with bio-systems to obtain cutting-edge materials and tools for both diagnosis and treatment [10], [11]. Nanostructures used in nanobiotechnology have high control properties [9]. Since the field of nanobiotechnology consists of the intersection of materials science, chemistry, physics and biology, there are many research studies published in this field [9], [10]. As a multidisciplinary approach, nanobiotechnology is a promising development stage for many research areas. Such developments in biotechnology will have a great impact in the fields of science and technology [9].

1.2. Classes of Nanomaterials

Nanomaterials (NMs) are defined as a collection of interconnected atoms with an average radius of 1 to 100 nm, usually consisting of 10^3 – 10^5 atoms [2], [12]. The mobility, chemical and physical properties of NMs in different systems are determined by the size, shape and composition of their crystal structures [12]. In nanobiotechnology, it is of great importance for the development of various applications as well as for the evaluation/comparison of cytotoxicity between different nanomaterials or studies [13]. Applications of nanomaterials in the fields of medicine and biotechnology are often used where traditional methods fail [12], [13]. Thanks to the new properties of nanomaterials, which are becoming more widespread as technology advances, complex biological functions are being given the opportunity to interact in new ways. [14]. In addition, nanomaterials offer remarkable solutions to traditional problems and their advanced properties are integrated into the production of cosmetics, biotechnological and pharmacological products, drugs or food additives. [13].

NMs are classified according to many different criteria (Fig. 1.2) [15]. These materials are generally characterized by their dimensionality (Zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D) and three-dimensional (3D) nanomaterials), chemical composition (inorganic, organic and mixed organic-inorganic nanomaterials) and uniformity (isometric and inhomogeneous or dispersed and agglomerated nanomaterials) [12], [15]. Considering their chemical composition, NMs can also be classified into other types, primarily single constituent nanoparticles (NPs) and nanocomposites [15].

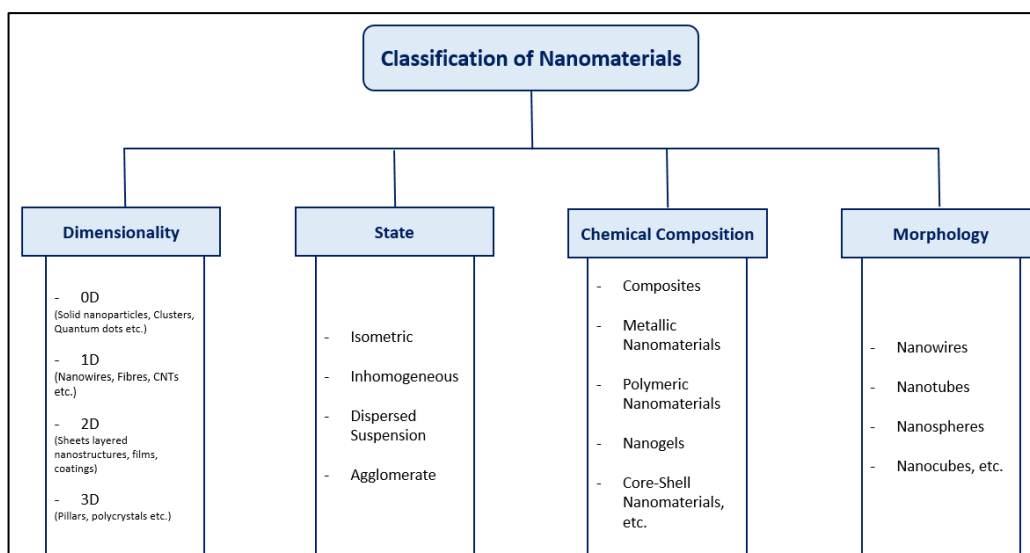


Figure 1. 2: Classification of nanomaterials according to different criteria.

Considering all these classifications, it is possible to classify NMs in five main groups in general: carbon NMs, metal oxide NPs, zero-valence metal NPs, quantum dots (QDs) and dendrimers [12].

Carbon NMs: A new molecular form of carbon (C₆₀) was discovered by Kroto et al. in 1985, and subsequently numerous studies have been conducted to develop carbon-based NMs [12], [16]. Carbon-based NMs play a key active role in a variety of disciplines with unique properties such as good chemical versatility and biocompatibility in addition to optical and electronic properties [15], [16]. These types of nanomaterials shed light on studies in areas such as conductive coatings, water treatment systems, orthopedic implants, adhesives, and sensors. [12], [16]. Graphene, a newer form of carbon, is very strong and conductive. For this reason, major microchip manufacturers believe in the potential of graphene as a base material for computers [12], [15].

Zero-valence Metals: Zero-valence metals are produced by reduction of solutions of metal salts [12]. The zero-valent metals were introduced by Sweeney and Fischer as the means of choice for abiotic dehalogenation reactions [17]. Control of its physical properties is achieved by changing the reduction conditions. Iron NPs, which are important for environmental applications of zero-valent metals, have been preferred for water purification, sediment removal, and soil improvement [12]. In the

production of consumer products using NPs at zero cost, silver NPs (Ag-NPs) are used due to their bactericidal properties, while gold NPs (Au-NPs) are also used in some bioanalytical and medical applications [12].

Quantum Dots (QDs): QDs are described as uniform small semiconductor nanocrystals that have a reactive core and control their optical properties [12], [18]. Strict control of crystal size and shape has implications for the conductivity and energy release to which QDs are made [12]. These materials are used in medical imaging, cancer treatment, skin care, solar cell manufacturing, photonics, and telecommunications [12], [18].

Dendrimers: Dendrimers have been described as branched, spherical, monodisperse, radially symmetric, and well-defined homogeneous three-dimensional (3D) nanostructures [12], [19]. Their formation, the chemical composition of the core, the internal branching, and the surface functions determine the size, shape, and reactivity of the dendrimers [12]. They consist of only one kind of branching segments around a small molecule or a linear nucleus [19]. Dendrimers obtained with gradually increasing dimensions from 1 nm to over 10 nm have applications such as drug delivery systems, polymeric materials, chemical sensors and modified electrodes, and DNA transfection agents [12].

Metaloxide NPs: Metal oxide nanoparticles (MONPs) are one of the commonly used nanomaterials [12]. Nanostructured metal oxides, which have been extensively studied, are described as a promising area of research in technology [20]. For many years, bulk materials such as zinc oxide (ZnO), titanium dioxide (TiO₂), aluminum oxides (Al₂O₃) and iron oxides were preferred by researchers, but nowadays the nanoscale structures of these materials have conquered the market and are included in various consumer products [12].

MONPs are used in various fields due to their important properties, e.g. as bactericidal agents, for the destruction of carcinogenic cells and for the delivery of drugs [21], [22]. Metal nanoparticles are developing in the field of nanoscience due to conditions such as optimization, optics, catalysis, and media recording [22]. In addition, such nanoparticles show promising progress in areas such as medicine, agriculture, information technology, biomedicine, optics, electronics, catalysis,

environment, energy, and sensor technology, as well as in materials chemistry research [20].

Metal oxides can have various geometric shapes with electronic properties that allow them to have metallic, semiconducting, or insulating properties [23]. In addition, they have unique properties such as low toxicity, high stability, simple preparation steps, easy engineering to achieve desired size, shape and porosity, no change in swelling, high stability, high selectivity, easy integration into hydrophobic and hydrophilic systems, easy functionalization by different molecules and negative surface charge [24], [20]. The antimicrobial activity of metal nanoparticles (silver (Ag), silver oxide (Ag₂O), titanium dioxide (TiO₂), silicon (Si), copper oxide (CuO), zinc oxide (ZnO), Au, calcium oxide (CaO) and magnesium oxide (MgO) etc.) has been demonstrated [25]. Moreover, the appropriate method to prepare MONPs with tunable structural parameters and expected properties such as monodispersity should include the optimization of defects in size, shape, morphology, and crystal structure [20], [21].

1.3. Zinc Oxide (ZnO) Nanoparticles

Nanoscale materials such as "nanoparticles" exhibit novel properties and functions that are very different from mass scale objects [14]. NPs have become a promising area of research as they bridge the gap between bulk materials and atomic or molecular structures [7]. Due to their unique properties such as small size, surface compatibility, improved solubility and multifunctionality, nanoparticles are opening up many new areas of research for researchers [14].

In recent years, rapid progress has been made in the biotechnology of NPs, supported by promising multiple applications [26]. The advent of nanotechnology and biotechnology has expanded the use of NPs and opened up new fields [1]. Nanotechnology is a rapidly developing field that increases the potential for diagnosis and treatment of diseases. Therefore, it is essential for the environmental health industry to create a harmless system for NP synthesis [1], [27]. As a result of nanoparticle size reduction, there is an increase in surface and interfacial atoms, strains or stresses, and adjacent structural distortions. The size of the nanoparticle affects its magnetic, conductive, chemical and electronic properties. As a result of this situation,

the observed changes in cell parameters can be attributed to size-related structural changes in nanoparticles such as CuO, ZnO, SnO₂, Al₂O₃, MgO, ZrO₂, AgO, TiO₂, CeO₂ [21].

ZnO, one of the most important semiconductor metal oxides for bulk and nano-sized applications, has properties such as ultraviolet (UV) light blocking, photocatalytic and antibacterial [23], [28]. These metal oxides occur in two crystalline forms (Figure 1.3), a zinc blend and wurtzite, which is more stable and has a refractive index of 2.3 to 2.0 [28], [29]. Furthermore, ZnO NP is a nanostructure of semiconductor materials belonging to the space group P6₃ [22].

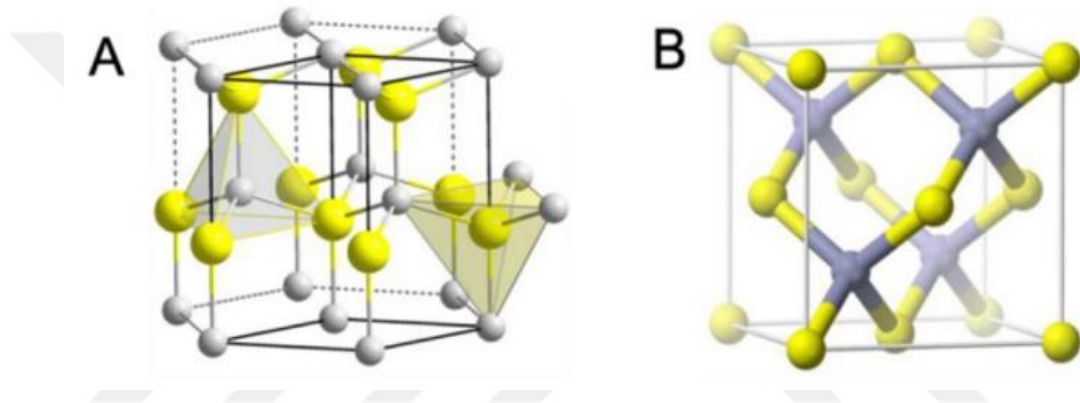


Figure 1. 3: Crystallographic structures of ZnO nanocrystals: hexagonal wurtzite (A) and cubic zincblende (B).

ZnO NPs show several advantageous properties such as good light scattering, high transparency, non-toxicity, natural occurrence and good photocatalysis [22]. ZnO NPs, which exhibit bactericidal activity against Gram-positive and Gram-negative bacteria and spores, resistant to high temperatures and high pressure, can be used for textiles and surfaces that come into contact with the human body due to their safety and compatibility with human skin [25]. ZnO NPs are cheaper and less toxic than other MONPs and have unique biomedical applications (e.g., anticancer, drug delivery, antibacterial, wound healing, and bioimaging) [28].

Table 1.1 represents physical properties of ZnO NPs. ZnO nanoparticles are II-VI semiconductor structures that have a broad bandgap energy of 3.3 eV and a high excitation energy of 60 eV [23], [30], [31]. Therefore, due to their energy values, these NPs can tolerate large electric fields, high temperatures and high powers [30]. ZnO, which functions in both electronic and optoelectronic applications with its wurtzite-

like structure, is widely used in applications such as gas sensors and varistors [21]. ZnO usually has a wurtzite crystal structure on the nanoscale and its lattice parameters are $a=0.3296$ nm and $c=0.52065$ nm. [30], [32].

Along the c-axis (in the direction of $\langle 0001 \rangle$), the wurtzite structure is a stack of alternating positively and negatively charged planes of zinc and oxygen atoms [33]. With the coordination of zinc and oxygen atoms, the tetrahedral structure comes into focus [30], [33]. Polarization occurs due to the non-centrosymmetric property of the wurtzite structure, and ZnO can be defined as a piezoelectric material with large piezoelectric coefficients [33]. In summary, the crystal structure of ZnO NPs gives rise to their piezoelectric properties, which makes them advantageous for acoustic wave resonators and acoustic-optic modulators. Moreover, the highest tensor among all semiconductors is associated with its centrosymmetric structure, which leads to a large electromechanical coupling capability [30].

Table 1. 1: Physical properties of ZnO nanoparticles.

Physical Appearance	White, colorless, odorless solid
Crystalline Structure	Wurtzite
Molecular Weight	81.38 g/mol
Electron Effective Mass	60
Bandgap Energy	3.3 eV
Melting Point	1.975 °C
Excitation Energy	60 eV
Density	5.47 g/cm ³
Standard Molar Entropy	49.9 J.K ⁻¹ mol ⁻¹
Standard Enthalpy of Formation	-348.0 kJ/mol
Space Group	$C_{6v}^4 - P6_3mc$

It has been reported that the UV spectrum of ZnO NPs shows the surface plasmon resonance (SPR) peak at 346 nm, which is a characteristic of these nanostructures [29]. ZnO NPs exhibit high photocatalytic properties that enhance their antimicrobial activity [25]. In addition, it can be used to destroy organic compounds in paints, losing UV absorbing ability due to photocatalysis of ZnO by visible and UV light [23]. As a result, the interest of scientific researchers is focused on ZnO NPs that are stable under UV radiation [22].

ZnO exhibits unique optical and piezoelectric properties as well as important electrical properties. As a semiconductor II-IV, ZnO generally exhibits n-type conductivity with high carrier mobilities of $100 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. In the context of the optoelectronic properties of ZnO, a thorough understanding of the band structure and the optical and electrical properties is crucial [32].

There are also some lesser known properties of ZnO, such as high radiation hardness, high thermal conductivity, and strong nonlinear optical behavior, which make it suitable for aerospace applications, thermoelectric generators, and nonlinear optical elements [32], [34], [35]. Due to all these novel and dominant properties, ZnO has great potential for nanoscience and nanotechnology research and application [29].

1.4. Applications of ZnO Nanoparticles

Due to the innovative electrical, optical and mechanical properties of nanocrystalline semiconductors, their use in optoelectronic devices, drug delivery devices, electrochromic devices and various applications such as cosmetics is becoming increasingly common [36]. ZnO NPs in this class are also attracting attention with their increasing applications and have a wide range of uses from medicine to agriculture, from paints to chemicals, from tires to ceramics, from solar cells to light-emitting devices and sensors [37], [38]. The graph in Figure 1.4 shows the numerical data of the universal consumption of ZnO [37].

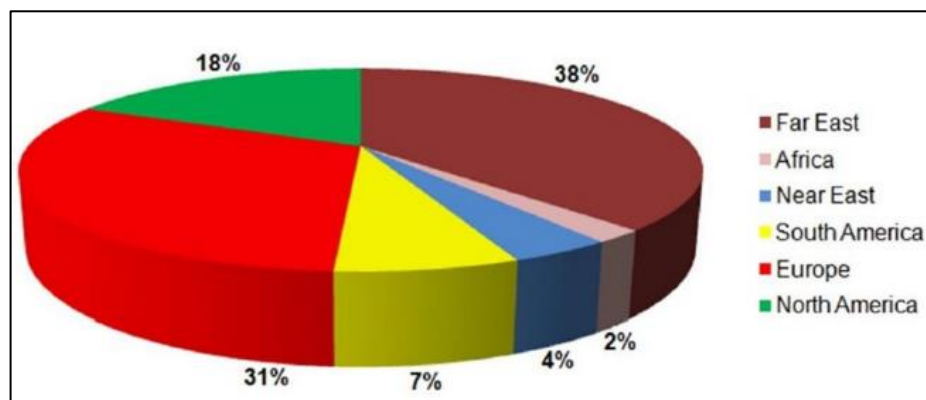


Figure 1. 4: Universal consumption of ZnO.

1.4.1. Agricultural Applications

Due to their eco- friendly properties, ZnO NPs are used in agricultural applications and have the potential to enhance food growth [37], [39]. ZnO NPs are not only a source of slow-release Zn that has a nutritive effect on plants, but are also used in laboratory, greenhouse, and field studies of crop plants because of their UV-protective and antimicrobial properties [39]. Studies in this field have shown that seeds obtained with different concentrations of ZnO NP improve seed dispersal, seed vigour and plant growth [37]. Unlike some other engineered nanoparticles, readily soluble ZnO NPs were observed to play an active role in growing parts of root stems and seeds [37], [39]. In addition, these NPs have the ability to reduce plant exposure to environmental stresses such as drought, temperature, metals, metalloids, and salt [39]. It has been reported that enzymatic activity increases rapidly when zinc oxide is used in high concentrations [37]. In summary, at suitable concentrations, ZnO NPs have an increasing effect on seed germination, growth, antioxidant activities and protein production, chlorophyll content and photosynthesis, oil and seed production, and essential element uptake [39].

1.4.2. Cosmetic Applications

Since they cause skin cancer in humans, it is important to avoid harmful radiation such as UV rays. Therefore, to protect the skin, it is necessary to add substances with UV-blocking properties to the formulations of cosmetic products [36]. ZnO NPs, which have good antifouling and antibacterial properties as well as being an effective UV blocker, are widely used in cosmetic applications such as the manufacture of sunscreens [28], [36], [40]. The use of ZnO in cosmetics is not limited to sunscreens [41]. Due to the high photocatalytic activity of ZnO, it is also possible to generate reactive oxygen species (ROS) that can oxidize the ingredients of the cosmetic formulation [36], [41].

1.4.3. Biomedical Applications

ZnO NPs are used in biomedical and proecological applications due to their low toxicity, biodegradability and biocompatibility [40]. They are also preferred for drug-delivery and bioimaging systems, antidiabetic agents, anti-cancer agents, antioxidants, antibacterial and anti-inflammatory activities [28], [40], [42]. In addition, for various areas of biomedical applications, it is important to produce controlled nanoparticles that are uniform in size, morphology, and functionality [28]. Due to its biocompatible nature, ZnO can be used directly in biomedical applications [38].

1.4.3.1. Anticancer Activity

For the past decade, chemotherapy, radiotherapy, and surgery have been among the typical treatments for cancer, which is defined as a condition of uncontrolled, malignant cell proliferation [28], [43]. Although all these treatments are quite successful in theory, they also have many serious side effects due to their non-selectivity. In recent years, studies have been conducted showing that nanomaterials can overcome the above side effects due to their high biocompatibility, easy surface functionalization, and ability to target cancer and deliver drugs [28].

ZnO NPs have great potential for biomedical applications due to their excellent anticancer activity [28]. ZnO NPs have been reported to exhibit selective cytotoxicity against cancer cells in vitro and in vivo compared to normal cells [24]. In addition, these NPs cause some cytotoxicity to cancer cells due to their high ion release and enhanced ROS induction, which is why their use is often included in cancer treatments [24]. In addition, ZnO NPs are considered attractive candidates for anticancer drug delivery due to their biodegradability [24]. Considering all this information, it should be noted that the wide use of ZnO NPs in cancer therapy and the induction of selective cytotoxic effect on cancer cell proliferation have been reported [28].

1.4.3.2. Diabetes Treatment

Diabetes mellitus (DM), a universal, dangerous, and important public health problem, is a metabolic disease resulting from the body's inability to produce insulin

or to effectively use the insulin it does produce. [28], [44]. According to global estimates, 642 million people will be affected by this problem by 2040 [44]. Zinc (Zn) maintains the structural integrity of insulin, plays an active role in insulin secretion from pancreatic cells, and is involved in the synthesis, storage, and secretion of insulin [24], [28]. Therefore, ZnO NPs have the potential to be an effective antidiabetic agent or to attenuate diabetic complications when stabilized or conjugated with certain substances [24]. Based on these unique properties of zinc, ZnO NPs were prepared as a new active ingredient and their antidiabetic potential was evaluated [28].

1.4.3.3. Anti-Inflammatory Activity

Inflammation, an adaptive immune response, aims to eliminate the original cause of cellular damage, the tissue damaged by the original damage, and the lethal cells [45]. Due to the significant biological activities of zinc ions, the anti-inflammatory effects of ZnO NPs are also the focus of attention [28].

Atopic dermatitis (AD) is a multifactorial chronic inflammatory skin disease caused by the interaction of immunological and environmental factors [46]. Textiles are the products that provide the longest and most intense touch with human skin [28]. In a study by Wiegand et al. AD patients were dressed with ZnO textiles overnight for 3 consecutive days and a remarkable improvement in AD itching and subjective sleep quality was observed [47]. This research result is due to the high antioxidant and strong antibacterial structure of ZnO textiles [28], [47]. Nagajyothi et al. and Thatoi et al. have shown in their studies that the anti-inflammatory effect of ZnO NPs is not limited to the treatment of AD, but also has a high potential for other inflammatory diseases [28].

1.4.3.4. Bioimaging

In addition to biomedical applications in anticancer, antibiotic, diabetes, anti-inflammatory, and drug delivery, ZnO NPs also have the potential for bioimaging activity [28], [40]. Low toxicity makes them a good candidate for bioimaging applications [48]. ZnO NPs show green or yellow emissions (near-UV radiation) associated with oxygen vacancies and effective blue emissions. Therefore, the

applications of these nanoparticles in the field of bioimaging are becoming broader. [28]. Considering the reported improved intrinsic fluorescence properties, nanoscale ZnO particles can also be referred to as potential candidates for cell imaging and pathological studies [48].

1.4.3.5. Antibacterial Activity

The presence of antibiotic-resistant pathogens is a health problem that requires attention, and various studies are being conducted to develop antimicrobial treatments to eliminate this problem [25]. Metal oxides, obtained by combining metallic elements with oxygen, interact with bacteria through electrostatic interactions that disrupt the prokaryotic cell wall and enzyme or DNA pathways by producing ROS [49]. Therefore, metal oxide nanoparticles of Zn metals such as Ag, Cu, Au, Ti are also included in applications as antimicrobial agents as their antimicrobial activities, potentials and activity spectra are well known in antimicrobial treatment studies [25], [49].

Zinc oxide, a food additive, is on U.S. Food and Drug Administration's (FDA) list of substances generally recognized as safe (21CFR182.8991) [49]. The nanoscale forms of these materials stand out as antibacterial materials due to their high specific surface area and superior ability to inhibit a variety of pathogens [28]. Although the crucial first step for the antibacterial mechanism of ZnO NPs is the binding of the NPs to the bacterial membrane, the exact antimicrobial mechanisms are unknown [28], [49]. However, electrostatic interaction with the membrane, formation of ROS and/or release of ions are thought to influence this effect. It is known that the main antibacterial activity of ZnO NPs is due to their ability to induce excessive ROS production, and Figure 1.5 illustrates this mechanism [49]. When the relationship between the antibacterial activity of ZnO NPs and particle size was investigated, the particles with the smallest particle size showed the best bactericidal response [24], [25], [49]. Moreover, the high photocatalytic properties of ZnO nanoparticles are effective in enhancing their antimicrobial activity [25]. In various studies, ZnO NPs showed antibacterial activity as well as antifungal activity and bactericidal effects on spores resistant to high temperatures and high pressures [24], [25]. In addition, due to

their antimicrobial activity, ZnO NPs have widespread application in food preservation, production stability and shelf-life extension of products [49].

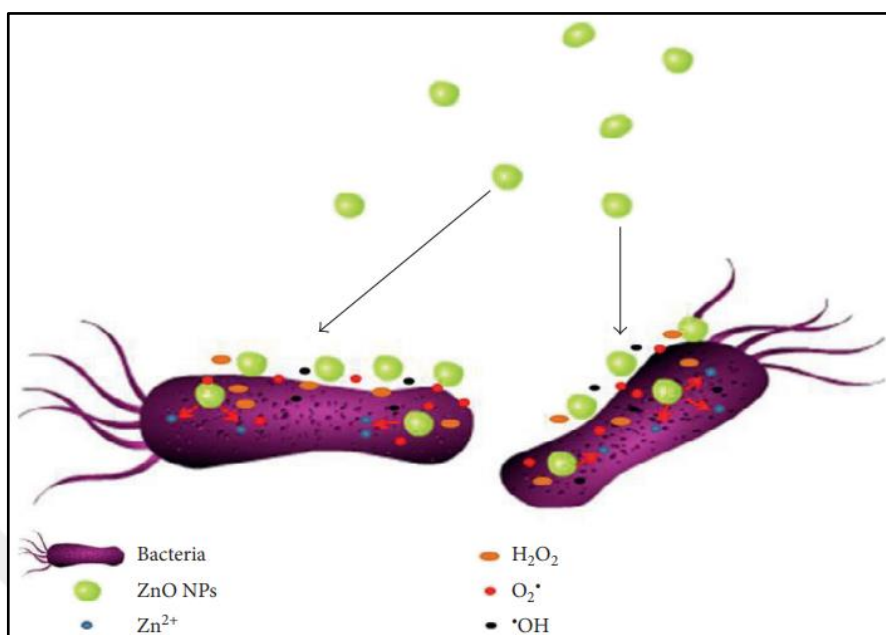


Figure 1. 5: Schematic illustration of antibacterial activity of ZnO NPs.

Due to the optical properties of ZnO NPs, faster and more efficient ROS generation occurs at shorter wavelengths (like light) [49]. In addition, hydrogen peroxide can enter the prokaryotic cell by formation, destroying various organelles [25], [49]. Another mechanism for the antibacterial effect of ZnO NPs is thought to be the deposition of the particles on the bacterial surface due to electrostatic forces [25]. In addition, lipid peroxidation may occur in the bacterial membrane, weakening membrane integrity and leading to cell lysis [49].

Another factor affecting the antimicrobial activity of ZnO NP is the bacterial species and the selection [49]. Generally, *Escherichia coli* (*E. coli*) as Gram-negative model bacteria and *Staphylococcus aureus* (*S. aureus*) as Gram-positive model bacteria are used to observe the antibacterial activity of ZnO NPs [28]. In addition, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Proteus vulgaris* (*P. vulgaris*), *Vibrio cholerae* (*V. cholerae*) and *Campylobacter jejuni* (*C. jejuni*) from other gram-negative bacteria, and *Bacillus subtilis* (*B. subtilis*) and *Enterococcus faecalis* (*E. faecalis*) from other gram-positive bacteria are also used in research [25], [28], [49]. In a study with *S. aureus* and *E. coli* bacteria, it was concluded that the ZnO material had higher sensitivity and higher susceptibility with Gram-positive bacteria than with Gram-

negative bacteria. Although there are several important factors underlying this situation, the main factors are the difference in membrane thickness and sensitivity to ROS [49]. Cell wall-forming components of Gram-positive bacteria are peptidoglycans, teichoic acid and lipoteichoic acid [50]. The cell walls of Gram-negative bacteria have a thicker and more complex structure than those of Gram-positive bacteria [49]. This is because Gram-negative bacteria have a cell wall that contains an outer lipopolysaccharide membrane and a peptidoglycan layer, while the membrane and cell wall of Gram-positive bacteria consist of peptidoglycans, teichoic acid and lipoteichoic acid, which are easier to penetrate [49], [50]. This leads to inhibition of the uptake of ROS and ions from the membrane into the cell [49].

1.5. Fabrication Techniques of ZnO Nanoparticles (NPs)

The methods used to produce various nanoparticles can be divided into 2 different categories: Top-down and Bottom-up [49]. The top-down process, which does not involve a chemical reaction process, refers to the process of forming the nanoscale material from the main macroscopic material (Figure 1.6) [21], [51]. Applications of this approach include adding or removing thin layers of bulk material, such as ball milling, mask etching, lithography, etc. [21], [30], [51]. In the bottom-up process, on the other hand, a chemical reaction is carried out to create larger stable structures from the basic units that constitute the starting material in order to produce another material on the nanoscale [21], [51]. This process is thus an approach that aims to organize atomic or molecular components into hierarchical nanocomplex structures [21]. Examples of nanostructures produced by bottom-up methods (sol-gel process, aerosol process, chemical vapor deposition (CVD), atomic condensation, gas-phase condensation, and supercritical fluid synthesis) include QDs or nanoparticles formed from colloidal dispersion [21], [51]. The bottom-up approach can be divided into three types: chemical, biological, and physical methods [21], [30].

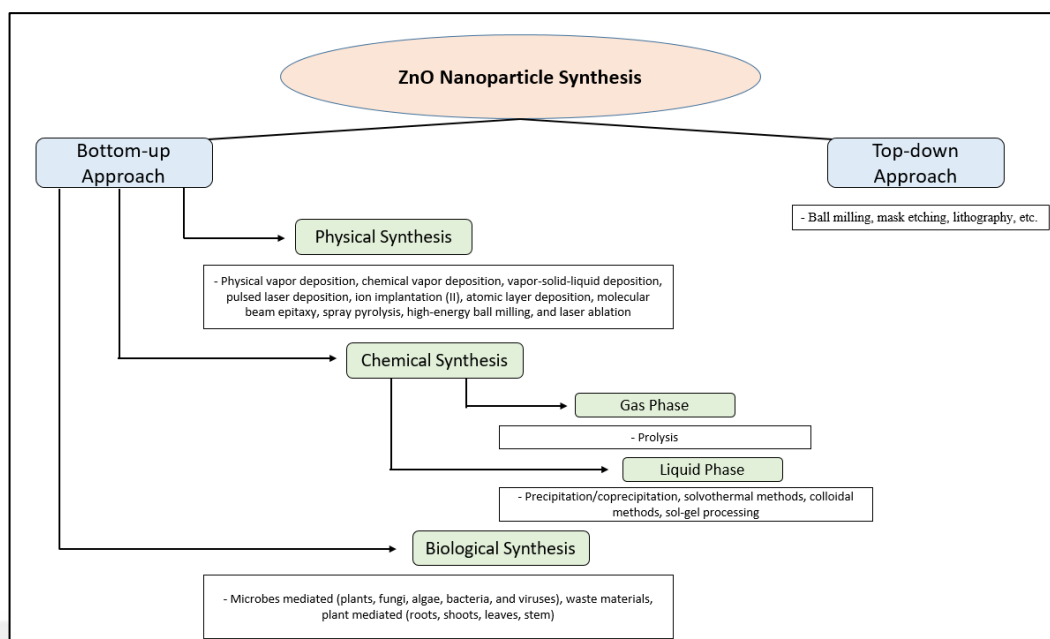


Figure 1. 6: The fabrication techniques of ZnO nanoparticles.

1.5.1. Physical Fabrication

Production methods for nanostructured materials include physical vapor deposition (PVD), chemical vapor deposition (CVD), vapor-solid-liquid deposition (VLS), pulsed laser deposition (PLD), ion implantation (II), atomic layer deposition (ALD), molecular beam epitaxy (MBE), spray pyrolysis (SP), high-energy ball milling, and laser ablation [21], [30]. Nanoparticles produced by physical processes are mostly used for industrial processes [30].

The technique of high-energy ball milling was developed by Salah et al. in 1961. It involves the production of fine metal NP as a result of high-energy collisions of stiff spheres of powdered material placed in a high-energy shaking mill [29], [30]. The advantage of this production method is that there is significant potential for scaling to obtain large amounts of material, while the disadvantage is the formation of irregularly shaped NPs, in addition to the risk of contamination from the grinding balls and/or the atmosphere [29], [37].

The laser ablation technique is based on the removal of metallic ions from a metal surface using a laser beam and immersion in a small volume of a liquid such as methanol, ethanol, distilled water [29], [30]. In addition, the size of nanoparticles can be controlled by manipulating the parameters of the laser flow and the nature of the liquid medium [20]. While the advantages of this method are that it is efficient, simple,

environmentally friendly, and safe, the effect of the pyrolysis by products produced during laser ablation in the presence of organic compounds on the stability of the colloidal system is not well known, and it is the disadvantage that this needs to be clarified [29].

The physical vapor deposition (PVD) technique, which describes various vacuum deposition methods, is a method of coating surfaces by depositing metals [30], [52]. Evaporation and sputtering techniques form the basis of PVD [30]. By means of these physical processes, a vapor in the form of atoms, molecules, or ions is generated from the coating material, which is then emitted from a target [52].

The basic principle of chemical vapor deposition (CVD) is the evaporation of the source in a chamber using resistive heat. This heat source can be electron or laser beams or radio frequencies. The resulting vapors are transferred from the heat chamber to a colder chamber containing inert gas, where they are collected for further consolidation. Coalescence and agglomeration of nanoparticles is the main drawback of the CVD method [30].

In the spray pyrolysis (SP) method, the precursor metal salt is obtained by heating the aerosol droplets with a flame and is often preferred [30]. In this method of preparation, which belongs to the cheap and relatively simple methods, the dispersion of the droplets in the gas is followed by their reduction in size by dehydration [21], [30]. Finally, the required material is separated and sintered [30].

1.5.2. Biological Fabrication

Biological production is a method of producing nanoparticles that does not produce toxic waste and prefers an enzymatic reaction to chemical processing, which is more environmentally friendly [20]. Furthermore, this method of producing NPs involves the use of various natural resources such as plants, fungi, algae, bacteria, and viruses [29], [30]. Among the advantages of these productions are that they do not produce large amounts of pollutants, are energy efficient, do not have high costs and are biocompatible [20], [21], [28], [30]. Biological production has disadvantages compared to chemical and physical methods, such as low efficiency of production, inability to obtain nanoparticles of the desired size and/or shape, slow production (the

process may take a few hours or even a few days), decomposition of the obtained nanoparticles after a certain time [20].

1.5.3. Chemical Fabrication

Chemical fabrication can also be divided into liquid phase fabrication and gas phase fabrication. While the subtitles of liquid phase fabrication are precipitation, co-precipitation method, colloidal methods, sol-gel method, water-oil microemulsions, hydrothermal synthesis, solvothermal, and sonochemical and polyol method, the subtitles of vapor phase production are pyrolysis and inert gas condensation methods [30].

1.5.3.1. Gas Phase Production

SP is one of the gas-phase synthesis methods that can be used to produce nanoscale structures if the solution and drying parameters are kept under control [32]. Not only is it one of the preferred methods, but it is also prepared by flame heating of aerosol droplets of the precursor zinc salt. The steps of this method are as follows: The droplets are dispersed in the gas, their size is reduced by dehydration, the desired material is decomposed and sintered [30].

1.5.3.2. Liquid Phase Production

A typical precipitation and coprecipitation method, in which a reducing agent (usually inorganic alkalis) is reacted with the zinc salt, has advantages such as simplicity, no high temperature requirements, and overall energy efficiency [29], [30]. A soluble or insoluble precipitate is obtained, which is then washed and calcined at various temperatures to synthesize nanoparticles with the expected morphology and properties [30]. The disadvantages of this method include the large amount of adsorbed water molecules that can affect the desired NP properties, difficulties in reproducibility, poor particle size distribution, and strong agglomeration [29].

Solvent precipitation or solvothermal methods are widely used methods for obtaining nano-structured powders. In the solvothermal method, the metal salt is added

to a solvent by adding water as another reactant to obtain a compound (base, alcohol, etc.) or a zinc hydroxide complex, usually with a hydroxyl (OH) group. The hydrothermal method, on the other hand, is a variant of the solvothermal method in which the solvent is water [32]. In solvothermal and hydrothermal processes, hot solvents or water are dissolved under moderate to high pressure (1-10,000 atm) and moderately high to high temperature (100-1000°C). [30].

The basis of colloidal methods was laid in 1857 by Faraday, who synthesized colloids of gold nanoparticles by reduction of HAuCl_4 with phosphorus and has the simplest and well-established chemistry for many years [30]. The sol-gel synthesis approach, which is mostly used for the preparation of metal oxide NP, is based on colloid chemistry [29], [30], [53].

When all these nanoparticle fabrication methods are explored, there are several methods to grow ZnO NPs in controlled structures that are uniform in size, morphology, and functionality and affect the biological activity of the NPs [21], [28], [30], [36]. The preparation and growth of various stable ZnO NPs by methods such as chemical precipitation, sol-gel, chemical vapor deposition and biosynthesis are available in the literature [28], [29], [31]. Among these methods, the sol-gel fabrication technique, which has novel properties in the production of ZnO NPs, has the best thermal stability ratio, the best solution resistance, higher mechanical stability, and the possibility of transformation simulation [28], [38].

1.6. Sol-Gel Method

The chemists J.J. Ebelmen and Graham opened up a new field in the production of inorganic ceramics and glass materials by the sol-gel process with their work begun in 1846 [54], [55]. These two pioneers of the sol-gel method have obtained SiO_2 , which they refer to as a "glass-like material," by hydrolysis of tetraethyl orthosilicate (TEOS), $\text{Si}(\text{OC}_2\text{H}_5)_4$, under acidic conditions [54]. In 1930, Geffcken and Berger of the Shott Company discovered that the sol-gel process could be used to deposit oxide layers on industrial glass using alkoxides [55], [56]. Later, in the 1950s and 1960s, D. Roy and co-workers proposed to process a single homogeneous powder from a sol-gel to obtain phase diagrams for ceramics [54], [56], [57]. Subsequently, phase diagrams were created by mineralogists, ceramicists, and glass scientists to conduct research

using the sol-gel method, while this method was used by nuclear physicists to produce fuels. Examples of the application of this method include the work on the production of Ti-SiO₂ reflector coating in 1959 and the production of SiO₂-B₂O-Al₂O₃-Na₂O-K₂O multicomponent glass in 1971 [55]. In the mid and late 1960s it was developed by the adoption of the sol-gel process by scientists such as H. Schroeder [56]. Sol-gel technology developed rapidly in the 1980s, driven by Dislich's compelling research [55], [56]. This development contributed significantly to the spread of sol-gel technology in a short period of time. At the same time, Dislich illuminated the sustained growth of sol-gel technology in the number of publications in the last years of the 20th century [56]. Today, it is used in the production of glass, oxide coatings, functional ceramic powders, especially nanooxide materials, functional materials and composites that are difficult to produce by conventional methods. Catalysts, microspheres, piezoelectric devices, waveguides, lenses, high-strength ceramics, superconductors, nanoparticles and insulating materials, and much more can be produced using sol-gel technology [55].

In the sol-gel process, a wet chemical method for preparing metal oxide materials, a metal alkoxide is used as a precursor. A sol is formed by the hydrolysis of this precursor and the condensation reaction, and finally a gel product is formed by a polycondensation reaction [55]. Moreover, the synthesis schemes generally used in the sol-gel method can be divided into 3 groups, depending on the first precursors: aqueous solutions of metal salts, metal alkoxide solutions, and mixed organic-inorganic precursors [56].

This technology is used for the synthesis of various metal oxide nanoparticles such as TiO₂, ZnO, MgO, CuO, ZrO₂ and Nb₂O₅ and many nanocomposites [20]. The sol-gel method is very well suited for the preparation of ZnO nanoparticles [40], [58]. The reason for this statement is that it has several attractive advantages over other methods, such as:

- Lower processing costs,
- ease and simplicity,
- good control over particle size,
- exceptional purity levels,
- high reliability,
- control of additive concentrations,

- low processing temperature (below 100°C),
- good repeatability, and
- the ability to produce perfectly homogeneous multicomponent compositions with different product shapes [37], [40], [59], [60].

In addition, it is important to use the sol-gel technique because it offers the best thermal stability ratio, solution resistance, higher mechanical stability and the possibility of transformation simulation [37]. Furthermore, it is a production method that allows effective control of the morphology, optical properties, and physical properties of the nanoparticles produced [40]. Sol-gel chemistry has attracted great interest from researchers due to its significant advantages in the production of products such as highly dispersible multicomponent ceramics, organic-inorganic hybrid materials, and other materials [55]. Because of all these unique properties, the sol-gel process can be used in extremely mild conditions (often at room temperature). In addition, products can be made in various shapes, sizes and formats, and the applications of this technology in many scientific and technical fields are increasing day by day [61].

1.6.1. Mechanism of Sol-Gel Fabrication

Sol-gel techniques based on colloidal chemistry used in the preparation of metal oxide NP involve a series of chemical reactions defined as the gradual transition of a homogeneous solution of a molecular reactant precursor (sol, e.g., a solution containing inorganic metal salts) into a solid phase (gel) [29], [30], [62].

The sol-gel process can be listed as follows:

- Preparation of the precursor solution,
- Hydrolysis of the organometallic precursor compounds,
- Polycondensation to form a metal hydroxide network,
- Aging and drying,
- Calcination [20], [61].

The chemical reactions used in this technique allow the control of the process from the precursor to the final product to realize the desired design and obtain the

stable phase. For the final product, each step of the sol-gel process is very important. Therefore, it is necessary to develop a process suitable for the characteristics of the desired product by studying each step in detail [61].

1.6.2. Mechanism of Hydrolysis and Condensation

The chemical process of the sol-gel method begins with the dispersion of organometallic precursors (such as alkoxides, chloride, beta-diketonate, or nitrate) in a solvent under aqueous conditions and continues with a hydrolysis reaction to obtain an active monomer and condensation reactions [55], [40]. As a result of polymerization of the active monomer, a sol is formed, followed by a gel with a specific spatial structure [55]. One of the factors affecting the rate of hydrolysis and condensation is the steric effect of the alkoxide groups. The longer the chain attached to the metal alkoxides, the more difficult it is to break the O-R bond, and the rate of hydrolysis decreases accordingly. Smaller OR groups tend to water molecules in shorter time [61].

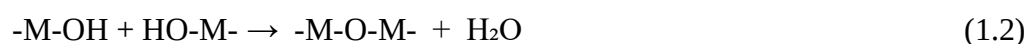
The reactions listed below explain the hydrolysis, condensation, and polymerization reactions [55], [61].

Hydrolysis: Non-ionizing molecular precursors (for example, metal alkoxide $M(OR)_n$) react with water [55].



Condensation: This step can be studied in two ways, based on the type of molecules removed.

Dehydration polycondensation:



Dealcoholization polycondensation:



1.6.3. Mechanism of Gelation

As a result of hydrolysis and condensation reactions, colloidal particles aggregate and form a gel [54], [61]. This process is thus directly influenced by all parameters that affect the hydrolysis and condensation reactions [61]. In gelation, which is defined as an event similar to the freezing process in which the liquid of a solution suddenly disappears and changes into an elastic solid form, the viscosity increases sharply [54], [61]. Gel can be defined as a rigid, interconnected network of polymer chains (longer than an average of one micrometre) with nanometer-sized pores [53], [54]. Although the gel contains a large amount of liquid, it is not liquid. Muscles, skin, cell membranes, blood vessel walls, hair, nails and cartilage in the human body are examples of this structure, which also has a remarkable influence on the composition of organisms [55].

1.6.4. Mechanism of Aging, Drying

Hydrolysis and condensation are followed by aging to obtain the desired material, solvent extraction and drying of the synthesized product [55], [40]. The expected time required for a gel to form a stable structure due to the reactions caused by the chemicals it contains is defined as the aging process [61]. With aging, the strength of the gel increases, while the porosity decreases due to progressive polycondensation [54]. In addition, when the interface of the gel becomes smaller, the pressure generated during the drying phase decreases [61]. In this way, you get stronger gel nets that prevent cracks that can occur during drying. [54], [61]. In the drying step, the liquid is removed from the interconnected pore network, usually by evaporation of the solvent or supercritical drying [54].

1.6.5. Mechanism of Calcination

It is explained that post-treatment annealing, carried out at suitable temperatures, prevents the accumulation of additives and eliminates them by controlling the defects [40]. The calcination temperature, time and environment play a decisive role in the

desired end product [61]. The gel is removed from the organic substance after aging, drying or heat treatment, and a porous metal oxide material is obtained [55].

The pH of the sol, the additives used (such as coating material, surfactants), the annealing temperature, and the effect of calcination have a remarkable importance in tailoring the morphology and optical properties of the nanoparticles prepared by the sol-gel technique to the desired application. There are many studies in the literature that investigate the effects of annealing and calcination temperature on the structural properties of ZnO nanoparticles prepared by sol-gel [40]. For example, Sangeetha et al. synthesized ZnO nanoparticles by the sol-gel method and calcined these NPs at 300°C, 500°C, 650°C, 700°C, and 750°C to observe the effects of calcination temperatures on the ZnO NPs [63]. In another study, Z. N. Kayani et al. investigated the relationship between the calcination temperature of ZnO NPs produced by the sol-gel method for the same purpose at different temperatures (200°C, 500°C, 700°C, and 900°C) [42].

2. MATERIALS AND METHODS

2.1. Schematic Representation of the ZnO Nanoparticles Synthesised by the Sol-gel Method

In this study, we aim to synthesise nanoscale ZnO particles using the sol-gel method and investigate the morphological and antibacterial properties of these ZnO nanoparticles. Figure 2.1 shows the schematic representation of ZnO Nanoparticles synthesised by the sol-gel method.

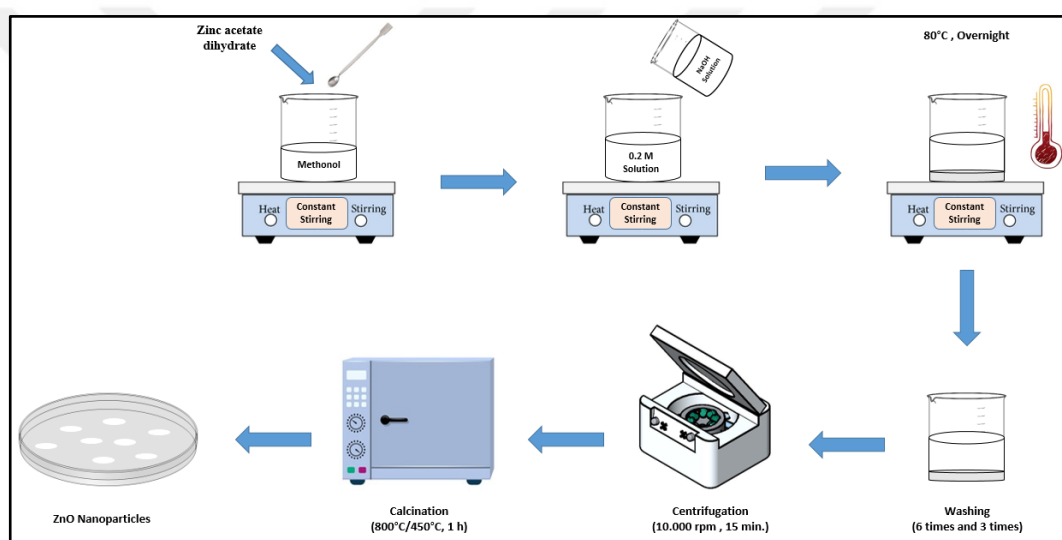


Figure 2. 1: A schematic view of ZnO nanoparticle synthezies via sol-gel.

2.2. Precursors

The chemicals used in this study were obtained from Sigma Aldrich and used without further purification. Zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) with 99% purity was preferred as zinc precursor. Sodium hydroxide (NaOH) was used to adjust the pH to 12 as a source of hydroxyl groups. Methanol (CH_3OH) with (99%) and deionised water (H_2O) were used as solvents. Methanol and sodium hydroxide help to prepare a stoichiometric solution for obtaining zinc oxide nanoparticles, as well as adjusting the homogeneity and pH value of the solution [64]. The properties of the chemicals used are listed in Table 2.1.

Table 2. 1: Chemical formula of substances for NPs.

Chemical Substance	Chemical Formula	Molecular Weight (g/mol)	Purity (%)
Zinc Acetate Dihydrate	$\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$	219.5	99.0 - 101.0%
Sodium Hydroxide	NaOH	40.00	97%
Methanol	CH_3OH	32.04	99%
Deionized Water	H_2O	18.02	99.9%

2.3. Synthesis of ZnO Nanoparticles

ZnO NPs were synthesized by the sol-gel method at pH=12. Two different numbers of washes (3 and 6 times) and two different calcination temperatures (800°C and 450°C) were applied as ZnO-800°C and ZnO-450°C, respectively. Flow diagrams of the synthesis steps of ZnO NPs using sol-gel are shown in Figure 2.2 for ZnO-800°C and Figure 2.3 for ZnO-450°C.

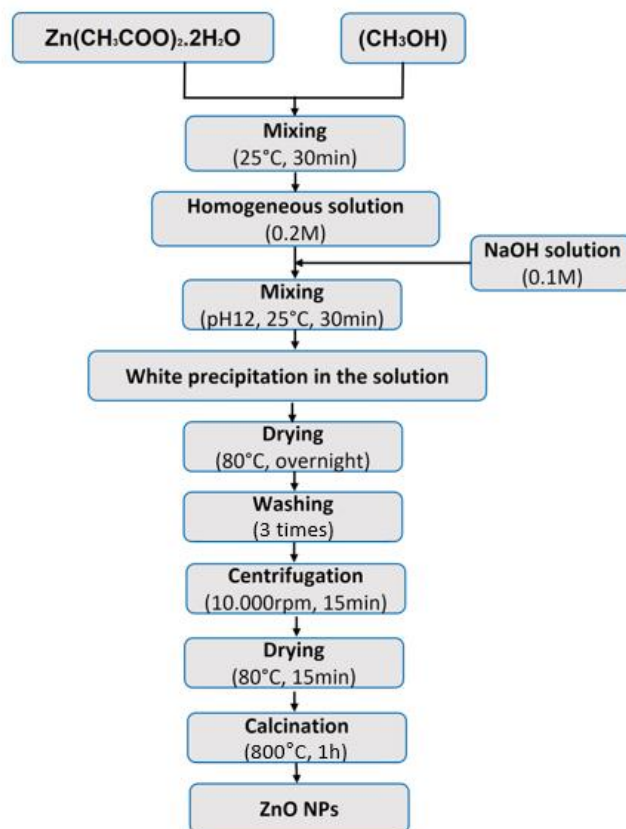


Figure 2. 2: Flowchart of ZnO NPs (ZnO-800°C).

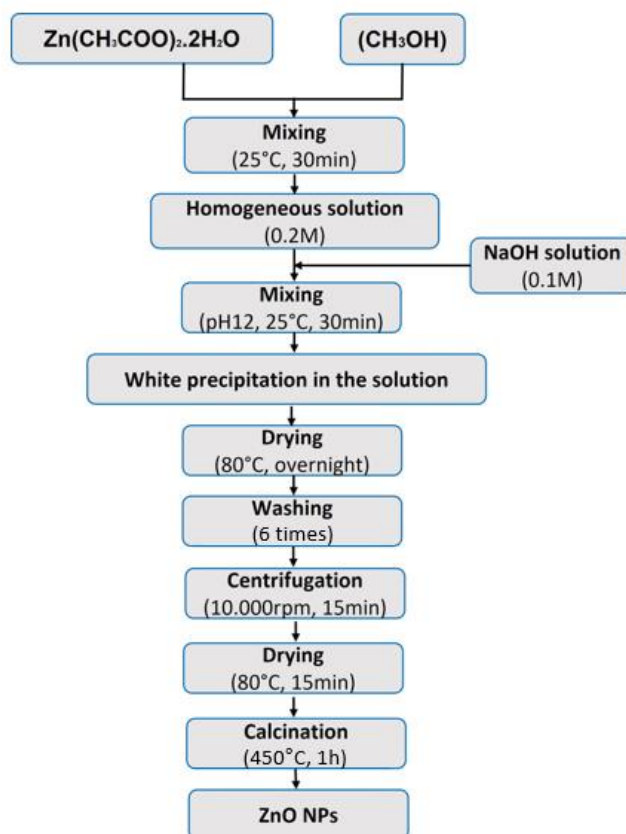


Figure 2. 3: Flowchart of ZnO NPs (ZnO-450°C).

In the synthesis of ZnO NPs, 4.39 g of zinc acetate dehydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) powder was dissolved in 100 ml of methanol (CH_3OH) to give a concentration of 0.2M. The homogeneous ZnO solution was obtained after stirring in a magnetic stirrer for 30 minutes. Separately, a homogeneous sodium hydroxide (NaOH) solution with a concentration of 1.0 M was prepared by dissolving 6 g sodium hydroxide in 150 ml deionised water after stirring at 25°C for 30 minutes. 1.0 M NaOH solution was added dropwise to the ZnO solution to adjust the pH to 12. The solution turned milky white and was stirred in a magnetic stirrer for 1 hour at constant room temperature with constant stirring. Considering the relationship between pH and precipitation time, the solution was dried in a magnetic stirrer at 80°C for 1 night to obtain a white precipitation. Then the white precipitate solution was filtered and washed with methanol 3 times for the ZnO-800°C experiment and 6 times for the ZnO-450°C experiment. It was then centrifuged at 10.000 rpm for 15 minutes to separate the white precipitate. After centrifugation, the resulting white, creamy sample was dried in a magnetic stirrer at 80°C for 15 minutes. Finally, the calcination temperature was

applied at 800°C for 1 hour to obtain ZnO-800°C NPs and at 450°C for 1 hour to obtain ZnO-450°C NPs.

2.4. Characterization Techniques

The crystallite size, morphology, structural and optical properties of the synthesized nanoparticles were investigated by Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Ultraviolet-Visible (UV-Vis-NIR) Spectrophotometer.

2.4.1. X-ray Diffraction (XRD)

The crystal structure of ZnO NPs samples were analyzed using X-ray diffraction (XRD-Rigaku Dmax 2200) with $\text{CuK}\alpha$ radiation ($\lambda = 1.5405 \text{ \AA}$) at a rate of $1^\circ/\text{min}$ in the range of $20\text{-}70^\circ$.

2.4.2. Scanning Electron Microscope (SEM)

Both the surface texture and the morphology and structure of the fabricated NPs were examined using a Phillips XL 30 SFEG scanning electron microscope. The surface morphology of the NPs is an important tool for studying their nanostructure. The surface images were used to search for micro/nano cracks, pores or crystallisations.

2.4.3. Energy Dispersive X-Ray Spectroscopy (EDS)

EDS analysis was performed with SEM for ZnO elemental analysis. The spectrum of EDS contains the frequency of X-rays received in counts for each energy level. In most cases, the spectrum shows the peaks corresponding to the energy levels from which the X-rays were received. Each of these peaks corresponds to a particular atom. It therefore indicates the characteristics of a particular element.

2.4.4. UV-VIS-NIR Spectrophotometer

The Schmadzu model UV-VIS-NIR spectrophotometer was used to study the optical properties of the nanoparticles. The optical properties of ZnO NPs were investigated using the UV-Vis-NIR spectrophotometer in the wavelength range of 200-800 nm. Reflectance spectra were recorded for all measurements.

2.5. Antibacterial Activity of ZnO NPs

In this study, the antibacterial activity of ZnO nanoparticles against *Escherichia coli* (*E. coli*) ATCC 25922 as Gram-negative bacteria and *Staphylococcus aureus* (*S. aureus*) ATCC 6538 and *Bacillus cereus* (*B. cereus*) as Gram-positive bacteria were examined under laboratory conditions.

2.5.1. Preparation of Bacterial Culture and Stock Solutions

The potential antimicrobial activity of ZnO NPs was investigated against three different strains of pathogenic bacteria. One strain is a Gram-negative bacterial strain (*E. coli*) and the other two are Gram-positive bacterial strains, namely *S. aureus* and *B. cereus*, a spore-forming bacterium. The characterized bacterial strains were obtained from the Industrial Biotechnology Laboratory of Gebze Technical University, Institute of Biotechnology.

2.5.2. Assessment of Antibacterial Activity of ZnO NPs by Agar Well Diffusion Method and Agar Disc Diffusion Method

The antibacterial activity of ZnO-800°C and ZnO-450°C NPs was determined using three different bacteria and two different methods such as agar well diffusion and agar disk diffusion. In all experiments, the ZnO NPs were suspended in distilled water. In the first and second experiments, the agar well diffusion method was used and 50000 ppm and 5000 ppm concentrations, respectively, of ZnO NPs both in ZnO-800°C and ZnO-450°C were chosen. In the third experiment, the agar disc diffusion method was used with the concentrations of ZnO NPs of 5000 ppm and 2500 ppm in

both ZnO-800°C and ZnO-450°C. A uniform colloidal suspension of ZnO NPs was obtained by sonication for 15 seconds. In addition, UV irradiation was performed for 15 minutes at both ZnO-800°C and ZnO-450°C in this experiment to observe the UV effect.

The 1st and 2nd antibacterial experiments were performed using the agar well diffusion method. While the reference strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538 were grown for 24 hours at 37°C in nutrient broth medium in the agar well method, the strain *Bacillus cereus* Cultured for 24 hours at 25°C in the same medium. The density of the bacterial isolates was adjusted to the optimal density of 0.5 McFarland standard using sterile saline. In the agar well diffusion method, 0.1 mL of the activated cultures of *E. coli* ATCC 25922, *S. aureus* ATCC 6538, and *B. cereus* were added to individually labeled Petri dishes. Separately, 20 mL of Brain Heart infusion containing a soft agar medium with 0.75% agar (w/v) was added to the cultures, mixed, poured into sterile Petri dishes, and allowed to stand in the refrigerator for 2 hours to solidify. In addition, distilled water was used as a negative control and chlorocomphenicol, an antibiotic, was used as a positive control in the experiment. Then, 8 mm diameter wells were opened on the solidified agar surface using sterile automatic pipette tips (1 mL), and 100 µL of the ZnO NPs samples to be dispersed (50000 and 5000 ppm concentrations of ZnO-800°C and ZnO-450°C), the negative control, and the positive control were added to each well. Petri dishes containing *E.coli* and *S.aureus* cultures were incubated at 37°C for 24 hours, and those containing *B.cereus* cultures were incubated at 25°C for 24 hours. After incubation, the colony-forming units (cfu) expected to form clear zones of inhibition around the wells were examined.

The 3rd antibacterial experiment was performed using the agar disk diffusion method. In the agar disk diffusion method, the reference strains of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538 were grown at 37°C and the reference strains of *Bacillus cereus* were grown at 25°C for 24 hours in a nutrient broth medium. Using sterile saline, these reference strains were adjusted to 0.5 MacFarland standard turbidity and applied to the Mueller-Hinton agar medium at a volume of 0.1 ml. Separately, ZnO NPs were dispersed by pretreatment with 15-second sonication. Moreover, 1 set of ZnO NPs was irradiated with UV light for 30 min to observe the effect of UV on antibacterial activity in this experiment. Nanoemulsions of ZnO-800°C and ZnO-450°C powders prepared with distilled water at different

concentrations (5000 and 2500 ppm) were imbibed into sterile blank paper disks with a diameter of 6 mm in 15 μ L and placed on the surface of the medium colonized by bacteria. Distilled water was used as a negative control and the antibiotic chlorphenicol as a positive control. Petri dishes containing the *E. coli* and *S. aureus* reference strains were incubated at 37°C and Petri dishes containing the *B. cereus* reference strains were incubated at 25°C for 24 hours. At the end of incubation, potential zones of inhibition were evaluated for antimicrobial activity.



3. RESULTS

The section "Results" is divided into two parts: the results of the microstructural and optical properties and the results of the antibacterial activity of the produced ZnO NPs.

3.1. Microstructural and Optical Properties of ZnO NPs Produced by Sol-Gel Method

3.1.1. XRD Analysis of ZnO NPs

X-ray diffraction (XRD) was used to characterize the ZnO NPs. The examination revealed the typical wurtzite hexagonal ZnO structure for ZnO-800°C and ZnO-450°C samples (JCPDS 00-036-1451) in Figure 3.1 and Figure 3.2, respectively. The data were taken with CuK α radiation ($\lambda=1.5056$ Å) in the range of $20^\circ \leq 2\theta \leq 70^\circ$. The XRD peaks of 31.84° , 34.50° , 36.34° , 47.65° , 56.73° , 63.01° , 66.54° , 68.12° and 69.26° corresponded to the orientations of ZnO (100), (002), (101), (102), (110), (103), (200), (112) and (201), respectively. No other peaks associated with the phases were observed, indicating that high purity ZnO NPs were produced. The main peak at a calcination temperature of 450°C is sharper and has a smaller full width at half maximum (FWHM) compared to 800°C.

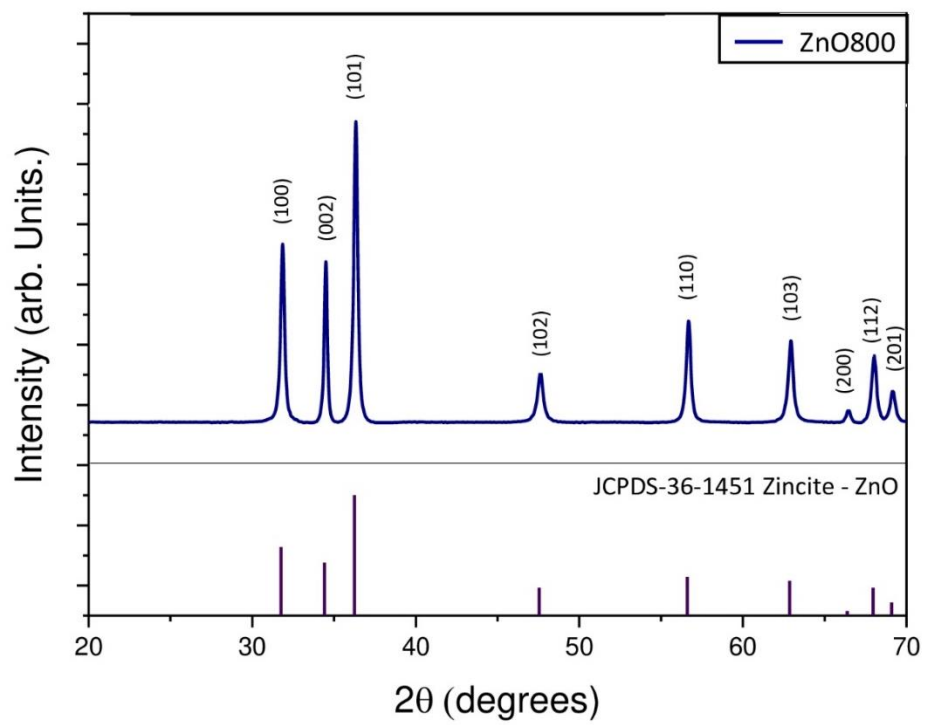


Figure 3. 1: XRD pattern of ZnO-800°C NPs.

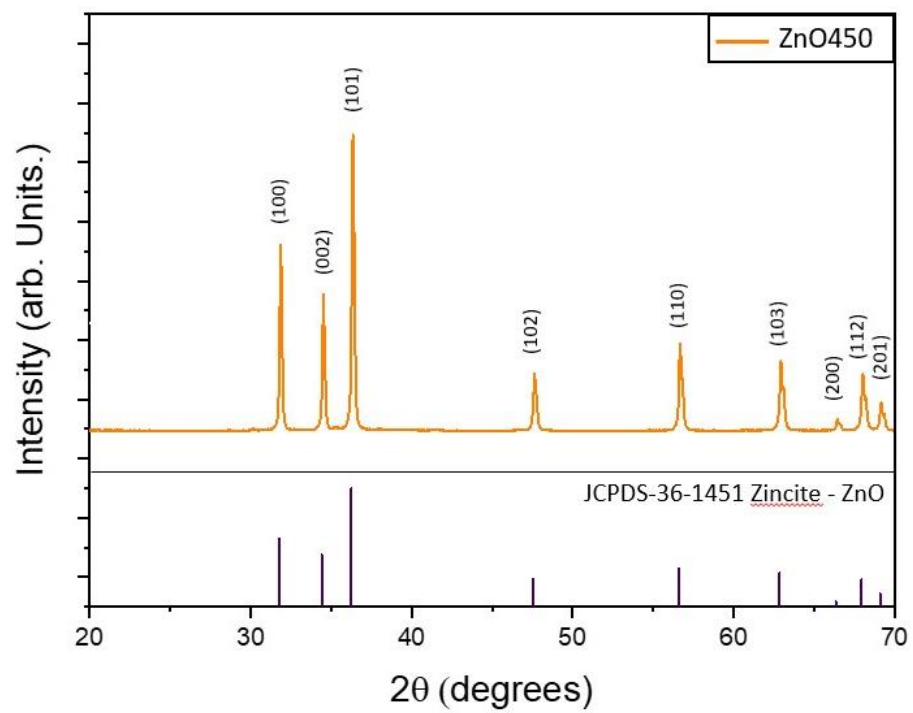


Figure 3. 2: XRD pattern of ZnO-450°C NPs.

The crystallite sizes of ZnO NPs were calculated using the Scherrer's formula from XRD data. The measurement of the crystal size of the material is related to the full width at half maximum (FWHM) at the angle at which the maximum intensity of the peak obtained as a result of X-ray diffraction is observed. This change is given by the Scherrer equation;

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (3.1)$$

Where D is the average crystallite size of the phase under investigation, K is the Scherrer constant (0.89), λ is the wavelength length which is constant (0.15406 nm) of X- ray beam used, β is the fullwidth half maximum (FWHM) of diffraction (in radians) and θ is peak position of diffraction (in radians).

The crystallite size of the ZnO NPs in ZnO-800°C ranged from 47.20 to 60.25 nm, and the crystallite size of the ZnO NPs in ZnO-450°C ranged from 18.82 to 31.11 nm. Therefore, the average crystallite size of ZnO nanoparticles was calculated to be 52.65 nm for ZnO-800°C and 25.11 nm for ZnO-450°C. As the calcination temperature decreased with increasing washing time, the crystallite size decreased as shown in Table 3.1. These results thus show that it is possible to synthesise nanoparticles with smaller crystallite sizes at lower calcination temperatures with increasing washing times.

Table 3. 1: Average crystallization size of the ZnO-800°C and ZnO-450°C.

	Washing times	Calcination Temperature	Average Crystallite Size (nm)
ZnO-800°C	3 times	800°C	52.65
ZnO-450°C	6 times	450°C	25.11

3.1.2. SEM Analysis of ZnO NPs

Figure 3.3(a-b) shows the SEM surface morphology of ZnO-800°C and ZnO-450°C at low and high magnifications of ZnO NPs synthesized using the sol-gel method. It was observed that the ZnO-800°C NPs have an irregular shape, while the ZnO-450°C NPs have more spherical particles.

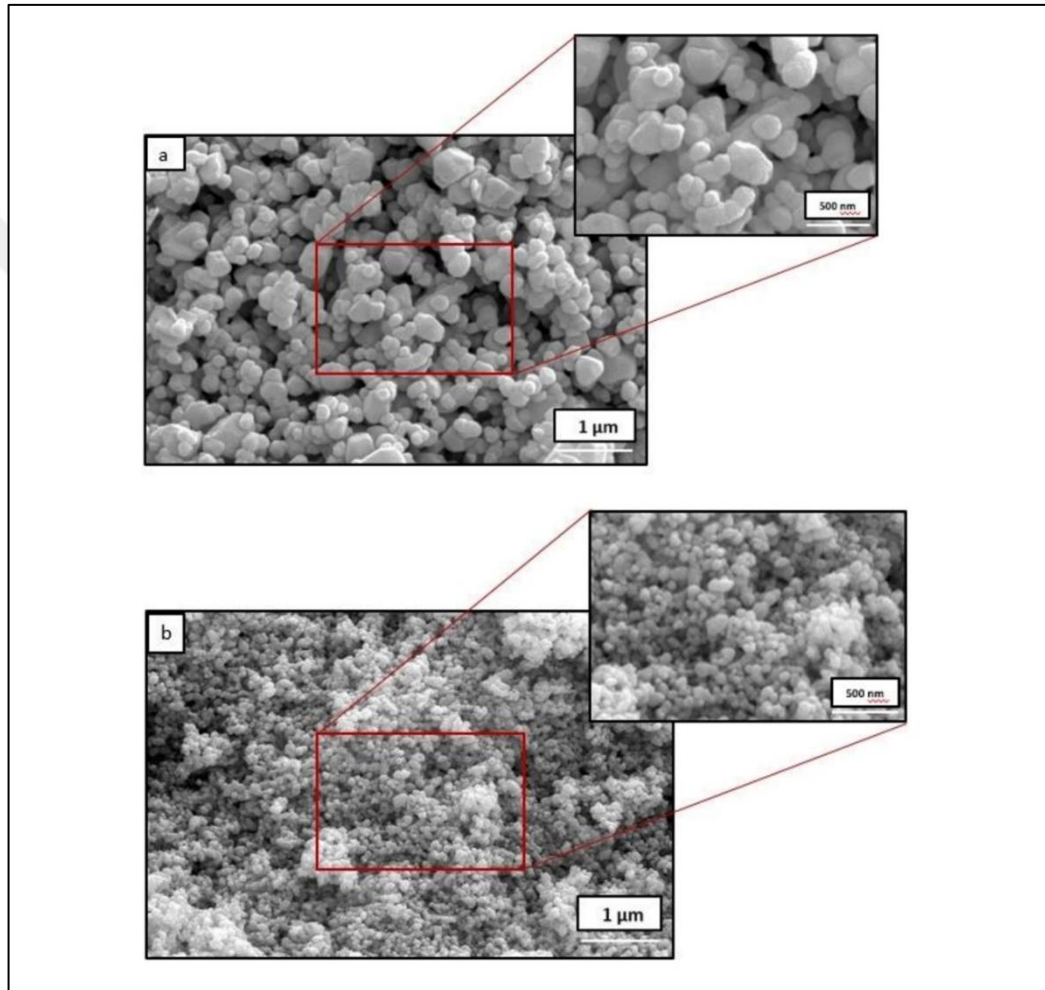


Figure 3. 3: SEM images of ZnO NPs synthesized by sol-gel method: a) ZnO-800°C b) ZnO-450°C.

3.1.3. Energy Dispersive X-Ray Spectroscopy (EDS) Analysis of ZnO NPs

Fig.3.4(a-b) shows the EDS spectra of ZnO-800°C and ZnO-450°C ZnO NPs. The ZnO nanoparticles consisted only of Zn and O. No evidence of other impurities was found and the ZnO nanoparticles were nearly stoichiometric. This observation was in good agreement with the XRD results, which confirmed the phase purity of the ZnO nanoparticles.

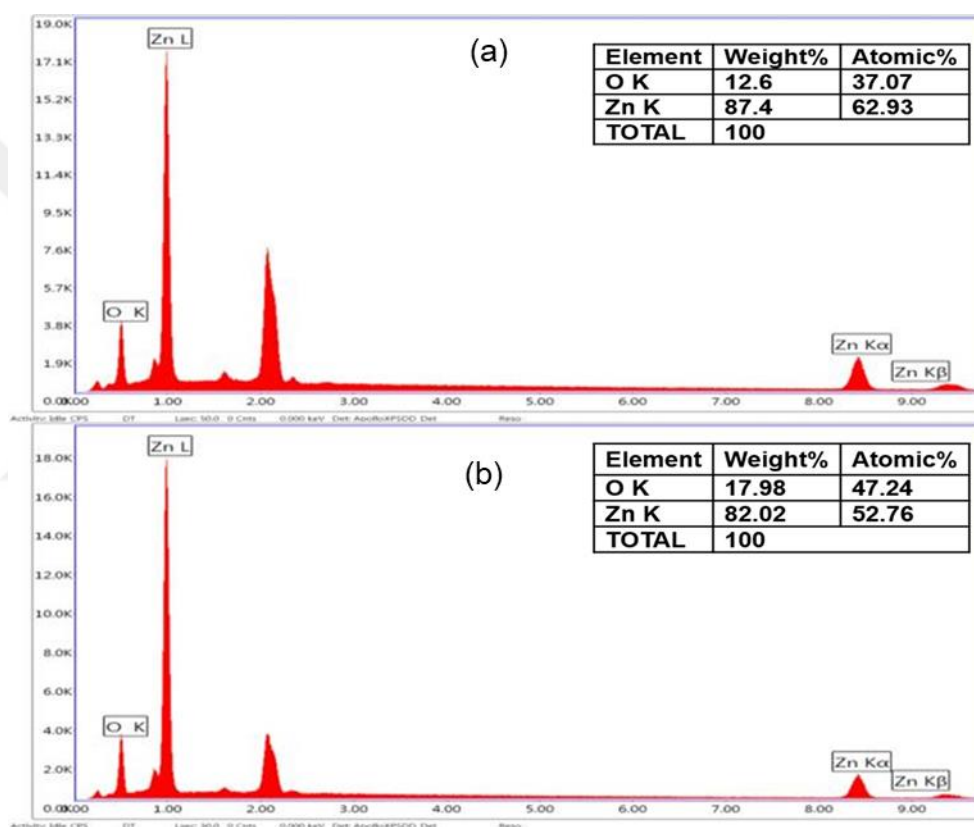


Figure 3. 4: EDS spectra for ZnO NPs a) ZnO-800°C b) ZnO-450°C.

3.1.4. UV-VIS-NIR Spectrophotometer Analysis of ZnO NPs

The reflectance spectra showed a sharp increase around 370 nm and the strong reflectance characteristic was observed around 460 nm for ZnO-800°C and around 420 nm for ZnO-450°C in Figure 3.5. It was observed that the absorption of ZnO nanoparticles was strongly influenced by the particle size. The energy of the band gap of the ZnO nanoparticles was calculated from the reflectance curve by UV-VIS-NIR

spectrophotometry using the Tauc relationship in Figure 3.6. The band gap was 3.26 eV for ZnO-800°C and 3.28 eV for ZnO-450°C, which increased with decreasing annealing temperature.

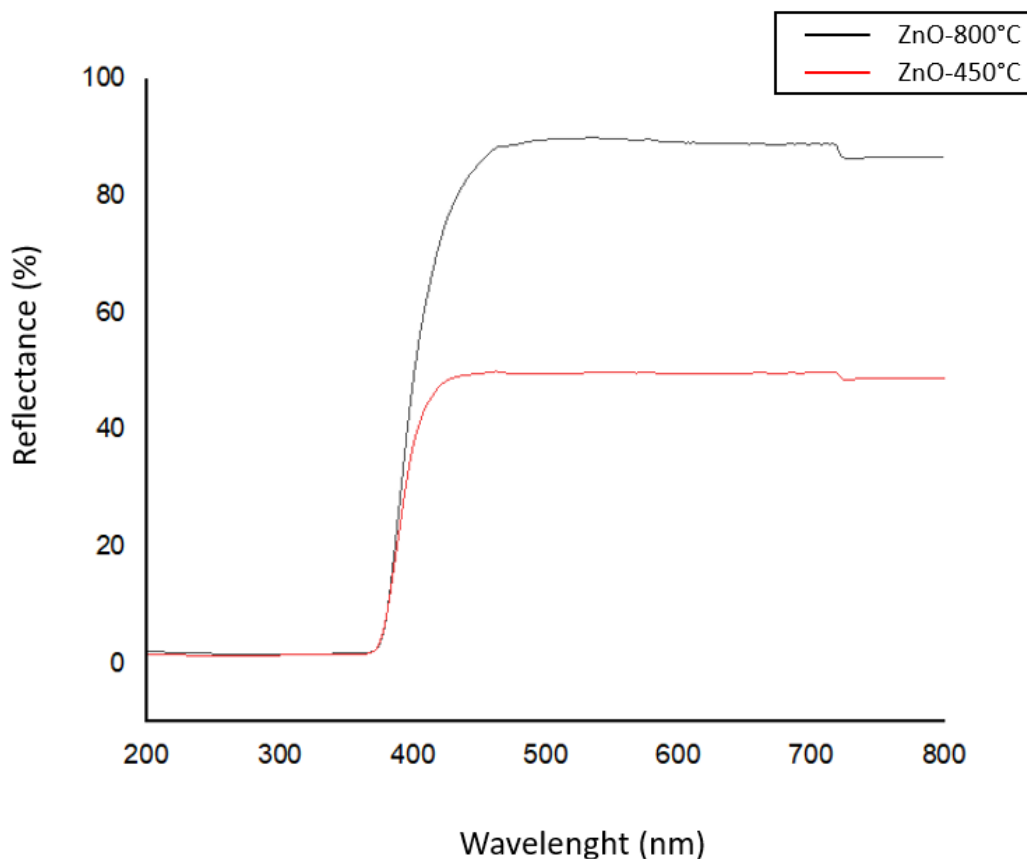


Figure 3. 5: Reflectance spectra of ZnO NPs calcined at 800°C and 450°C.

The average band gap was observed in a range from 3.28 to 3.26 eV (Figure 3.6), where the size of ZnO NPs (ZnO-450°C and ZnO-800°C, respectively) produced by annealing changed from 25.11 nm to 52.65 nm.

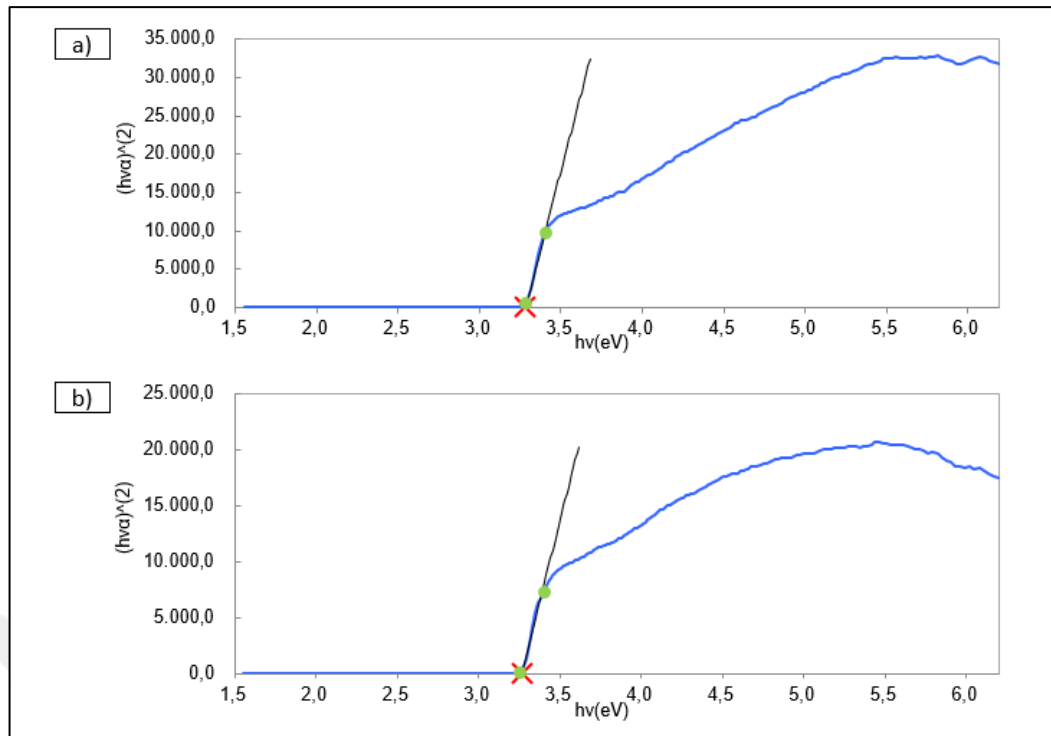


Figure 3. 6: Band gap energy of ZnO NPs a) ZnO-800°C b) ZnO-450°C.

3.2. Antimicrobial Activity of ZnO NPs

The antibacterial activity of ZnO NPs prepared as ZnO-800°C and ZnO-450°C by the sol-gel method was investigated in 3 different species of against positive (*S. aureus* and *B. cereus*) and negative (*E. coli*) pathogenic bacteria. Antibacterial activities of the produced ZnO NPs were determined using agar disc diffusion and agar well methods. In addition to the two different methods, the effect of different concentrations (50000, 5000 and 2500 ppm) and UV on the antibacterial activity of ZnO NPs was also investigated.

In the first experiment, the agar well diffusion method was used. The high concentration of ZnO NPs (50000 ppm) in both ZnO-800°C and ZnO-450°C showed no antibacterial effect on pathogenic Gram-positive and pathogenic Gram-negative bacteria, as shown in Figure 3.7 (a-c), because no zone was formed in the inhibition zone.

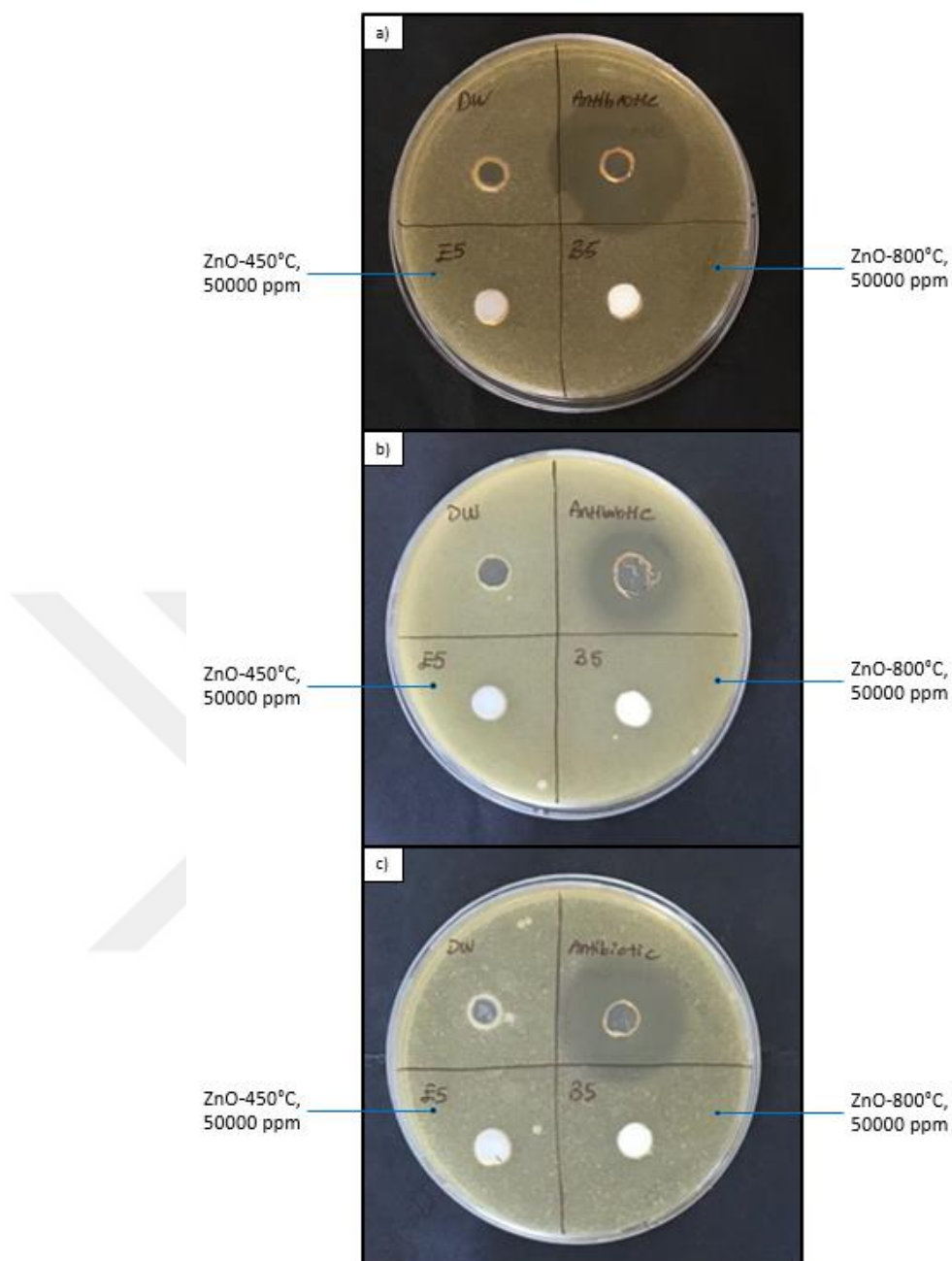


Figure 3. 7: Comparison of bacterial activity of ZnO-800°C and ZnO-450°C on a) *B. cereus* b) *E. coli* c) *S. aureus* in the 1st experiment.

In the second experiment, the same agar well diffusion method was used, but the lower concentration of ZnO NPs (5000 ppm) was performed in both ZnO-800°C and ZnO-450°C. No zone formation was observed against the pathogenic Gram-positive bacteria in Figure 8.8 (a-c) and the pathogenic Gram-negative bacteria in Figure 3.8 (b) for both the ZnO-800°C and ZnO-450°C NPs. The result was that both the higher concentration of 50000 ppm and the lower concentration of 5000 ppm ZnO NPs in

both ZnO-800°C and ZnO-450°C NPs had no effect on the antibacterial activity using the agar well diffusion method.

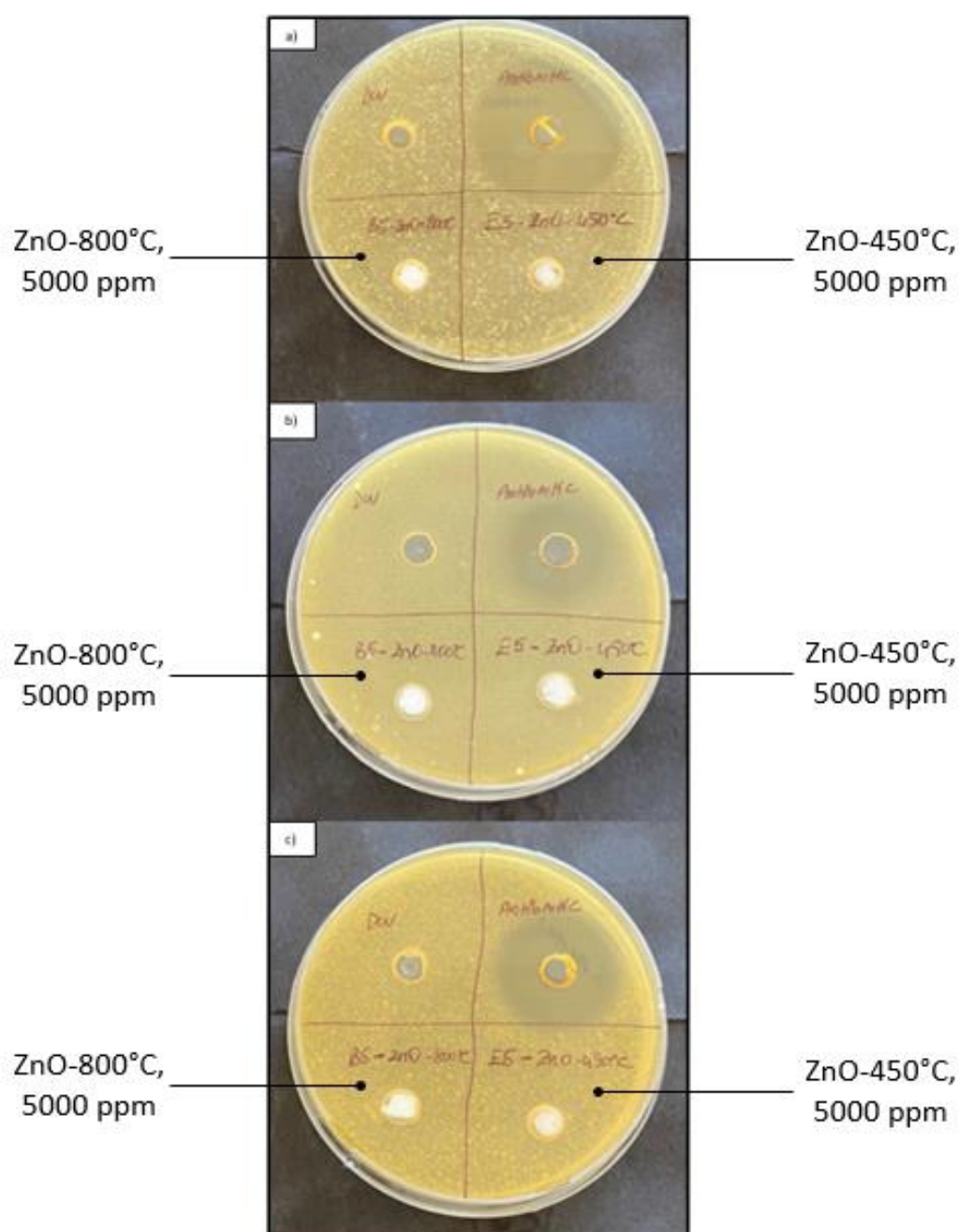


Figure 3. 8: Comparison of bacterial activity of ZnO-800°C and ZnO-450°C on a) *B. cereus* b) *E. coli* c) *S. aureus* in the 2nd experiment.

In the third experiment, two concentrations were performed: 2500 ppm, a lower concentration than in the previous study, and 5000 ppm as in the previous experiments, to observe the different methods on antibacterial activity. Therefore, the method was changed to the agar disk method. Additionally, UV illumination was applied to

enhance the ZnO NPs-bacteria interaction. In contrast to the previous two experiments, some differences were found in the results of the third experiment. Although both ZnO-800°C and ZnO-450°C NPs showed no antibacterial effect on gram-positive bacteria *B.cereus* and gram-negative bacteria *E.coli* (Figure 3.9 - 3.10), unexpectedly during the experiment, the sliding of the disks in the Petri dish containing the gram-positive bacteria *S.aureus* showed that the ZnO NP powder solutions greatly slowed the growth of bacteria in the areas where the disks slid, as shown in Figure 3.11. The results show that the ZnO NPs have a bacteriostatic effect against Gram-positive bacteria *S. aureus*. The most significant slowdown in bacterial growth for Gram-positive bacteria *S.aureus* was observed for ZnO-450°C NPs with a smaller crystallite size of 25.11 nm exposed to UV irradiation.



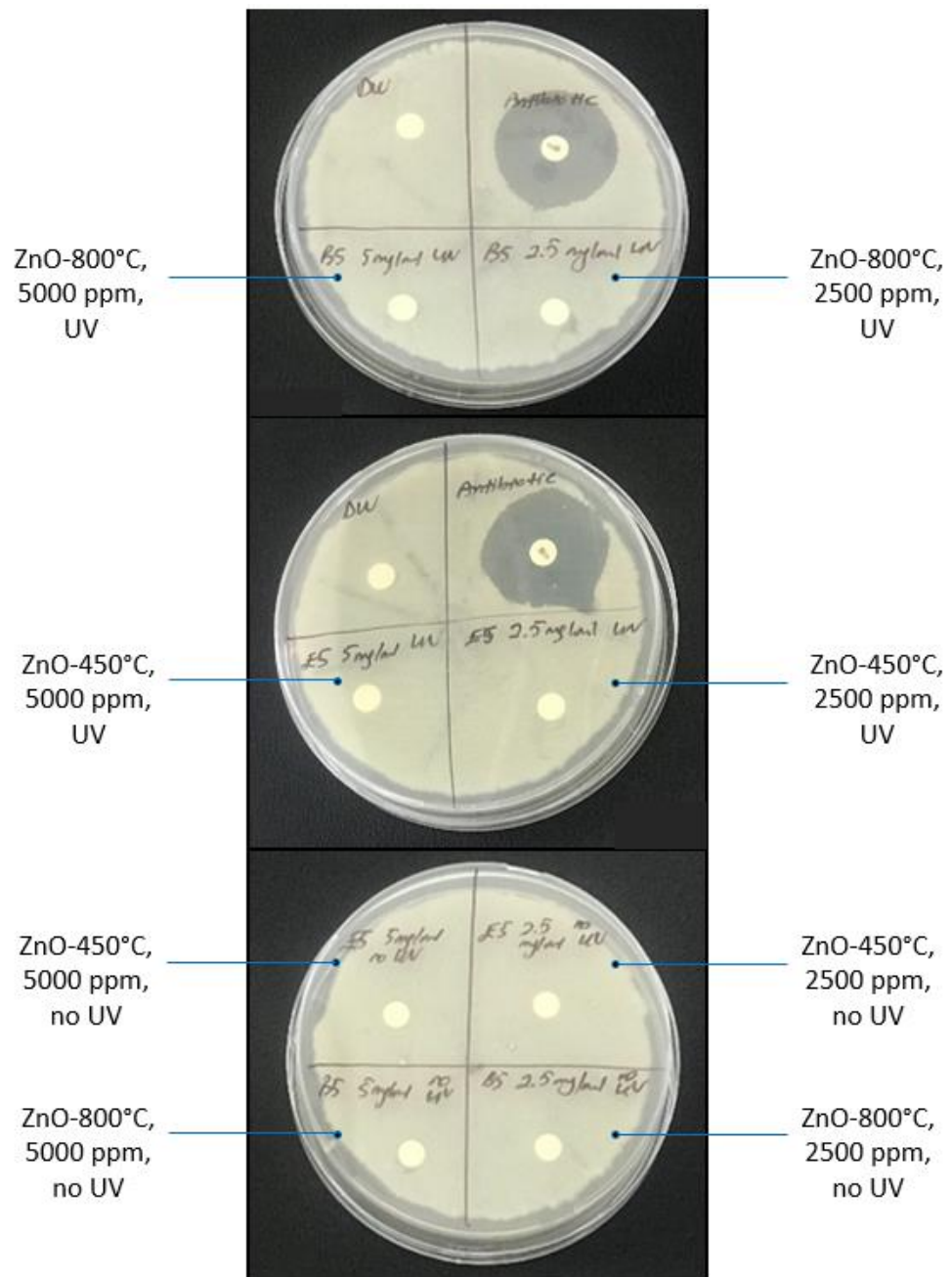


Figure 3. 9: Comparison of bacterial activity of ZnO-800°C and ZnO-450°C on *B. Cereus* in the 3rd experiment.

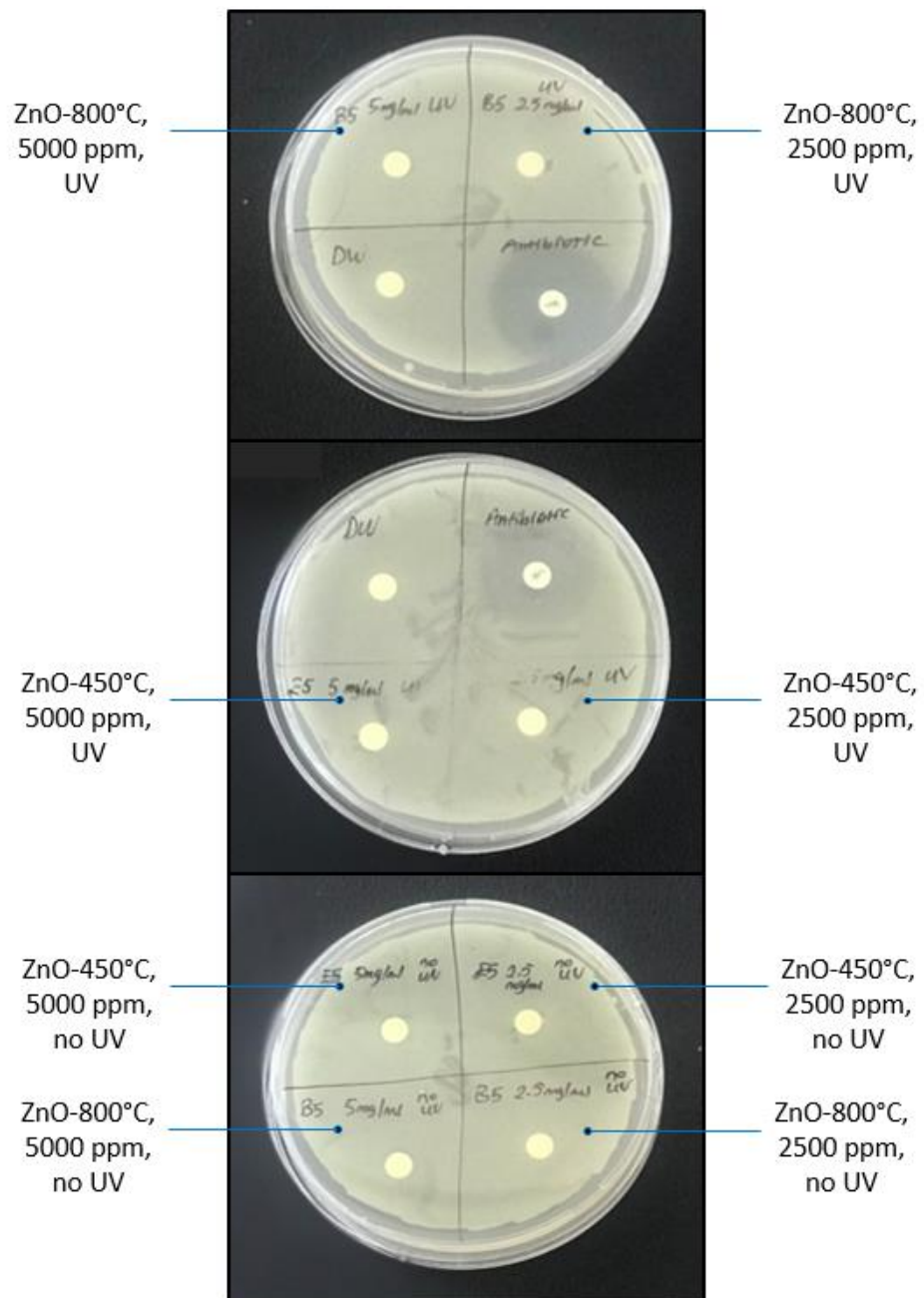


Figure 3. 10: Comparison of bacterial activity of ZnO-800°C and ZnO-450°C on *E.coli* in the 3rd experiment.

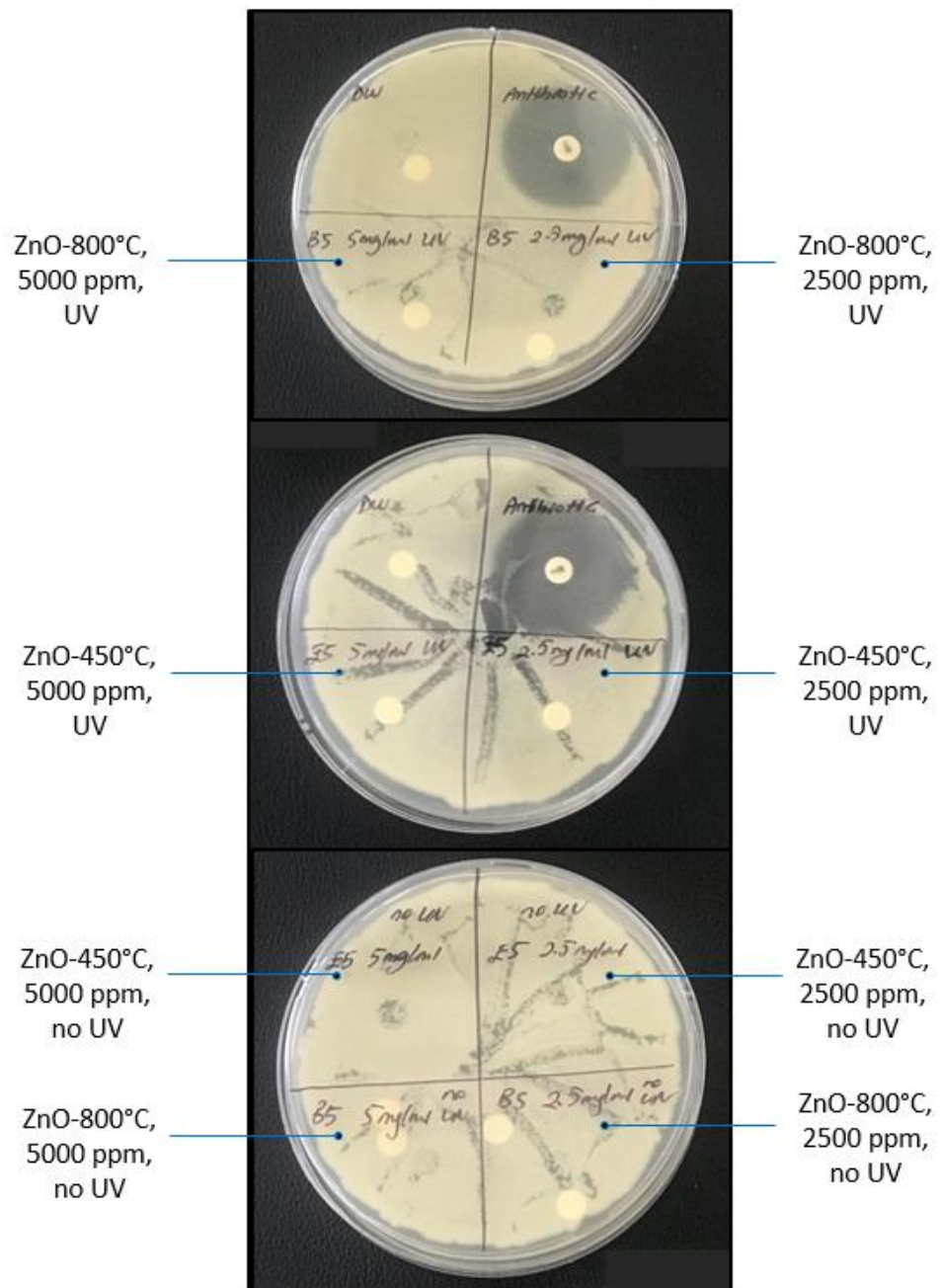


Figure 3. 11: Comparison of bacterial activity of ZnO-800°C and ZnO-450°C on *S. aureus* in the 3rd experiment.

4. DISCUSSION AND CONCLUSION

Zinc oxide nanoparticles designated ZnO-800°C and ZnO-450°C were successfully prepared by a simple sol-gel method. All ZnO nanoparticles were studied in terms of their surface morphology (e.g., surface shape and elemental ratio), structural parameters (e.g., average crystal size), and optical properties (e.g., band gap energy and reflectivity). The characterization of the prepared ZnO NPs was carried out by different techniques such as XRD, SEM, EDS, UV-VIS-NIR spectrophotometer. The results showed that XRD and SEM confirmed the presence of ZnO NPs. The antibacterial activity of ZnO NPs was tested in 3 separate experiments.

According to the XRD results, the studied powdered nanoparticles (ZnO-800°C and ZnO-450°C) are polycrystalline and consist of ZnO nanocrystals in the typical wurtzite hexagonal zincite phase. The smaller particle size was achieved by increasing the washing time to 6 with decreasing annealing temperature to 450°C. The average crystallite size of the ZnO NPs was calculated to be 52.65 nm for ZnO-800°C and 25.11 nm for ZnO-450°C using Scherrer's equation.

In the results of SEM, it was found that the calcination temperature and the size and agglomeration of ZnO nanoparticles are directly proportional. The ZnO-800°C nanoparticles had an irregular shape and were larger in size, while the ZnO-450°C nanoparticles had a more spherical shape and a smaller size. These characterization results show that the calcination temperature and ZnO nanoparticle sizes are directly proportional. The particles in both SEM are arranged in bundles due to agglomeration. The size of ZnO NPs decreased and the agglomeration decreased with decreasing calcination temperature to 450°C [65]. Moreover, the results of EDS, showed that the prepared ZnO nanoparticles consisted only of Zn and O.

UV-Vis-NIR spectrophotometer has been used both to measure the optical properties of ZnO NPs produced by sol-gel method. Parra and Haque explained that the photons that lack the necessary energy to interact with electrons or atoms have a high probability of reflection [66]. The optical properties of the ZnO NPs were affected by the particle size of the ZnO NPs. The UV-VIS reflectance spectrum has less effect on scattering than on absorption. The sudden decrease in reflectance at a certain wavelength, corresponding to the optical band gap, is interpreted as a nearly uniform distribution of particles in the sample [67]. The band gap energy was determined to be

3.26 eV for ZnO-800°C and 3.28 eV for ZnO-450°C, which increased with decreasing annealing temperature. The increase in bandgap energy with decreasing particle size is the result of the effects of quantum size [67]. Compared to the ZnO bulk (3.37 eV), the optical absorption edge shifted slightly to longer wavelengths, which could be due to the increase in particle size at higher calcination temperature [66]. These results are in agreement with the calculations of the average crystal size of 52.65 nm for ZnO-800°C and of 25.11 nm for ZnO-450°C from the XRD.

The antibacterial activity of ZnO-800°C and ZnO-450°C NPs was investigated against three different bacterial strains (gram-positive bacteria *B.cereus* and *S.aureus* and gram-negative bacteria *E.coli*) using two different methods (agar well diffusion and agar disc diffusion). In the two experiments, there was no antibacterial activity. It was thought that the ZnO NPs were not well dispersed and the ZnO NPs were agglomerated due to the very high concentration of ZnO NPs. In addition to the high concentrations, Ismail et. al. showed that the calcination temperature of 800°C increases the agglomeration with large size and showed the best results of antibacterial activity of ZnO NPs at a calcination temperature of 300°C. Since no zone of inhibition formed in these experiments, it can be presumed that there was no direct interaction between the ZnO NPs and the bacterial cell membrane and no indirect interaction with the release of Zn^{+2} , and also no production of active ROS as hydrogen peroxide (H_2O_2) in the cells [65].

The significant results were observed in the third experiment performed by agar disc diffusion method after irradiating the ZnO NPs with UV light just before the experiment. Although both ZnO-800°C and ZnO-450°C NPs showed no antibacterial effect on gram-positive bacteria *B.cereus* and gram-negative bacteria *E.coli*, unexpectedly during the experiment, the sliding of the disks in the Petri dish containing the gram-positive bacteria *S.aureus* showed that the ZnO NP powder solutions greatly slowed the growth of bacteria in the areas where the disks slid. The results show that the ZnO NPs have a bacteriostatic effect against Gram-positive bacteria *S. aureus*. The most significant slowdown in bacterial growth for Gram-positive bacteria *S.aureus* was observed for ZnO-450°C NPs with a smaller crystallite size of 25.11 nm exposed to UV irradiation. Sirelkhatim et al. have explained the UV illumination effect on the antibacterial activity of ZnO NPs by the fact that ZnO absorbs UV light to a high extent and shows a better response to UV light, which dramatically increases its conductivity. This property significantly activates the interaction of ZnO with bacteria. The

photoconductivity remains long after the UV light is turned off and is attributed to a region of electron depletion on the surface that is strongly associated with negative oxygen species (O_2^- , O_2^{2-}) adsorbed on the surface. UV illumination causes rapid desorption of this loosely bound oxygen from the surface. This photoinduced oxidation process can damage and inactivate organisms [68]. Furthermore, ZnO-450°C NPs has smaller particle size that increased the efficiency in inhibiting the growth of bacteria and decreased the energy of band gap (3.28 eV for ZnO-450°C NPs). The lower the energy of the band gap in the electronic structure of ZnO, the more easily the energy of the photons is absorbed, so that the electrons in the structure move from VB (valence band) to CB (conduction band). The holes formed in VB and the free electrons generated in the CB reactions are related to the antibacterial activity of ZnO, which leads to the production of H_2O_2 molecules that penetrate the membrane and cause lethal damage [65], [68]. According to this result, it can be said that the method of antibacterial activity test, the irradiation of ZnO NPs with UV light, the particle size and the concentration of ZnO NPs all have an influence on the antibacterial activity of ZnO NPs.

A number of developments closely related to ZnO NPs in various fields such as medical imaging, drug delivery, electronic devices, and antibacterial activity have proven the bright future of nanoscience and nanotechnology. Future continuous research efforts should focus on the fabrication and application of new devices or products. With further efforts to optimize and improve, the development of devices based on ZnO such as nanogenerators and solar cells, as well as products based on ZnO NPs in the cosmetic and biomedical fields, has the potential to change lifestyles.

ZnO NPs, which are nanocrystalline semiconductors, are attracting more and more attention for the development of devices, sensors and products such as medicine, agriculture, chemicals, cosmetics and solar cells due to their unique innovative electrical, optical and mechanical properties. These results suggest that the fabricated ZnO NPs may be good candidates for various biotechnological applications. In this context, these simple, low-cost and antibacterial nanoparticles can be easily integrated into sensors, products or devices and preferred as bioagents with antibacterial activity.

REFERENCES

- [1] Moradpoor H., Safaei M., Mozaffari H. R., Sharifi R., Imani M. M., Golshahe A. and Bashardoustf N., (2020), "An overview of recent progress in dental applications of zinc oxide nanoparticles," RSC Advances, 11 (34), 21189–21206.
- [2] Nasrollahzadeh M., Sajadi S. M. and Mohaddeseh Sajjadi Z. I., (2019), "An Introduction to Nanotechnology," 1st Edition, Academic Press.
- [3] Silpa S., (2016), "Nanotechnology-Present Revolutionary Biotechnology," International Journal of Pharma Research and Health Sciences, 4 (4), 1261-1267.
- [4] Selvanayaki R., Rameshbabu M., Muthupandi S., Razia M., Florence S. S., Ravichandran K. and Prabha K., (2022), "Structural, optical and electrical conductivity studies of pure and Fe doped ZincOxide (ZnO) nanoparticles", Materials Today:Proceedings, 49 (7), 2628-2631.
- [5] Milojević S., (2012), "Multidisciplinary Cognitive Content Of Nanoscience And Nanotechnology," Journal of Nanoparticle Research, 14 (1), 685.
- [6] Amin R., Hwang S., Park S. H., (2011), "Nanobiotechnology: An Interface Between Nanotechnology And Biotechnology," Nano: Brief Reports and Reviews, 6 (2), 101–111.
- [7] Rana K. L., Kour D., Yadav N. and Yadav A. N., (2020), "Microbial Endophytes: Prospects for Sustainable Agriculture", 1st Edition, Elsevier.
- [8] Tartaj P., Morales M. P., González-Carreño T., Veintemillas-Verdaguer S. and Serna C. J., (2005), "Advances in magnetic nanoparticles for biotechnology applications," Journal of Magnetism and Magnetic Materials, 290-291 (1), 28-34.
- [9] Chavda V. P., (2019), "Applications of Targeted Nano-Drugs and Delivery Systems", 1st Edition, Elsevier.
- [10] Shen W. Z., Cetinel S. and Montemagno C., (2018), "Application of biomolecular recognition via magnetic nanoparticle in nanobiotechnology," Journal of Nanoparticle Research, 20 (5), 130.
- [11] Steffy K., Shanthi G., Maroky A. S. and Selvakumar S., (2018), "Synthesis and characterization of ZnO phytonanocomposite using Strychnos nux-vomica L. (Loganiaceae) and antimicrobial activity against multidrug-resistant bacterial strains from diabetic foot ulcer", Journal of Advanced Research, 9, 69-77.

- [12] Farre´ M. and Barcelo´ D., (2012), "Comprehensive Analytical Chemistry", 1st Edition, Elsevier.
- [13] Renero-Lecuna C., Iturrioz-Rodríguez N., González-Lavado E., Padín-González E., Navarro-Palomares E., Valdivia-Fernández L., García-Hevia L., Fanarraga M. L. and González-Legarreta L., (2019), "Effect of Size, Shape, and Composition on the Interaction of Different Nanomaterials with HeLa Cells," *Journal of Nanomaterials*, 28, 1-11.
- [14] Parashar U. K., Saxena P. S. and Srivastava A., (2008), "Role of Nnanomaterials In Biotechnology," *Digest Journal of Nanomaterials and Biostructures*, 3 (2), 81 - 87.
- [15] Saleh T. A., (2020), "Nanomaterials: Classification, properties, and environmental toxicities," *Environmental Technology & Innovation*, 20 (11), 1-16.
- [16] Xu J. and Wang L., (2019), "Nano-Inspired Biosensors for Protein Assay with Clinical Applications", 1st Edition, Elsevier.
- [17] Warren K. D., Arnold R. G., Bishop T. L., Lindholm L. C. and Betterton E. A., (1995), "Kinetics and mechanism of reductive dehalogenation of carbon tetrachloride using zero-valence metals", *Journal of Hazardous Materials*, 41 (2-3), 217–227.
- [18] Liu Y., Li F., Huang H., Mao B., Liu Y. and Kang Z., (2020), "Optoelectronic and photocatalytic properties of I–III–VI QDs: Bridging between traditional and emerging new QDs", *Journal of Semiconductors*, 41 (9), 1-13.
- [19] Munavalli B. B., Naik B. B., Torvi A. I. and Kariduraganavar M. Y., (2019), "Functional Polymers", 1st Edition, Springer.
- [20] Stankic S., Suman S., Haque F. and Vidic J., (2016), "Pure and multi metal oxide nanoparticles: synthesis, antibacterial and cytotoxic properties", *Journal of Nanobiotechnology*, 14 (73), 1-20.
- [21] Chavali M. S. and Nikolova M. P., (2019), "Metal oxide nanoparticles and their applications in nanotechnology", *SN Applied Sciences*, 1 (607), 1-30.
- [22] Madhumitha G., Elango G. and Roopan S. M., (2015), "Biotechnological aspects of ZnO nanoparticles: overview on synthesis and its applications", *Applied Microbiology Biotechnology*, 100 (2), 571-581.
- [23] Girigoswami K., (2018), "Cellular and Molecular Toxicology of Nanoparticles", 1st Edition, Springer.

- [24] Nikolova M. P. and Chavali M. S., (2020), "Metal Oxide Nanoparticles as Biomedical Materials", *Biomimetics*, 5 (27), 1-47.
- [25] Dizaj S. M., Barzegar-Jalali M., Zarrintan M. H. and Adibkia K., (2014), "Antimicrobial activity of the metals and metal oxide nanoparticles", *Materials Science and Engineering: C*, 44, 278–284.
- [26] Palchetti S., Pozzi D., Capriotti A. L., La Barbera G., Zenezini Chiozzi R., Digiacoio L., Peruzzi G., Caracciolo G. and Laganà A., (2017), "Influence of dynamic flow environment on nanoparticle-protein corona: From protein patterns to uptake in cancer cells", *Colloids and Surfaces B: Biointerfaces*, 153, 263-271.
- [27] Rehman S., Jermy B. R., Akhtar S., Borgio J. F., Azeez S. A., Ravinayagam V., Jindan R. A., Alsalem Z. H., Buhameid A. and Gani A., (2019), "Isolation and characterization of a novel thermophile; *Bacillus haynesii*, applied for the green synthesis of ZnO nanoparticles", *Artificial Cells, Nanomedicine, and Biotechnology*, 47 (1), 2072–2082.
- [28] Jiang J., Pi J. and Cai J., (2018), "The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications", *Bioinorganic Chemistry and Applications*, 2018 (3), 1-18.
- [29] Cruz D. M., Mostafavi E., Vernet-Crua A., Barabadi H., Shah V., Cholula-Díaz J. L. and Webster T. J., (2020), "Green nanotechnology-based zinc oxide (ZnO) nanomaterials for biomedical applications: a review", *Journal of Physics: Materials*, 3 (3), 1-38.
- [30] Haq A. N. U., Nadhman A., Ullah I., Mustafa G., Yasinzaï M. and Khan I., (2017), "Synthesis Approaches of Zinc Oxide Nanoparticles: The Dilemma of Ecotoxicity", *Journal of Nanomaterials*, 2017 (12), 1–14.
- [31] Fan Z. and Lu J. G., (2005), "Zinc Oxide Nanostructures: Synthesis and Properties", *Journal of Nanoscience and Nanotechnology*, 5 (10), 1561-1573.
- [32] Borysiewicz M. A., (2019), "ZnO as a Functional Material, a Review", *Crystals*, 9 (10), 1-29.
- [33] Consonni V. and Lord A. M., (2021), "Polarity in ZnO nanowires: A critical issue for piezotronic and piezoelectric devices", *Nano Energy*, 83, 105789.
- [34] Koike K., Aoki T., Fujimoto R., Sasa S., Yano M., Gonda S. I., Ishigami R. and Kume K., (2012), "Radiation hardness of single-crystalline zinc oxide films", *Physica Status Solidi (c)*, 9 (7), 1577–1579.
- [35] Yuldashev S. U., Yalishev V. S., Cho H. D. and Kang T. W., (2016), "Thermal Conductivity of ZnO Single Nanowire", *Journal of Nanoscience and Nanotechnology*, 16 (2), 1592–1595.

- [36] Vaseem M., Umar A. and Hahn Y. B., (2010), "Metal Oxide Nanostructures and Their Applications", 1st Edition, American Scientific Publishers.
- [37] Azeez H. H., Barzinjy A. A., Hamad S., (2020), "Structure, Synthesis and Applications of ZnO Nanoparticles: A Review", Jordan Journal of Physics, 13 (2), 123-135.
- [38] Azem N. F. A., Birlik I., (2018), "Optimization of ZnO Nanoparticles Prepared by Sol-Gel Technique", Dokuz Eylul University-Faculty of Engineering Journal of Science and Engineering, 20 (58), 121-127.
- [39] Šebesta M., Kolencík M., Sunil B. R., Illa R., Mosnáček J., Ingle A. P., Urík M., (2021), "Field Application of ZnO and TiO₂ Nanoparticles on Agricultural Plants", Agronomy, 11 (11), 1-13.
- [40] Arya S., Mahajan P., Mahajan S., Khosla A., Datt R., Gupta V., Young S. J., Oruganti S. K., (2021), "Review—Influence of Processing Parameters to Control Morphology and Optical Properties of Sol-Gel Synthesized ZnO Nanoparticles", ECS Journal of Solid State Science and Technology, 10 (2), 023002.
- [41] Oprea O., Andronescu E., Fikai D., Fikai A., Oktar F. N., Yetmez M., (2014), "ZnO Applications and Challenges", Current Organic Chemistry, 18 (2), 192-203.
- [42] Kayani Z. N., Saleemi F., Batool I., (2015), "Effect of calcination temperature on the properties of ZnO nanoparticles", Applied Physics A, 119 (2), 713–720.
- [43] Baskar R., Dai J., Wenlong N., Yeo R., Yeoh K. W., (2014), "Biological response of cancer cells to radiation treatment", Frontiers in Molecular Bioscience, 1 (24), 1-9.
- [44] Al-Lawati J. A., (2017), "Diabetes Mellitus: A Local and Global Public Health Emergency!", Oman Medical Journal , 32 (3), 177–179.
- [45] Kim M. H., (2016), "Biological effects of zinc oxide nanoparticles on inflammation", Cellmed Orthocellular Medicine and Pharmaceutical Association, 6 (4), 1-6.
- [46] Akan A., Mısırlıoğlu E. D., Kocabaş C. N., (2015), "Recent Improvements in the Pathogenesis of Atopic Dermatitis", The Journal of Current Pediatrics, 13 (3), 194-200.
- [47] Wiegand C., Hipler U. C., Boldt S., Strehle J., Wollina U., (2013), "Skin-protective effects of a zinc oxide-functionalized textile and its relevance for atopic dermatitis", Clinical, Cosmetic and Investigational Dermatology, 6, 115–121.

- [48] Eixenberger J. E., Anders C. B., Wada K., Reddy K. M., Brown R. J., Moreno-Ramirez J., Weltner A. E., Karthik C., Tenne D. A., Fologea D., Wingett D. G., (2019), "Defect Engineering of ZnO Nanoparticles for Bioimaging Applications", *ACS Applied Materials & Interfaces*, 11 (28), 24933–24944.
- [49] Gold K., Slay B., Knackstedt M., Gaharwar A. K., (2018), "Antimicrobial Activity of Metal and Metal-Oxide Based Nanoparticles", *Advanced Therapeutics*, 3 (1), 1700033.
- [50] Malanovic N., Lohner K., (2016), "Gram-positive bacterial cell envelopes: The impact on the activity of antimicrobial peptides", *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1858 (5), 936-946.
- [51] Siswanto S., Rochman N. T., Akwalia P. R., (2017), "Fabrication and characterization of Zinc Oxide (ZnO) nanoparticle by sol-gel method", *Journal of Physics: Conference Series*, 853 (012041), 1-5.
- [52] Rane A. V., Kanny K., Abitha V. K., Thomas S., (2018), "Synthesis of Inorganic Nanomaterials: Advances and Key Technologies", 1st Edition, Elsevier Science & Technology.
- [53] Kouhpanji M. R. Z., Stadler B. J. H., (2020), "A Guideline for Effectively Synthesizing and Characterizing Magnetic Nanoparticles for Advancing Nanobiotechnology: A Review", *Sensors*, 20 (9), 1-37.
- [54] Hench L. L., West J. K., (1990), "The sol-gel process", *Chemical Reviews*, 90 (1), 33–72.
- [55] Wang X., (2020), "Preparation, synthesis and application of Sol-gel method", Vidyasirimedhi Institute of Science and Technology: Rayong.
- [56] Dimitriev Y., Ivanova Y., Iordanova R., (2008), "History of Sol-Gel Science and Technology (Review)", *Journal of the University of Chemical Technology and Metallurgy*, 43 (2), 181-192.
- [57] Sakka S., (2013), "Handbook of Advanced Ceramics: Materials, Applications, Processing, and Properties", 2nd Edition, Elsevier Academic Press Inc.
- [58] Vishwakarma A., Singh S. P., (2020), "Synthesis of Zinc Oxide Nanoparticle by Sol-Gel Method and Study its Characterization", *International Journal for Research in Applied Science & Engineering Technology (IJRASET)*, 8 (4), 1624-1628.
- [59] Pintarić L. M., Škoc M. S., Bilić V. L., Pokrovac I., Kosalec I., Rezić I., (2020), "Synthesis, Modification and Characterization of Antimicrobial Textile Surface Containing ZnO Nanoparticles", *Polymers*, 12 (6), 1210, 2020.

- [60] Tseng Y. K., Chuang M. H., Chen Y. C., Wu C. H., (2012), "Synthesis of 1D, 2D, and 3D ZnO Polycrystalline Nanostructures Using the Sol-Gel Method", *Journal of Nanotechnology*, 2012 (12), 1-8.
- [61] Toygun Ş., Köneçoğlu G., Kalpaklı Y., (2013), "General Principles of Sol-Gel", *Journal of Engineering and Natural Sciences*, 31 (4), 456-476.
- [62] Jeevanandam J., Chan Y. S., Danquah M. K., (2016), "Biosynthesis of Metal and Metal Oxide Nanoparticles", *ChemBioEng Reviews*, 3 (2), 55–67.
- [63] Sangeetha A., Jaya Seeli S., Bhuvana K. P., Abdul Kader M., Nayak S. K., (2019), "Correlation between calcination temperature and optical parameter of zinc oxide (ZnO) nanoparticles", *Journal of Sol-Gel Science and Technology*, 91 (2) , 261-272.
- [64] Manikandan B., Endo T., Kaneko S., Murali K. R., John R., (2018), "Properties of sol gel synthesized ZnO nanoparticles", *Journal of Materials Science: Materials in Electronics*, 29 (2), 9474–9485.
- [65] Ismail A. M., Menazea A. A., Kabary H. A., El-Sherbiny A. E., Samy A., (2019), "The influence of calcination temperature on structural and antimicrobial characteristics of zinc oxide nanoparticles synthesized by Sol-Gel method", *Journal of Molecular Structure*, 1196, 332-337.
- [66] Parra M. R., Haque F. Z., (2014), "Aqueous chemical route synthesis and the effect of calcination temperature on the structural and optical properties of ZnO nanoparticles", *Journal of Materials Research and Technology*, 3 (2), 363-369.
- [67] Samuel M. S., George L. B. K., (2009), "Optical Properties of ZnO Nanoparticules", *SB Academic Review*, 16 (1-2), 57-65.
- [68] Sirelkhatim A., Mahmud S., Seenii A., Kaus N. H. M., Ann L. C., Bakhori, H. Hasan S. K. M., Mohamad D., (2015), "Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism", *Nano-Micro Letters*, 7 (3), 219–242.

BIOGRAPHY

Büşra Eren graduated from Üsküdar University, Faculty of Engineering and Natural Sciences, Department of Bioengineering, which she started in 2014, with a first degree in 2019. She started her graduate program at Gebze Technical University Biotechnology Institute in the fall semester of the same year. She presented a poster in the 4th International Conference on Photonics Research (INTERPHOTONICS) 2022 namely “Influence of The Size of ZnO Nanoparticles on Their Optical Properties”.

