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ANTIBACTERIAL ACTIVITIES OF NICKEL DOPED ZnO THIN FILMS PREPARED BY SILAR METHOD

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ANTIBACTERIAL ACTIVITIES OF NICKEL DOPED ZnO THIN FILMS PREPARED BY SILAR METHOD

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June 2023

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Sarah HASAN ALI ISMAEL

ABSTRACT

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Master of Science in Physics

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June 2023

ZnO thin films are widely utilized in various technological applications due to their low cost, wide bandgap, high electrical conductivity, and optical transparency. This thesis investigates the structural and surface morphology, antibacterial properties, bacterial adhesion, and organism survival count of nickel (Ni) doped ZnO thin films grown on FTO substrates produced by the SILAR method. The nickel (Ni) doping ratio in the ZnO thin films was varied from 1 % to 3 %. SEM-EDS analysis showed that nano-flower structures were formed on the surface of the ZnO thin films. It was observed that the structures formed were dependent on the nickel doping. Among the produced samples, FTO-Ni 2 % exhibited the highest antibacterial activity against E. coli ATCC 35218, while no activity was observed against S. aureus ATCC 25923. In addition, the highest bacterial adhesion rate was observed on the sample doped with Ni 2 %.

2023, 51 pages

Keywords: SILAR method, Zinc oxide, Ni doped, Thin films

ÖZET

SILAR YÖNTEMİYLE HAZIRLANAN NİKEL KATKILI ZnO YARIİLETKEN İNCE FİLMLERİN ANTİBAKTERİYEL AKTİVİTELERİ

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ZnO ince filmler düşük maliyeti, geniş bant aralığı, elektriksel iletkenliği ve optik geçirgenliği yüksek olmasından dolayı teknolojik açıdan pek çok yerde kullanılırlar. Bu tez çalışmasında, SILAR yöntemi üretilen FTO alltaş üzerine büyütülen Nikel katkılı ZnO ince filmlerin yapısal, yüzeysel morfolojisi, antibakteriyel özellikleri, bakteriyel yapışma ve hayatta kalma organizma sayımı özellikleri incelenmiştir. Katkılı ZnO ince filmlerde Nikel (Ni) katkılama oranı % 1'den % 3'e değiştirilerek elde edilmiştir. SEM-EDS analizlerinden üretilen ZnO ince filmlerin yüzeyde nano-çiçek yapıların oluşturmuştur. Oluşan yapılar Nikel katkısına bağlı olarak değiştiği gözlendi. Üretilen numunelerden FTO-Ni % 2 numunesinin E. coli ATCC 35218'e karşı en yüksek antibakteriel aktiviteye sahip olduğunu, S. aureus ATCC 25923'e karşı hiçbir aktivite gözlenmediğini gösterdi. Ayrıca numuneler üzerine bakteri organizmalarının yapışma oranı en yüksek Ni % 2 katkılı numunede en yüksek olduğu görülmüştür.

2023, 51 sayfa

Anahtar Kelimeler: SILAR metodu, Çinko oksit, Ni katkılama, İnce film

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LIST OF SYMBOLS

A	Absorbance value
α	Absorption coefficient
Å	Angstrom value
θ	Bragg angle
k_B	Boltzman constant
β	Half peak width
°C	Centigrade degree
Cs	Crystal size
N	Complex refractive index
δ	Dislocation density
d_{XRD}	Experimental distance
E_g	Energy gap
k_o	Extinction coefficient
eV	Electron volt
ν	Frequency value
d_{hkl}	Interplaner distance
$M_{(hkl)}$	Miller indices
M	Molar value
nm	Nanometer value
h	Planck's constant
E_{ph}	Phonon energy
I_R	Reflected intensity
n_o	Refractive index
R	Reflectance
d_{CARD}	Standard distance
I_o	Standard (Incidence) intensity
K	Shape factor
I	Transmittance intensity
T	Transmittance value
TCO	Transparent conductive oxides
λ	Wavelength

LIST OF ABBREVIATIONS

EDS	Energy dispersive X-ray spectroscopy
FESEM	Field emission scanning electron microscope
UV-VIS	Ultraviolet-visible
XRD	X-ray diffraction

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1. INTRODUCTION

1.1 Overview of Nanotechnology

One of the most important scientific endeavours of the early 21st century, nanotechnology takes use of the unique properties of atomic and molecular assemblages built at the nanoscale. Our ability to manipulate the physical, chemical, and biological characteristics of nanoparticles has allowed for their rational design and usage in the delivery of drugs, as image contrast agents, and in diagnostics. The integration of these newly acquired abilities, together with breakthroughs in imaging, bioinformatics, and systems biology, has considerable potential for tackling some of biology's most challenging biochemical and genetic challenges. While many current technological advances are beneficial to biology, nanotechnology may have themost far reaching and far-reaching impact on basic research, drug development, and clinical care.

As nanotechnology functions on the nanoscale level the same scale as biomolecules it presents an enormous pool of resources and applications. In the near future, these nanoscale probes may be used to track the motion of cells (Reddy *et al*. 2007), molecules (Johari and Rana 2012), and atoms (Chowdhuri *et al*. 2009). Other potential applications include chip based nanolabs with monitoring capabilities, enhanced image contrast agents, and drug-delivery platforms. This unprecedented capability to watch and change complex systems in vivo and in real time significantly expands the arsenal of techniques now available for medication administration and noninvasive drug monitoring. This data is utilised to gain insight into the underlying molecular and cellular processes that contribute to the onset of illness. Constructions that can integrate several functions into one tiny item are now conceivable thanks to nanotechnology, and this in turn allows scientists to monitor and identify cellular and molecular alterations associated with illness (Kitamura *et al*. 2007). Because of its versatility, a single nanoparticle may be used to target a specific tissue or cell type, transport a contrast agent for noninvasive imaging, and deliver a therapeutic payload. To ensure that the payload has been delivered and is having the intended therapeutic effect, nanoparticles may also contain a reporter, such as an apoptotic marker. Such combinatorial nanostructures hold promise for realizing

'personalised medicine,' in which medication administration is tailored to each patient's unique reaction. Although it seems like something from the future, many companies have already built multifunctional nanodevices and are testing them in in vitro and in vivo settings (Bratovčić *et al.* 2015). It is difficult to explore the significance of nanotechnology to biologists without first addressing language. To describe nanotechnology, It's refer to the NNI's definition: "research and development at the atomic, molecular, or macromolecular scale that culminates in the carefully planned design and implementation of structures, devices, and systems with a length scale of 1 nanometer to 100 nanometers" (nm). Owing to their compact size, these buildings also require novelcapabilities.

Gold nanoshells and carbon nanotubes are two examples of different kinds of nanomaterials. macroscopically display a unique set of physical characteristics in comparison to carbon (Chaubey and Malhotra 2002) or gold (Ali *et al.* 2017). Dendrimers (Frey 2015), liposomes (Sandstead 2012), and semiconducting quantum dots are all instances of nanotechnology (Kinumoto *et al.* 2014). DNA, bacteriophages, and monoclonal antibodies (mAbs) may all be nanometer-sized particles, on the other hand. Nanotechnology has allowed the creation of several materials that might be useful to biologists (Ma and Sun 2002).

In order to serve as biosensors, molecular-scale fluorescent tags, imaging agents, targeted molecular delivery vehicles, and other useful biological tools, nearly all of these materials have been designed with surfaces that can bind a variety of ligands. Nanotechnology's revolutionary promise lies in its capacity to allow for the unparalleled creation and modification of nanomaterials to target cells, chaperone medications, picture biomolecular processes, detect and signal molecular reactions to therapeutic agents, and guide surgical operations.

1.2 Define Nanoscience and Nanotechnology

Research in nanoscience and nanotechnology is cutting edge. In addition, it has been hailed as the revolution of the twenty-first century (Minami 2008). Numerous authors and

research institutions argue that nanoscience and nanotechnology are not synonymous. Can be defined in order to characterize them. Nanoscience and nanotechnology for the world's resources You'll often hear both words used together. Yet it's common knowledge that those two concepts are very different from one another (Lewis and Paine 2000). In nanoscience, the concept of materials with atomic, molecular, and macromolecular dimensions is introduced. Their distinct behavior in certain contexts is the subject of the scientific discipline known as (Mageshwari and Sathyamoorthy 2013) Specifically, nanoscience is the study of phenomena that occur between 1 and 100 nanometers in size. There are several specialized areas within this scientific discipline (Rai *et al.* 2019).

The application of nanotechnology It provides a large database of concepts that may be used to This time around, though, Yet, developing nanotechnology entails incorporating novel nanostructures into robust systems, such as submicron-sized atoms and molecules operating at a range of physical, chemical, and biological length scales. It's shorthand for a domain where system applications and nano production are common (Rehana *et al.* 2019). In its simplest form, nanotechnology may be thought of as engineering at the atomic level. defines itself (Rhouati *et al.* 2013).

Nanotechnology is the study and manipulation of matter on an atomic and molecular scale, having applications in the organization of nanostructures, the fabrication of electronics, and the coating of surfaces. It was built with scanning and processing in mind. Nanostructured materials are one-dimensionally nonporous and have unique melting points, optical absorption, chemical and physical features including fluorescence, catalytic activity, electrical and thermal conductivity, and magnetism compared to their larger-sized counterparts. Materials are classified as nano layers or nanotubes depending on the number of dimensions they occupy (Marcelo *et al.* 2020). When nanotechnology and biotechnology are combined, the resulting field is called nano biotechnology. There's new growth in the field. Exploring and adjusting living organisms with the help of nanotechnology Nano technology is the application of nanoscale ideas and techniques to (Wasa *et al.* 2004).

1.3 A Brief Overview of Nanoparticle and Nanomaterial Manufacturing

Nanoparticles may be made in two distinct ways: bottom-up and top-down. Top-down means that the material is mechanically crushed by a milling process. Bottom-up planning prioritizes the use of chemical reactions to assemble structures (Wilkins and Atanasov 1996). Figure illustrates how the nanoparticles' chemical makeup and the traits that the researchers want to see in the particles dictate which procedure is chosen (Figure 1.1).

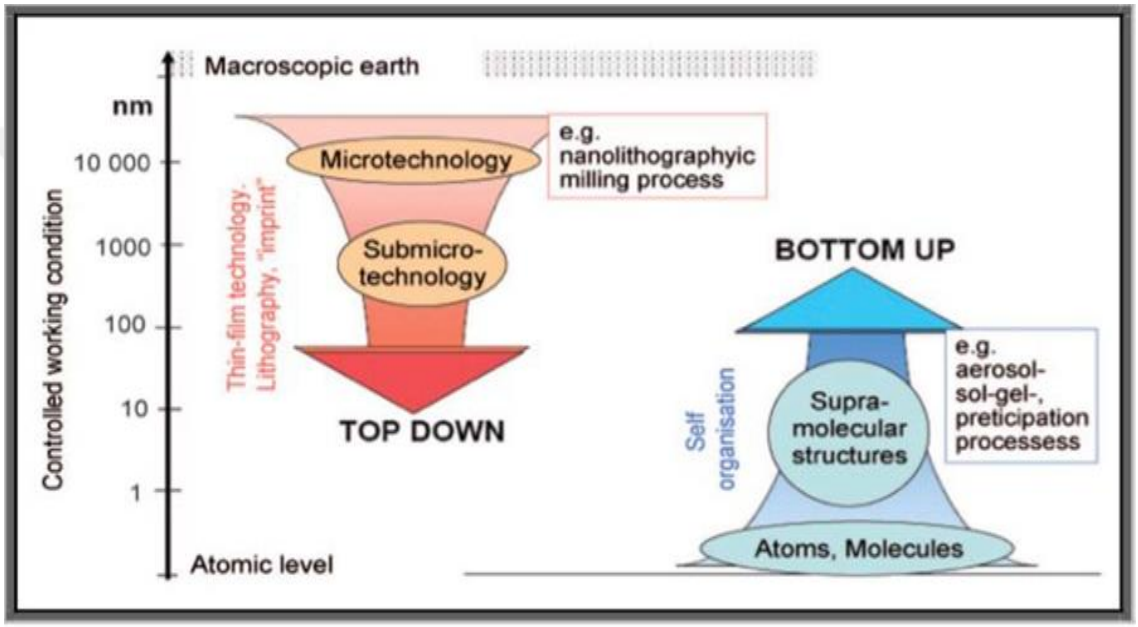

Figure 1.1 Production techniques for nanoparticles (Marcelo *et al.* 2020)

Top-down techniques use principles from microsystem technology to create mechanical-physical particles. In the traditional mechanical-physical crushing processes, numerous milling operations are utilised to produce nanoparticles (Wilkins and Atanasov 1996). As can be seen in Figure 1.2, this approach does not allow for precise manipulation of particle form.

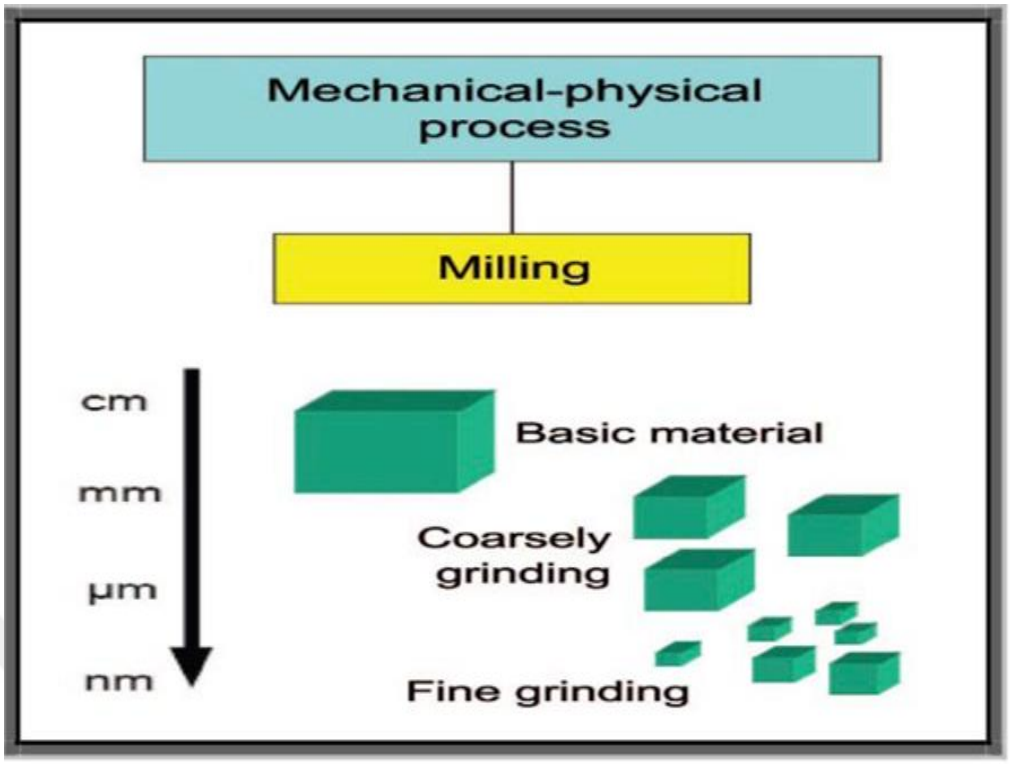

Figure 1.2 Mechanical and physical steps in nanoparticle synthesis are depicted (Minami 2008)

Bottom-Up Approach The strategies stem from ideas of molecular or atomic self-organization, which have their roots in physics and chemistry. This technique allows for more precise manipulation of atoms and molecules, allowing for the construction of complex structures. Sol-gel and aerosol precipitation processes make up this process.

1.4 Sol-Gel Method

This procedure uses a chemical starting ingredient, namely a solution and The term "sol-gel" refers to the fact that a gel made from the solution is employed in this process. Unlike conventional techniques, this one just requires 100 to 600 degrees Celsius in order to complete. Widespread application includes inorganic thin film coatings (Kay and Grätzel 2002). Sol is a term for a suspension of particles in a liquid. As electrical repulsion forces are more potent than gravitational forces, we may ignore the Van der Waals and Gravitational forces between molecules. The sky's the limit. As a result, the sol's constituent parts will not sink. In the sol-gel method, metal organic compounds are first

hydrolyzed after being dissolved in an alcohol. Then, the polymerized solution is de hydrolyzed to produce a sol-gel solution (Figure 1.3). Sol-gel solution's fundamentals Coatings are applied to the materials via dipping, spraying, or spinning. The amorphous film is annealed between 500 and 800 °C, where it crystallizes and becomes denser. The first stage in making a sol-gel is to combine a number of precursors with the right solvents is to dissolve it into a uniform solution. Sol-gel is made by a series of steps following hydrolysis, including polymerization, condensation, gelation, washing, and ageing. (Mehrotra 2016).

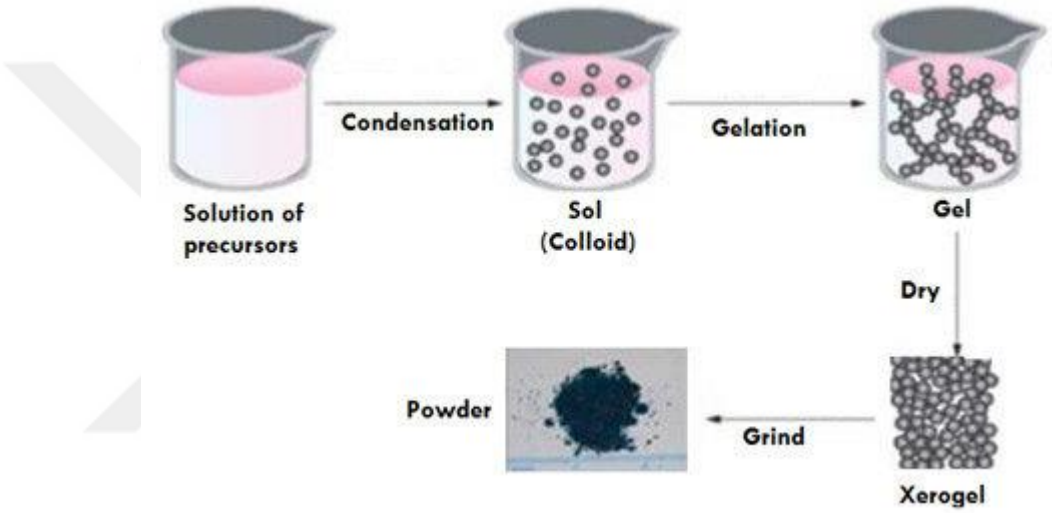

Figure 1.3 Schematic representation of Sol-Gel process (Mehrotra 2016)

1.5 Biosensors

Bioelectronics is the study of how electronics can be used to improve healthcare and biological research. In the field of bioanalysis, biosensors are a type of bioelectronics device that is widely used. When it comes to converting an input variable into a signal that can be measured, a sensor is sometimes referred to as the "primary element in a measurement chain." Several significant technical breakthroughs over the past decade have given us with the resources to build biosensor devices. The sensitivity, selectivity, and multiplexing abilities of modern biosensors have come a long way since the invention of the Clark oxygen electrode sensor. Incorporating a biological or biologically derived

sensing element into or closely linked to a physicochemical transducer, a biosensor is a miniature analytical instrument.

Biosensors rely on "biological recognition" and "sensing" as two of their primary modes of operation. A biosensor, then, is a device with three main pieces that are connected in series: (1) a biological recognition system, often known as a bio receptor; (2) a transducer; and (3) microelectronics. The basic concept of a biosensor is to locate this chemical recognition and convert it, via transducer, into a different type of signal. A high level of selectivity for the analyze being measured is a primary objective of the recognition system. The effect caused by the interaction of the analyze with the bio receptor is measured by the transducer, which converts the information into a measurable effect such as an electrical or visual signal.

Biosensors are defined by the International Union of Pure and Applied Chemistry (IUPAC) as "independently integrated receptor transducer devices that may provide selected quantitative or semi quantitative analytical information employing a biological recognition element" (Pradhan *et al.* 2015). The purpose of a biosensor is to provide information about an analyze rapidly, in real time, with high accuracy, and reliability. It would be ideal if the device could respond in a continuous, reversible manner without compromising the sample. It is anticipated that biosensors will play a significant analytical role in the sectors of medicine, agriculture, food safety, bioprocessing, environmental monitoring, and industrial monitoring.

1.6 A Basic Biosensor Concept

Biosensors are sensors that detect biological molecules such as enzymes, antibodies, or nucleic acids using a transducer and an a biological element. The bio element interacts with the analyze of interest, and the transducer then translates the resulting biological reaction into an electrical signal. The biological element of a biosensor is the "sensor," whereas the electrical element is responsible for detecting and transmitting the signal. As a result of immobilization, the biological material can be brought into touch with the transducer. The electronic response is generated when the analyze binds to the biological

material, creating a bound analyze. It is possible that the creation of a byproduct of the analyses' breakdown involves the liberation of heat, gas (oxygen), electrons, or hydrogen ions. The transducer then transforms the mechanical or thermal changes associated with the product into electrical signals that can be amplified and analyzed. The sensor is known as an affinity sensor if the bio element binds specifically to the analyze of interest. A metabolic sensor is one in which the interaction between the bio element and the analyze results in a chemical change that may be utilized to determine the concentration of a substrate. A catalytic sensor is one in which the biological component combines with the analyze but does not chemically alter it, instead converting it to an auxiliary substrate. The fundamental biosensor concept is shown in Figure 1.4.

1.7 Biosensor Components

Bio receptor, transducer, and signal processing system are the three major components of a biosensor (David *et al.* 2008). Biosensors may be broken down into bio receptor and transducer categories, respectively. Biosensors are categorized in accordance with the bio receptors and transducers they employ.

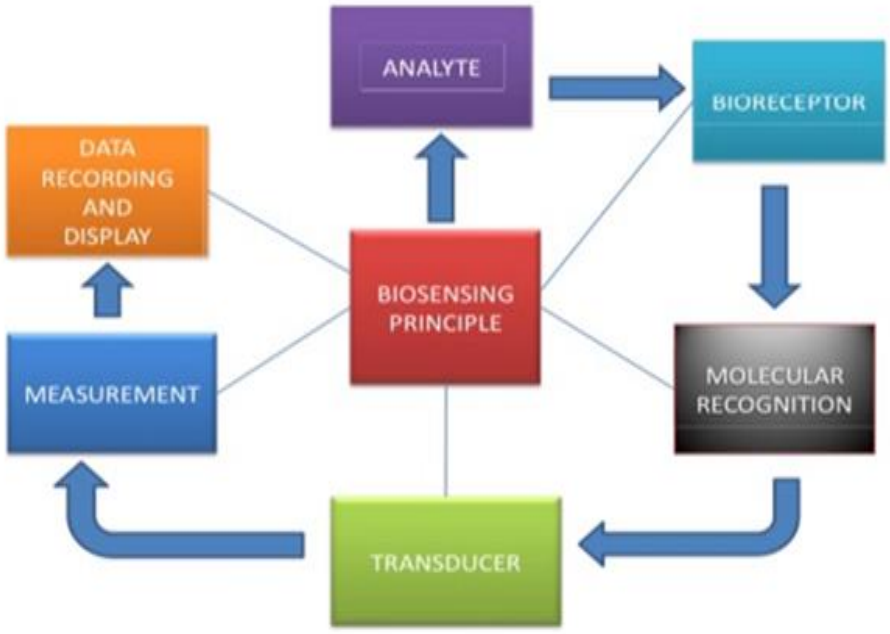

Figure 1.4 Basic principle of biosensors(Kitture *et al.* 2015)

1.7.1 Bio receptors

The biological recognition components, or bio receptors, are the secret to the biosensor technologies' specificity. The key characteristic that sets a biosensor apart from other devices is its bio receptor, or biological recognition component. The bio receptor is made up of a sensor's recognition system for the target analyze. A molecular species that relies on a biochemical recognition process is referred to as a bio receptor. They are in charge of securing the analyze of measuring interest to the sensor surface. The five main kinds of bio receptors are enzyme, antibody/antigen, nucleic acid/DNA, cellular structure/cell, and biomimetic. A biosensor's sampling component has a bio sensitive layer that may be made of covalently bonded bio receptors to the transducer or may contain bio receptors. The most common forms of bio receptors used in bio sensing are based on:

1. Antibody antigen interactions

2. Nucleic acid interactions

3. Enzymatic interactions

4. Cellular interactions (i.e., microorganisms)

5. Interactions using biomimetic materials (i.e., synthetic bio receptors)

The enzymes and antibodies are the main classes of bio receptors that are widely used in biosensor application.

1.7.2 Biosensors that respond to enzymes

Enzymes are the most often used kind of bio receptor molecule in biosensors. Due to their specific binding capabilities, enzymes typically serve as bio receptors. In addition to the catalytic role they play. In bio catalytic recognition systems, the detection is amplified by a catalytic process; with a few exceptions, all enzymes are proteins. Just the amino acid residues in some enzymes are necessary for their activity. Cofactors are required for the proper functioning of many enzymes; they may be one or more inorganic ions like Fe_2, Mg_2, Mn_2, or Zn_2, or they can be coenzymes, which are more complicated organic or organometallic molecules. The catalytic activity of enzymes allows for much lower

detection limits compared to traditional binding approaches. It stands to reason that the catalytic activity of an enzyme relies on the integrity of its native protein structure. If an enzyme is denatured, fragmented, or reduced to its amino acid building blocks, its catalytic activity is lost. Enzyme-coupled receptors may also be used to alter the recognition procedures. For instance, the binding of a ligand to a receptor may change the enzyme's rate of activity. Its enzymatic activity is sometimes greatly enhanced by an enzyme cascade, which in turn triggers complex cellular reactions. Biosensors often use enzymes because they are the only naturally occurring proteins capable of converting a given substrate molecule into a desired product without getting depleted in the process. Enzymes may be utilized in conjunction with various transduction processes, are more selective and sensitive than chemical reactions, and work relatively quickly. These bio receptors function by either (1) transforming the analyze into a sensor-detectable product, (2) identifying analyses that block or activate enzymes, or (3) assessing how enzyme characteristics change in response to the analyze.

1.7.3 Biosensors that respond to antibodies

The antibodies in an immunosensor are what allow the biosensor to "see" a certain antigen. Immunoassays, which are among the most accurate analytical procedures due to their low detection limits, are applicable to a broad range of substances. Proteins may be identified and measured in quantity with the use of such assays (Puneeth *et al.* 2021). Complete instruments, such as immunoreactions-based biosensors, are referred to as immunosensors, while tests based on immunoreactions are called immunoassays. Immunosensors may be built using either polyclonal or monoclonal antibodies. As polyclonal antibodies may detect several epitopes (the small place on an antigen to which a complementary antibody may exactly bind) on their target antigens, they are highly sensitive but less discriminating and prone to cross-reactivity. On the other hand, monoclonal antibodies have an identical structure and are highly selective since they are all produced by the same kind of immune cell and all bind to the same epitope of their respective antigen. Monoclonal antibodies are superior than polyclonal antibodies due to their specificity, making them a great choice as the main antibody in an immunoassay or for detecting particular antigens in the presence of interfering molecules.

Biosensors for nucleic acids: DNA and RNA hybridization is another bio recognition method. From its inception in the early 1950s, the use of nucleic acid sequencing for diagnostic purposes has grown steadily (Paddle 1996). Affinity bindings with a high degree of specificity. Nucleic acid-based biosensors use the strand-exchange process between two strands of DNA (ssDNA) to create a dsDNA molecule for biological identification.

DNA (geno sensors) (geno sensors):DNA biosensors based on nucleic acid recognition mechanisms are being rapidly developed with the goal of offering rapid and inexpensive diagnoses for viral and genetic disorders. DNA biosensors have attracted a lot of attention because of their potential applications in gene analysis, clinical diagnostics, forensic research, and other fields of medicine. A geno sensor is a biosensor that uses electricity to detect the individual nucleotides that make up a strand of DNA. Geno sensors that operate automatically pave the way for rapid, damage-free sequencing of DNA molecules. Geno sensors, also known as DNA biosensors, are devices that use single-stranded DNA (ssDNA) as a biological recognition agent in conjunction with a transducer. The former is what makes this instrument so selective, while the latter is what makes it so sensitive. The hybridization process is used by a geno sensor to locate a target DNA sequence. Humans, animals, bacteria, and viruses serve as the basis for determining nucleic acid sequences used in the investigation of a wide range of problems. Examining microbial contamination in food and water, diagnosing genetic disorders, matching tissues for transplants, using DNA in forensics, etc. A geno sensor is a substrate modified with oligonucleotides (probe DNA) that hybridizes with and identify target DNA. Optical, piezoelectric, and electrochemical geno sensors are the three main types. Based on the kind of transduction performed, these types may be categorized. Electrochemical methods for DNA detection provide a variety of benefits over other methods, including being easy to use, quick, cheap, and sensitive. They're great for making gadgets that don't break the bank and can go everywhere. To detect the hybridization event, electrochemical geno sensors rely on electrochemical transduction. The oxidation signal of DNA electroactive bases may be measured directly, DNA electroactive indicators can be utilized to create complexes with DNA nitrogenous bases, and enzyme-labeled oligonucleotides can be used to follow sequence-specific hybridization processes. The

electrochemical technique of measuring DNA hybridization known as impedance spectroscopy is rapidly developing. The interfacial characteristics (capacitance, electron transfer resistance) of changed electrodes may be effectively probed by means of impedance spectroscopy. The ability to detect DNA without the use of labelled oligonucleotides is a significant benefit of this method. Biosensors using "label-free" impedance sensing have been shown to be very effective.

1.7.4 Apt sensors

Biosensors known as apt sensors rely on aptamers for identification. Apt sensors, thanks to the specific properties of aptamers, will be more accurate and better able to adapt to the circumstances of real samples. Aptamers are molecules of single-stranded RNA or DNA that attach to their targets with great specificity and affinity. There are many different applications for aptamers. Using proteins as biological recognition components in biosensors has the potential to greatly simplify and speed up the process of protein detection. Aptamers may be used in a number of situations where antibodies are also viable options. As compared to other bio recognition molecules like antibodies or enzymes, aptamers are very compact (30-100 nucleotides in size). This allows for very dense immobilizations of aptamers. Aptamers are more amenable to mass production, miniaturization, integration, and automation in biosensors than antibodies. After aptamers have been selected, they may be synthesized with high levels of consistency and purity. The many types of aptamers include:

- Aptamers, probes based on either DNA or RNA
- Aptamers peptides
- DNA aptamers are very chemically stable, which allows the biosensors to be reused.
- In RNA aptamers, on the other hand, may be broken down by the body's own ribonucleases.

Frequent in plasma and cell lysates. Because of this, RNA aptamer biosensors. In a biological setting, bio recognition components can only be utilised for one-time

observations. Chemical modifications to DNA and RNA aptamers allow for analyte-dependent conformational alterations.

Apt sensors have been developed using a number of different detecting strategies: Surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) measurements are two examples of label-free techniques Electrochemistry, fluorescence, chemiluminescence, and field-effect transistors are all examples of labelled techniques.

Typically, an in vitro procedure is used to separate aptamers from combinatorial libraries. Systematic ligand evolution by exponential enrichment is the name of this kind of evolution (SELEX).

1.7.5 Biosensors made of microbes

A microbial biosensor is an analytical technique that uses microorganisms immobilized on a transducer to detect specific analyses. Biosensors may be used with microorganisms like bacteria and fungus to detect certain substances or the "state" of the environment. The costly and time-consuming process of purifying enzyme-based biosensors is unnecessary for microbial biosensors. Most of the biochemical components of bacteria are enzymes. Enzymes in living organisms may react selectively and precisely to analyses. Microorganisms have been integrated with ampere metric, potentiometric, calorimetric, conduct metric, colorimetric, lumino metric, and fluorescent transducers to construct biosensor devices. Proteins contained in cells may also operate as bio receptors to detect certain analyses.

1.8 Biosensor Types and Properties

Each ideal biosensor need to have a number of characteristics. The biosensor's efficiency is proportional to how well these factors are optimized (Naresh and Lee 2021).

1.8.1 Sensitivity

A biosensor's sensitivity is measured in terms of its limit of detection (LOD), which is defined as the minimum detectable concentration of analyze. Several factors of biosensor operation and design contribute to the sensor's sensitivity. The sensitivity of a detection system depends on a number of factors, including the shape of the transducer surface, the surface density of the immobilized coating, the molecular diffusivity of the free analyze in the sample solution, and the reaction kinetics. The surface area to volume ratio is inversely proportional to a biosensor's sensitivity (Kasap *et al.* 2007).

1.8.2 Selectivity

To be able to separate the target molecule (analyze) from other molecules in a sample is to have high selectivity. Selectivity is especially important in biological samples because the concentration of the target molecule may be much lower than the concentration of other biomolecules already present in the sample. The interaction between an antigen and an antibody is a prime example of selectivity. Antibodies are often affixed to transducer surfaces to act as bio receptors. The transducer is then incubated with a solution containing the antigen (often a saline buffer), where the antibodies attach specifically to the antigens. This guarantees that the device is a sensor tailored to the target analyze (Kanazawa and Cho 2009).

1.8.3 The Timing of a reply

The speed at which a biosensor can react is also very important. It is the quickest time required for a biosensor to locate enough biomolecules for an analyze to be identified. The method relies heavily on the target's density. biomolecules and their physical movement towards the surface of the sensor. The reaction time is affected by the biomolecules' (target molecules') diffusion through the solution and subsequent capture at the sensor surface. Equation (1.1) (Fang *et al.* 2009) demonstrates that response time (ts) is proportional to analyze concentration in the sample:

$$\rho = N\ V \tag{1.1}$$

The analyze concentration, the number of molecules of the analyze, and the volume of the sample are denoted by, *N*, and *V*, respectively. Very low concentrations of the analyze will result in a prolonged response time (ts). According to this formula, raising the value of N raises the value of. The expensive and labor-intensive instrumentation approaches, such as commercial testing or polymerase chain reaction, were thus stated (PCR). Moreover, these methods need extensive processing time and experienced personnel compared to biosensors.

1.8.4 Reproducibility

Reproducibility is defined as the consistency with which a biosensor yields results when the same set of tests is repeated. Precision (repeatable findings from the same sample) and accuracy set it apart (the degree of closeness between measured value and the true value). Inferring from a biosensor's response requires a signal that can be reproduced with great reliability and resilience (Fang *et al.* 2009).

1.8.5 Stability

Biosensor applications that need continuous monitoring necessitate a level of stability. The stability of a bio sensing device is defined as its resistance to internal and external perturbations. The bio receptor's affinity (the rate at which the analyze binds to the bio receptor) and its long-term degradation are two factors that influence stability. The reliability of biosensors, for instance, may be affected by environmental factors such as temperature, which may affect the performance of transducers and electronics. Equation (1.2) (Aminuzzaman *et al*. 2008).

$$y = m.\ c \tag{1.2}$$

2. LITERATURE REVIEW

2.1 Zinc Oxides

For monitoring the environment and providing medical care, biosensors have demonstrated considerable promise. The presentation the matrix material, which is the layer between the recognition layer of the biomolecule and the transducer, is a key factor in determining the stability and sensitivity of biosensors. and the biosensor's shelf life. Due to their biocompatibility, chemical stability, high isoelectric point, electrochemical activity, high electron mobility, ease of synthesis by a variety of methods, and high surface-to-volume ratio, zinc oxide (ZnO) nanostructures and thin films have recently attracted a lot of interest as materials for biosensors. ZnO nanostructures have demonstrated that proteins can bind to them in specific orientations with improved conformation and strong biological activity, improving their sensing properties. Additionally, ZnO nanostructures are a good contender for future compact integrated biosensor devices due to their compatibility with complementary metal oxide semiconductor technology for building integrated circuits.

Nanoscale materials, such "nanoparticles," have new characteristics and perform entirely different tasks than mass-scale things (Guo *et al.* 2015). NPs have emerged as a Considering that they connect bulk materials to atomic or molecular structures, this field of study is promising (Baruah and Dutta 2009). Nanoparticles are expanding the range of possible research topics for scientists because of their special qualities, including small size, surface compatibility, increased solubility, and multi functionality (Parashar *et al.* 2008),With the help of numerous prospective applications, nanoparticles biotechnology has advanced quickly in recent years (Guo *et al.* 2015). The development of nano and biotechnology has increased the usage of NPs and created new applications (Ashfold *et al.* 2004). The potential for disease diagnosis and therapy has increased thanks to the rapidly evolving science of nanotechnology. Hence, developing a safe mechanism for nanoparticles synthesis is crucial for the environmental health sector (Ashfold *et al.* 2004). The increase in surface and interfacial atoms, strains or stresses, and nearby structural deformities are all effects of nanoparticle size reduction. The magnetic,

conductive, chemical, and electrical properties of the nanoparticle are influenced by its size. Due to this circumstance, One of the most significant semiconductor metal oxides, ZnO exhibits characteristics like UV light blocking, photocatalysis, and antibacterial activity (Ashfold *et al.* 2004). Figure 2.1 a zinc blend and wurtzite, which is more stable and has a refractive index of 2.3 to 2.0 (Guo *et al.* 2015).

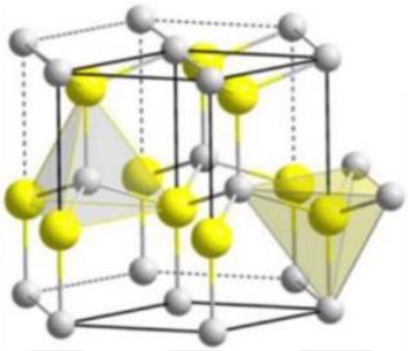

Figure 2.1 Crystallographic structures of ZnO nanocrystals: hexagonal wurtzite (Guo *et al.* 2015)

Many beneficial characteristics of ZnO include good light scattering, high transparency, non-toxicity, natural occurrence, and effective photo catalysis (Hanley *et al.* 2009). Due to their safety and compatibility with human skin, ZnO, which exhibit bactericidal activity against Gram-positive and Gram-negative bacteria and spores and are resistant to high temperatures and high pressure, can be used for textiles and surfaces that come into contact with the human body (Castillo *et al.* 2004). ZnO have special biomedical uses (such as anticancer, drug delivery, antibacterial, wound healing, and bio imaging) and are less expensive and hazardous than other MONPs (Caruthers *et al.* 2018). ZnO nanoparticles are IIVI semiconductors with large excitation energies of 60 eV and 3.3 eV, respectively (Ashfold *et al.* 2004). Hence, because of their energy content, these High temperatures, high voltages, and strong electric fields may all be tolerated by NPs. With its wurtzite-like structure, ZnO works in both electrical and optoelectronic applications and is commonly employed in devices like gas sensors and varistors (Cachet 2005). On the nanoscale, ZnO typically exhibits a wurtzite crystal structure, with lattice values of a=0.3296 nm and c=0.52065 nm. (Chaubey and Malhotra 2002). The wurtzite structure is a stack of alternating positively and negatively charged planes of zinc and oxygen

atoms along the c-axis (in the direction of 0001 >) (Chaubey and Malhotra 2002). Tetrahedral structure is brought into focus by the coordination of zinc and oxygen atoms (Chaubey and Malhotra 2002). Wurtzite's non-centrosymmetric characteristic causes polarization, and ZnO is known as a piezoelectric material with high piezoelectric coefficients. In conclusion, ZnO piezoelectric capabilities, which are useful for acoustic wave resonators and acoustic-optic modulators, result from their crystal structure. Moreover, its centrosymmetric structure is linked to the highest tensor of any semiconductor, which results in a significant improvement in electromechanical coupling (Bojorge Ramírez *et al.* 2009). According to a publication, ZnO exhibit the surface plasmon resonance (SPR) peak at 346 nm in their UV spectra, which is a feature of these nanostructures (Castillo *et al.* 2004). High photocatalytic capabilities in ZnO contribute to an increase in their antibacterial activity (Guo *et al.* 2015). As a result of photocatalysis of ZnO by visible and UV light, it can also be utilized to degrade organic molecules in paints, losing their ability to absorb UV light (Castillo *et al.* 2004).

Hence, ZnO nanoparticles that are stable under UV radiation are of particular interest to scientists (Baruah and Dutta 2009). ZnO possesses distinctive optical, piezoelectric, and significant electrical capabilities. ZnO often shows n-type conductivity as a semiconductor II-IV with high carrier mobilities of 100 cm^2 V^{-1} s^{-1}. Understanding the band structure, as well as the optical and electrical properties, in the context of ZnO's optoelectronic properties is essential (Aminuzzaman *et al*. 2008). ZnO has other, less well-known characteristics that make it ideal for thermoelectric generators, nonlinear optical components, and aerospace applications (Cheon and Lee 2008). These characteristics include high radiation hardness, high thermal conductivity, and strong nonlinear optical behavior. ZnO has a lot of promise for research and use in nanoscience and nanotechnology because of all these distinctive and dominating features (Aminuzzaman *et al*. 2008).

2.2 Applications of ZnO

Nano crystalline semiconductors' novel electrical, optical, and mechanical properties make them ideal for use in optoelectronic devices, medication delivery systems,

electrochromic devices, and a variety of other applications, including cosmetics. becoming more wide spread (Chaubey and Malhotra 2002). With more and more usage in fields such as medicine, agriculture, paints, chemicals, ceramics, tires, solar cells, and light-emitting devices and sensors, ZnO in this class are also garnering interest (Aminuzzaman *et al*. 2008).

2.2.1 Agricultural applications

ZnO nanoparticles are employed in agricultural applications because they are environmentally safe and have the potential to improve food growth (Bala *et al.* 2016), (Šebesta *et al*. 2021). Because of their UV-protective and antibacterial qualities, ZnO are employed in lab, greenhouse, and field investigations of crop plants in addition to being a source of slow-release Zn that has a nutritional effect on plants. Research in this area have demonstrated that seeds produced with various concentrations of ZnO-NP enhance germination, seed vigor, and plant growth (Aminuzzaman *et al*. 2008).

2.2.2 Cosmetic applications

It's crucial to stay away from dangerous radiation like UV rays because they can lead to skin cancer in people. Thus, it is vital to incorporate UV-blocking ingredients into the formulas of cosmetic products in order to protect the skin (Roselli *et al.* 2003) ZnO NPs are frequently employed in cosmetic applications, such as the production of sunscreens, because they have high antifouling and antibacterial capabilities in addition to being an efficient UV blocker (Balan *et al.* 2016). Sunscreens are not the only cosmetics that include ZnO. It is also possible to produce reactive oxygen species (ROS), which can oxidize the constituents in the cosmetic formulation, due to the strong photocatalytic activity of ZnO (Aminuzzaman *et al*. 2008).

2.2.3 Biomedical applications

Due to their low toxicity, biodegradability, and biocompatibility, reactive oxygen species (ROS) that can oxidize the components of cosmetic ZnO NPs are employed in biomedical and pro ecological applications (Sethi 1994). Also, they are favored for medication delivery and bio imaging systems, anti-diabetic, anti-cancer, antioxidant, and antibacterial/anti-inflammatory drugs. Moreover, it's crucial to create regulated nanoparticles that are homogeneous in size, shape, and functioning for a variety of biological applications (Sigurdsson *et al.* 1997). ZnO can be employed directly in biomedical applications since it is biocompatible.

2.2.4 Anticancer activity

Chemotherapy, radiation, and surgery have been the most common forms of treatment for cancer during the past ten years, which is characterized by unchecked, aggressive cell proliferation (Jawad and Hamooshy 2021). In theory, all of these treatments are highly effective, but because they are nonselective, they also have a lot of negative side effects. Recent research has demonstrated that nanomaterials' great biocompatibility, ease of surface functionalization, and capacity to target tumors and deliver medications allow them to avoid the aforementioned negative effects (Jawad and Hamooshy 2021).

2.2.5 Diabetes treatment

Diabetes mellitus (DM), a common, deadly, and significant public health issue, is a metabolic disorder brought on by the body's failure to create enough insulin 12 or to utilize the insulin that is produced efficiently (Weiss *et al.* 2006). By 2040, this issue will affect 642 million individuals worldwide, according to projections (Choi *et al.* 2018). In addition to being active in the synthesis, storage, and secretion of insulin from pancreatic cells, zinc (Zn) preserves the structural integrity of insulin (Das *et al.* 2018).

2.2.6 Bio imaging

ZnO-NPs have the potential for bio imaging activity in addition to medicinal applications in anticancer, antibacterial, diabetic, anti-inflammatory, and drug delivery (Dutta *et al.* 2013). They are a suitable choice for bio imaging applications due to their low toxicity. ZnO-NPs exhibit yellow or green emissions (near-UV radiation) oxygen vacancies and efficient blue emissions are connected. As a result, theuses for these nanoparticles in the field of bio imaging are expanding.

2.2.7 Antibacterial activity

Many investigations are being undertaken to create antimicrobial remedies to solve the issue of antibiotic-resistant bacteria, which is a health issue that needs to be addressed. By mixing metallic elements with oxygen to create metal oxides, bacteria are subjected to electrostatic interactions that disrupt the prokaryotic cell wall and enzyme or DNA pathways and generate reactive oxygen species (ROS) (Sajjadi 2005).

As their antibacterial activities, potentials, and activity spectra are well recognized in antimicrobial treatment research (Gajendiran and Rajendran 2015.), metal oxide nanoparticles of Zn metals such Ag, Cu, Au, and Ti are also included in applications as antimicrobial agents. The U.S. Food and Drug Administration (FDA) has listed zinc oxide as a food additive on its list of chemicals generally regarded as safe (Sajjadi 2005). The high specific surface area and superior capacity to inhibit a range of diseases make the nanoscale versions of these materials stand out as antibacterial materials (Jiang *et al.* 2018). Although attachment of the NPs to the bacterial membrane is the essential first step in the antibacterial mechanism of ZnO nanoparticles, the precise antimicrobial mechanisms remain unknown (Shukla 2012). However, it is believed that this effect is influenced by electrostatic contact with the membrane, the production of ROS, and/or the release of ions. the primary antibacterial activity of ZnO nanoparticles is believed to result from their capacity to cause excessive ROS generation (Soleymani and Li 2017). The particles with the lowest particle size had the highest bactericidal response when the link between the antibacterial activity of ZnO nanoparticles and particle size was examined

(Sudhagar *et al.* 2011). Moreover, ZnO nanoparticles' strong photocatalytic qualities are useful in boosting their antibacterial activity (Vinotha *et al.* 2019). ZnO nanoparticles demonstrated antibacterial, antifungal, and bactericidal effects on spores resistant to high temperatures and high pressures in a number of investigations. Additionally, because ZnO NPs are widely used in food preservation, production stability, and product shelf-life extension due to their antibacterial properties (Sudhagar *et al.* 2011). ZnO nanoparticles' optical characteristics allow for faster and more effective ROS formation at shorter wavelengths, such as light. Hydrogen peroxide can also enter prokaryotic cells through their creation and kill a variety of organelles (Vinotha *et al.* 2019). The deposition of the particles on the bacterial surface as a result of electrostatic forces is suggested to be another mechanism for the antibacterial activity of ZnO nanoparticles. Furthermore, the bacterial membrane may experience lipid peroxidation, compromising membrane integrity and resulting in cell lysis (Vinotha *et al.* 2019). The bacterial type and selection are another factor influencing ZnO nanoparticles antibacterial effectiveness. To study the antibacterial activity of ZnO nanoparticles, Escherichia coli (*E. coli*) as a gram-negative model bacteria and Staphylococcus aureus (*S. aureus*) as a gram-positive model bacteria are typically utilized (Vigneshvar *et al.* 2016). Moreover, other gram-negative bacteria such as *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Vibrio cholera*, and *Campylobacter jejune*, as well as gram-positive bacteria such *as Bacillus subtilis*, *Enterococcus* facials, and *Bacillus* subtilis are also employed in research (Vigneshvar *et al.* 2016). 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 ROS. Cell wall-forming components of Gram-positive bacteria are peptidoglycans, teichoic acid and lipoteichoic acid (Vigneshvar *et al.* 2016). Compared to Gram positive bacteria, Gram negative bacteria have thicker and more complex cell walls (Sudhagar *et al.* 2011). This is because the cell walls of Gram-positive bacteria are made up of peptidoglycans, teicoic acid, and lipo teicoic acid, which are easier to penetrate, while the cell walls of Gram-negative bacteria comprise an outer lipopolysaccharide membrane and a peptidoglycan layer. As a result, the cell's ability to absorb ROS and ions from the membrane is inhibited (Sudhagar *et al.* 2011).

2.3 Using of ZnO in Biosensor Applications

Zinc oxide (ZnO) is known as a useful substance with a wide range of biomedical and biochemical uses thanks to its biocompatibility, nontoxicity, and potent adsorption capacity. ZnO's high isoelectric nine point five (9.5) and strong sensing capabilities make it possible to detect biomolecules like proteins, enzymes, and antibodies. When compared to their bulk counterparts, nanostructured ZnO materials have a higher active surface area for protein binding and enzyme entrapment because they maintain the physiological activity and stability of biomolecules without denaturing them. A special material with enhanced structural, electrical, and optical properties is needed for the biosensor platform's effective signal conversion of biological interactions into physical signals. Because they possess the necessary physical characteristics (conductivity, luminescence, and absorption), metal oxides are very appealing for biosensor applications (Jawad and Hamooshy 2021). In addition to being a metal-oxide semiconductor and having a relatively large bandgap (Eg=3.37 eV), ZnO also has excellent chemical, thermal, electrical, and optical characteristics. The attachment of biomolecules, increasing the surface to volume ratio, and mechanical protection of the sensing probe are just a few of the functions that nanostructured ZnO materials, which are frequently used as thin layers, fulfill in biosensors (Roselli *et al.* 2003). Due to their similar size to biological molecules, ZnO nanostructures present prospects for research in biomedical applications, particularly biosensors. These ZnO properties show that it is excellent and suitable for transducer applications in a range of biosensors (Dutta *et al.* 2013). Molecular beam epitaxy (MBE), spray pyrolysis, chemical vapor deposition, RF magnetron sputtering, and sol-gel processing are a few of the methods that have been utilized to produce ZnO thin films. Controlling the concentration of dopants, which are purposely added impurities and are responsible for the electrical and optical properties, is crucial.

ZnO's properties can be used to create any device technology. The dopants control whether electrons or holes transport the current, which ultimately affects how the gadget processes information. Semiconducting oxides often allow for one of these categories but not both. Because they bring energy levels that are close to one of the permitted energy

bands of material 26 and are thus easily ionized, dopants are also known as shallow level impurities (Chaubey and Malhotra 2002).

2.5 SILAR Method

The SILAR technique is one of the most recent solution methods for producing thin films (Vinotha *et al.* 2019). SILAR is a technology that uses successive reactions at the substrate-solution interface in an aqueous solution. Aqueous solutions containing ions of each species are created by grinding thin sheets. By laying the material on the foundation material after sinking it in a specific order. It is an easy method to construct The SILAR method offers a wide range of precipitation applications and is cheap, straightforward, and easy to use. The reaction occurs at or near room temperature. Because to the surrounding temperatures and pressure that surrounds the solutions, it can be done with a variety of base materials, including insulators, semiconductors, metals, and temperature-sensitive materials (like polyester). The technique uses low temperatures, which prevents corrosion and base material oxidation. The primary goal of the SILAR process is to produce thin films of high quality. It is necessary to control the preparation conditions, such as adsorption, reaction, and rinse time, as well as the concentration of the precursors, the counterions, and the pH of the precursor solutions. Despite its simplicity, the SILAR process, one of the most recent solution methods for thin film creation, has many benefits. They are:

i. Film, which may be added in any ratio by simply converting it to a cationic solution in any form. Adding is enough, and it's a pretty easy way.
ii. Evaporation in a vacuum. As opposed to other procedures, SILAR does not require a vacuum at any point, making it unique. This is the method's commercial advantage. It offers a significant benefit when used.
iii. The amount of precipitation and the movie by altering the precipitation cycle, the thickness can be easily managed across a large range. Anything is possible.
iv. Using treatments at ambient temperature on healthier materials The movie can be made bigger.

Contrary to high energy approaches, material precipitation does not result in hazardous heating and the substrate's composition, size, and surface profile. There are hardly any limitations on Also, it is affordable, straightforward, and helpful for large-area deposition. Possible in glass beakers. Beginning supplies are frequently affordable and easily accessible. are substances. There is a large range of substrates available because it is a chemical technique. Thus, any insoluble surface that the solution can easily penetrate will make for a good precipitation substrate. Precipitation that is stoichiometric is simple to achieve.

As ions rather than atoms are the primary construction blocks, it is possible to simply manipulate the preparation factors and get the optimal orientation and particle structure (Vinotha *et al.* 2019).

2.6 Parameters Affecting Thin Film Growth in SILAR Technique

Useful solution concentrations (molarity), pH readings, the number of SILAR cycles, and immersion and shaking times are a few crucial variables that determine the formation of thin films using the SILAR process.

2.6.1 Solution concentration

The concentration is one of the most crucial variables in the SILAR method. It is crucial to employ cationic and anionic solutions at the correct concentration. If the solutions' concentrations should be The films might not develop to the intended quality if it is less than the value. This also entails producing thin films with an amorphous structure rather than a crystalline one. The concentration of ions rises along with the concentration of the solutions. Like this picture, growth happens more quickly. In this instance, a sturdier construction It is impossible for foreign atoms to penetrate a stable structure since they are trying to do so from the outside. The gaps between the particles get smaller as the solution concentration rises, which lowers the resistivity even more. Thin films of high quality are attainable. Unsuitable if the solution concentration is too high In the event of an excessive solution concentration, films are also an option. Amorphous films rather

than crystalline films are produced due to overgrowth and repetition. Excessive film buildup causes the base material to become a surface sediment as a result.

2.7.2 pH value of solutions

As is common knowledge, pH is a scale that indicates how acidic or alkaline a solution is the unit is. pH=-log [H^+] is the minus logarithm of the hydrogen ion in solution. pH [H^+] with the ion [OH^-] the direct ratio of the concentrations of theionsit depends. The solution is acidic if the concentration of H^+ is more than the concentration of OH; hence, the pH value is lower than 7. our solution is basic if the OH concentration is more than the concentration of H^+, meaning that the pH value is greater than 7. If sufficient amounts of OH and H^+ ions are present, the substance has a pH of 7 and is neutral. Hydrogen and hydroxyl ions are free in both acids and bases. It is feasible to determine the other by knowing the other since the relationships between the hydrogen and hydroxyl ions in a given solution are fixed. Anionic solutions for the SILAR technique must be basic, while cationic solutions must be acidic. While preparing a solution, the pH values should be adjusted to the best possible level. pH of solutions The initial solution's concentration is unaffected by the addition of solutions during adjustment. There should be consideration. In order to build films, the selection of an appropriate pH is crucial.

Since metals have an affinity for the hydroxyl ion, the solution becomes basic as the pH rises. The metals' affinity for the hydroxyl ion will grow as they accumulate, and the hydroxyl ion may coalesce and precipitate as a result. The effect of these metal ions on the base material causes them to lose interest as this interest rises, which makes them less likely to hang onto the base material. This will prevent the films from developing. Basic pH is crucial in anionic solutions, such as Na_2S solution. Because the solution's acidic quality increases as the pH lowers The Na_2S solution's sulfur ions are eliminated as you gain, forming H_2S in the process. The likelihood of escaping is fairly great. This is as a result of the films' lack of sulfur will lead to resulting flaws. a surplus of cationic solutions Make sure acidic and anionic solutions don't exhibit overly basic characteristics ought to be done.

2.7.3 SILAR cycle count

The SILAR cycle number is one of the variables that can be controlled in the SILAR method. The film thickness can be effectively managed by adjusting the number of cycles. As a percentage of cycles. The film gets thicker and thicker. Because there are fewer cycles, the film thicknesses are considerably Amorphous films will be produced since it will be very thin. A more stable structure will be produced as the number of cycles and film thickness rise. Its stable structure does not permit entry from the outside by foreign atoms. The spaces between the particles get smaller as the film thickness gets thicker. As a result, additional thin films of high quality can be produced. Also, a high SILAR cycle count is inappropriate. Since this time the ions are not residue when the film thickness reaches a particular value. They will start to build up on the surface as a degraded layer.

3. MATERIALS AND METHODS

In this section, the growth technique of ZnO using the SILAR method, the devices utilized in the analysis, and the performed measurements are described.

3.1 Scanning Electron Microscope (SEM)

Scanning Electron Microscope (SEM) is an advanced imaging tool that uses an electron beam to produce high-resolution images of the surface of a sample. The SEM is widely used in various fields such as materials science, physics, chemistry, biology, and geology. This article aims to provide an overview of the SEM, including its basic principle of operation, types, and applications.

The SEM has a wide range of applications in various fields. In materials science, the SEM is used to study the microstructure and morphology of materials, such as metals, ceramics, and polymers. In biology, the SEM is used to study the surface of biological samples, such as cells, tissues, and organs. In geology, the SEM is used to study the mineralogical and petrological properties of rocks.

The SEM works based on the interaction between an electron beam and the sample surface. When the electron beam is directed towards the sample, it interacts with the atoms in the sample, causing the emission of secondary electrons. These secondary electrons are then detected by a detector, which produces an image of the sample surface.

The Scanning Electron Microscope is a powerful imaging tool that has a wide range of applications in various fields. Its high resolution and ability to observe samples in various environments make it a valuable tool for researchers and scientists. With ongoing technological advancements, the SEM is expected to continue to play a crucial role in advancing our understanding of the world around us (Figure 3.1).

Figure 3.1 Hitachi SU 5000 field emission scanning electron microscope (Vinotha *et al.* 2019)

3.2 Annealing Oven

Annealing is a process that involves heating a material to a specific temperature and then cooling it slowly to make it more ductile, reduce internal stresses, and increase its strength. Annealing ovens are used for this process, and they are commonly found in various industries such as metallurgy, electronics, and glass manufacturing. In this article, we will discuss the basic principle of operation, types, and applications of annealing ovens.

Annealing ovens work based on the principle of heat treatment. The material to be annealed is placed in the oven, and then the temperature is increased to the desired level. The oven maintains the temperature for a specific amount of time, allowing the material to reach a homogeneous temperature throughout. After the annealing process is complete,

the oven slowly cools the material to room temperature. The cooling rate is critical, and it must be controlled carefully to ensure the desired results are achieved.

Annealing ovens are used in a wide range of applications in various industries. In metallurgy, annealing ovens are used to soften metal, improve its ductility, and reduce internal stresses. In electronics, annealing ovens are used to remove stress and improve the quality of semiconductors. In glass manufacturing, annealing ovens are used to cool glass slowly, making it stronger and more durable.

There are several types of annealing ovens, including batch, continuous, and vacuum ovens. Each type of oven is suitable for different applications, depending on the material being annealed. The continued development of annealing ovens is essential for the growth of various industries that rely on this technology (Figure 3.2).

Figure 3.2 Annealing oven (Dutta *et al.* 2013)

3.3 SILAR Method

The successive ionic layer adsorption and reaction (SILAR) method is a simple and cost-effective technique used for the deposition of thin films. The method is based on the alternate exposure of a substrate to a cationic and anionic precursor solution, resulting in the formation of thin layers on the surface of the substrate. The SILAR method has gained significant attention in recent years due to its versatility, scalability, and potential applications in various fields (Figure 3.3).

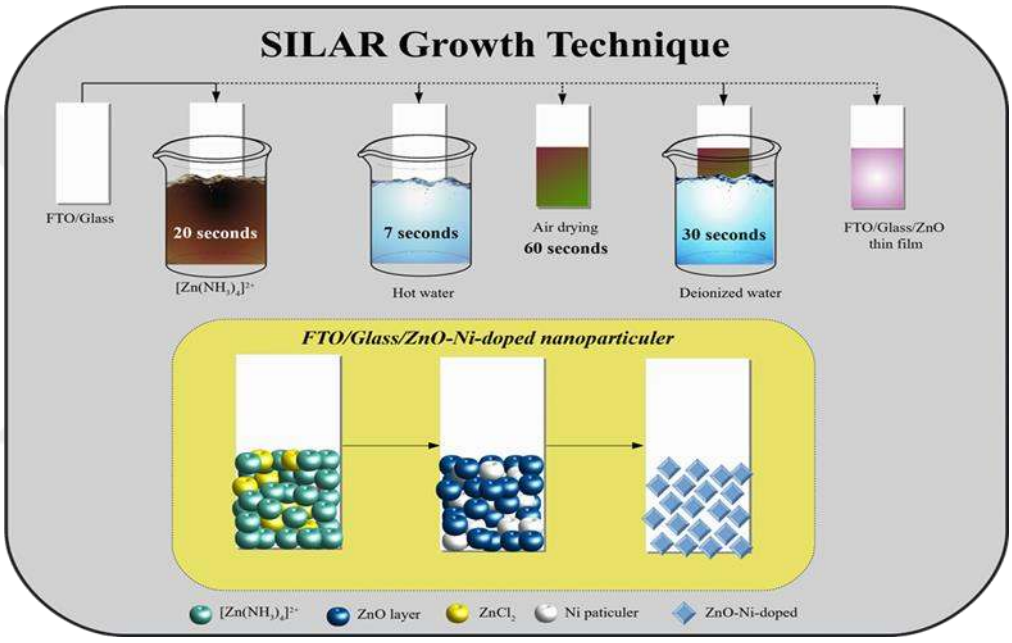

Figure 3.3 SILAR growth technique (Wang 2004)

The SILAR method involves the deposition of thin films through the alternate exposure of a substrate to cationic and anionic precursor solutions. The substrate is first dipped into a cationic precursor solution, and the excess solution is removed by rinsing with a solvent. Next, the substrate is dipped into an anionic precursor solution, and the excess solution is again removed by rinsing with a solvent. This process is repeated several times, resulting in the formation of thin layers on the substrate surface. The process can be repeated until the desired thickness of the film is achieved. The SILAR method has several advantages over other deposition techniques. The method is simple, cost-effective, and does not require any expensive equipment. The method can also be used to deposit a wide range

of materials, including oxides, sulfides, and selenides. The SILAR method can also be easily scaled up, making it suitable for large-scale production. Additionally, the method allows for the deposition of films with controllable thickness and uniformity.

Despite its advantages, the SILAR method also has some limitations. The method can result in the formation of non-uniform films due to the adsorption of excess precursor solution on the substrate surface. The deposition rate can also be slow, resulting in longer deposition times. Additionally, the method is sensitive to environmental conditions such as temperature and humidity, which can affect the quality of the deposited films.

The SILAR method has potential applications in various fields, including solar cells, sensors, and catalysts. In solar cells, the SILAR method can be used to deposit thin films of metal chalcogenides, which are efficient absorbers of sunlight. In sensors, the method can be used to deposit thin films of metal oxide semiconductors, which are sensitive to gases and other analytes. In catalysts, the method can be used to deposit thin films of metal sulfides, which are efficient catalysts for various chemical reactions.

The SILAR method is a versatile and cost-effective technique used for the deposition of thin films. The method is based on the alternate exposure of a substrate to cationic and anionic precursor solutions, resulting in the formation of thin layers on the surface of the substrate. The method has several advantages, including simplicity, cost-effectiveness, and scalability. However, the method also has some limitations, including non-uniform film formation and slow deposition rates. The method has potential applications in various fields, including solar cells, sensors, and catalysts.

3.4 Formation of ZnO films

Liquid ammonium is added slowly to make the 0.1 M Zn $(NO_3)_2$ solution used as a zinc source alkaline. This reaction is expressed as follows Equation (3.1).

$$Zn(NO_3)_2 + 2NH_4OH \rightarrow Zn(OH)_2 + 2NH_4^+ + 2NO^- \quad (3.1)$$

However, when enough NH_4OH is added, Zn^{+2} ions in the environment are reduced and Zn $(NH_3)_4^{+2}$ is formed. Thus, precipitation in the solution is avoided. In addition, the solution takes on a clean and transparent appearance, Equation (3.2):

$$Zn(OH)_2 + 4NH_4^+ \rightarrow Zn(NH_2)_4^{+2} + 2H_2O + 2H^+ \quad (3.2)$$

Glass bases, one surface of which is film-coated, are prepared for measurement Equation (3.3) and Equation (3.4):

$$H_2O_2 + H_2O \rightarrow 2OH^+ + 2H^+ + 1/2O_2 \quad (3.3)$$

$$Zn(NH_3)_4^{+2} + 2OH^- \rightarrow ZnO + 4NH_3 + H_2O \quad (3.4)$$

To produce Ni-doped ZnO samples, a FTO substrate was created, and subsequently, the FTO substrate was cleaned. After cleaning, solutions with the right additives were made, and then the SILAR method was used to create Ni-doped ZnO thin films on FTO. Here are a few of the chemical alterations that take place during the development stage as described in Equations (3.5), (3.6), (3.7), and (3.8).

$$ZnCl_2 + 2NH_4OH \leftrightarrow Zn(OH)_2 + 2NH_4^+ + 2Cl^- \quad (3.5)$$

$$Zn(OH)_2 + 4NH_4^+ \leftrightarrow [Zn(NH_3)_4]^{2+} + 2H_2O + 2H^+ \quad (3.6)$$

$$NiCl_2 + 2NH_4OH \leftrightarrow Ni(OH)_2 + 2NH_4^+ + 2Cl^- \quad (3.7)$$

$$Ni(OH)_2 + 4NH_4^+ \leftrightarrow [Ni(NH_3)_4]^{2+} + 2H_2O + 2H^+ \quad (3.8)$$

Based on the 1%, 2%, and 3% Ni-doping ratios as well as the Ni-doped ZnO thin films, the $[Zn(NH_3)_4]^{+2}$ and $[Ni(NH_3)_4]^{2+}$ complexes are combined in the right amounts. On a SnO_2:F/Glass (FTO) substrate, it was developed in stages (Figure 3.3).

4. RESULTS AND DISCUSSION

In this chapter, we examine the findings from research on the optical and characteristics of Ni-doped ZnO films made using the SILAR method as well as the impact of these films' antimicrobial activity qualities.

4.1 Morphological and Elemental Analysis of Thin Films

In this study, samples which were growing into the FTO surface (FTO/ZnO, FTO/Ni 1 %, FTO/Ni 2 %, and FTO/Ni 3 %) samples were used. The scanning electron Emitted electrons are used in a microscope (SEM/EDS).

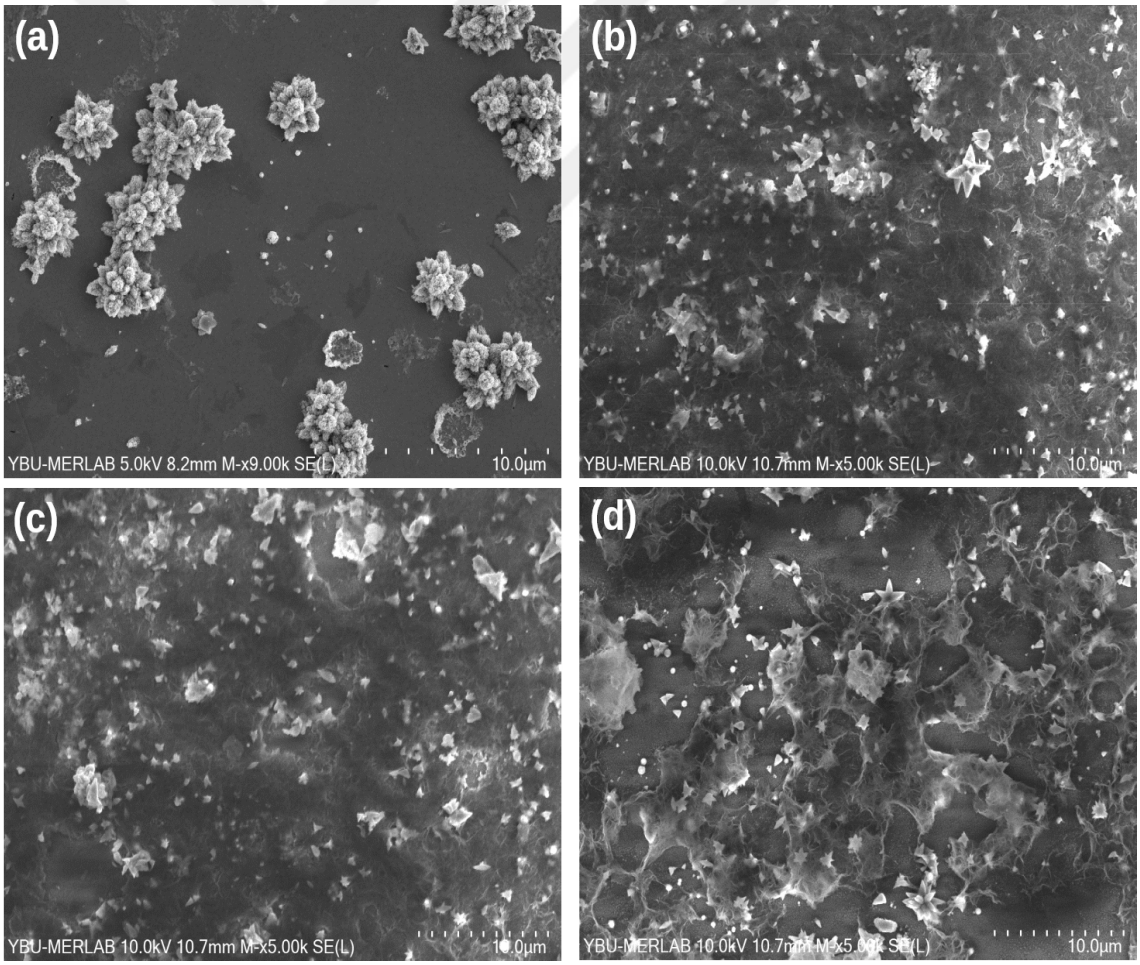

Figure 4.1 SEM analysis of thin films of (a) FTO/ZnO, (b) FTO/ZnO/Ni 1 %, (c) FTO/ZnO/Ni 2 %, (d) FTO/ZnO/Ni 3 %

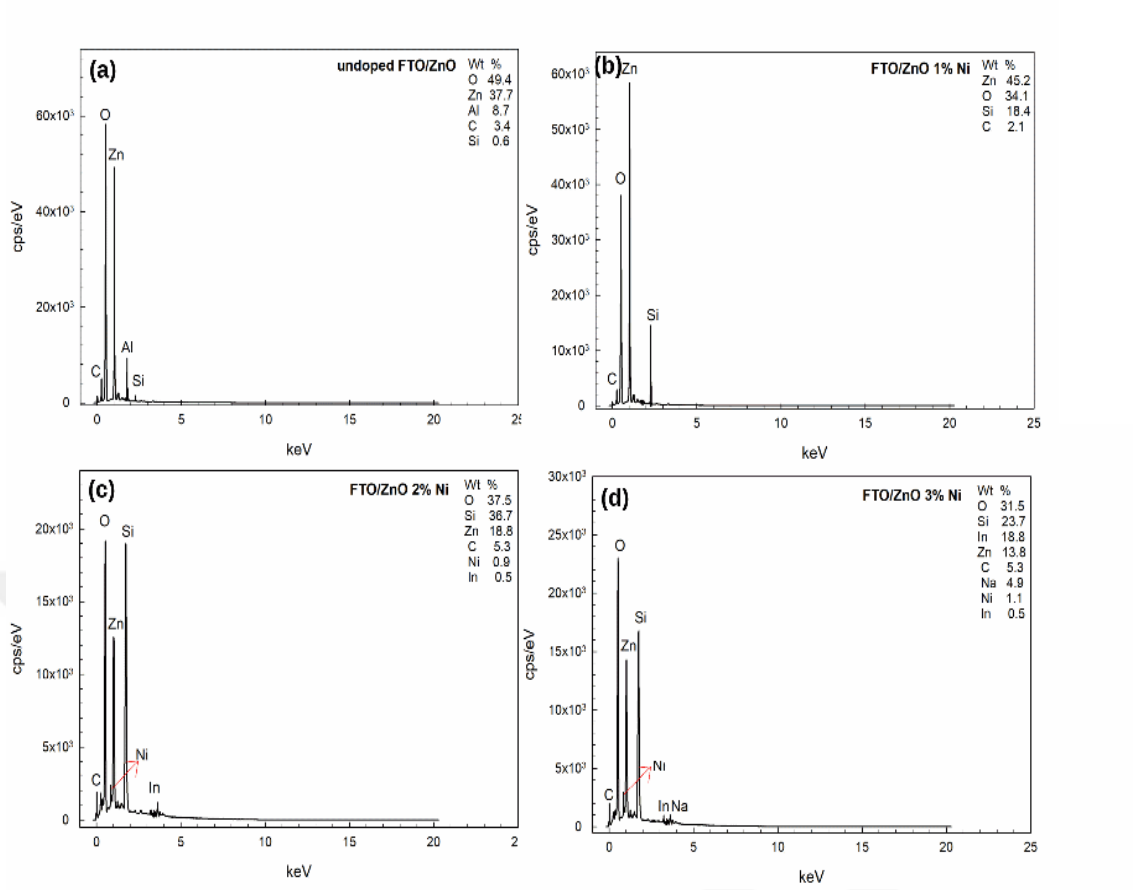

Figure 4.2 EDS analysis of thin films of (a) FTO/ZnO, (b) FTO/ZnO/Ni 1 %, (c) FTO/ZnO/Ni 2 %, (d) FTO/ZnO/Ni 3 %

The surface morphology of the undoped ZnO/FTO thin film samples shown the growth of nano-flower structures on the surface, according to SEM examination (Figure 4.1). Small clusters of these nanoflower formations combined to form larger ones. Ni-doped ZnO/FTO thin films' surface morphology analysis revealed that the size of the nano-flower formations decreased. EDS analysis showed that in the undoped ZnO sample, the most dominant peak after the O peak belonged to the Zn peak (Figure 4.2). Additionally, the peaks belonging to C and Si elements were observed to be impurity atoms. In the Ni-doped ZnO sample, the peak of the Ni element, which is the doping element, was observed in the EDS analysis. Furthermore, the peaks of Na, In, and Si elements were found to be impurity atoms.

4.2 Biosensor Analysis of Thin Films

4.2.1 Samples used in the study

Samples which were growing into the FTO surface (FTO/ZnO, FTO/Ni % 1, FTO/Ni % 2, and FTO/Ni % 3) that were combined with FTO surface samples were used in this study.

4.2.2 Determination of Antimicrobial Activity

The samples' antimicrobial efficacy was assessed using two separate techniques. First, the agar well diffusion method was used to test the antibacterial properties of the powder samples against gram-negative (*Escherichia coli* ATCC 35218) and gram-positive (*Staphylococcus aureus* ATCC 25923) pathogenic bacteria (Figure 3.2). In Nutrient broth (Merck), test bacteria were cultured, and their density was regulated to McFarland 0.5. 100 L of each test bacterium were added to the Nutrient solid medium, and the inoculation bacteria were evenly distributed throughout the medium. The solid medium was then divided into wells that were 7 mm in diameter. The wells drilled in various plates were filled with 100 L of samples at various concentrations (10, 25, 50, 100, 250, 500, 1000, 1500, 2000, 3000, 4000, and 5000 g/mL) and then incubated at 37 °C for 24 hours. The diameter of the zones that had formed around the well was measured using a calliper at the conclusion of the incubation, and the results were given in millimeters. Three times were used for each study (Figure 4.3).

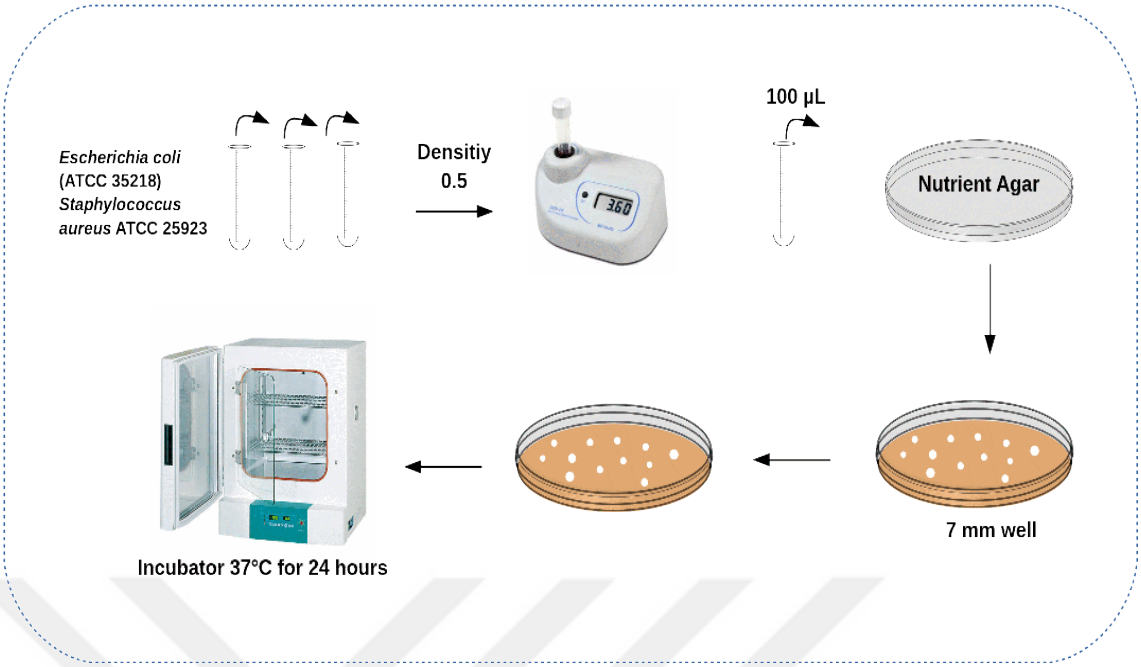

Figure 4.3 Agar well diffusion antimicrobial activity method steps (Sudhagar *et al.* 2011)

The test bacteria density (*E. coli* ATCC 35218, *S. aureus* ATCC 25923) was adjusted to McFarland 0.5 in the second approach, which is comparable to the first method. 100 L of each test bacterium was put to the nutrient solid medium and evenly distributed. The test bacteria were then placed on a glass surface that had been chopped into small pieces and integrated with various metals (FTO/ZnO, FTO/Ni % 1, FTO/Ni % 2, and FTO/Ni % 3). cultivated for 24 hours at 37 °C.

4.3 MIC and MLC/MBC Analysis

The smallest concentration required to stop bacterial growth is known as the Minimum Inhibitory Concentration (MIC). The minimum concentration at which no growth took place in the medium is known as the Minimum Lethal/Bactericidal Concentration (MLC/MBC) (Al-Mijalli *et al.* 2021). The density of the bacteria samples was adjusted to McFarland 0.5 (around 1.5x108 CFU/mL) after they had been cultured in nutrient broth. The preparation of solutions containing metal powder at various concentrations (10–5000 g/mL). Inoculating 50 liters of bacteria, 50 liters of metal, and 100 liters of nutrient media into 96-well plate wells, they were then incubated at 37 °C for 24 hours.

10 L were collected from the wells and dropped onto a plate at the conclusion of the incubation time Nutritional agar. It was again incubated for 24 hours at 37 °C. Three times were used for each study. Metal served as the study's negative control, while bacteria served as the study's positive control.

4.4 Survival Organism Counting Method

Coarse blotting paper was used to cover the Petri dish lid, and water was used to moisten it. The glass-surfaced metal samples were put in a normal petri dish with wet filter paper on the top. Glass samples were broken up into little pieces, and 100 L of the bacterial solution was applied. The samples were then incubated at 37 °C for 24 hours (Castillo *et al.* 2004). In order to count the number of viable bacteria on the surfaces, the samples were placed into a tube containing phosphate-buffered saline (PBS) solution (0.02 % KCl, 0.144 % Na_2HPO_4, 0.8% NaCl, 0.024% KH_2PO_4, pH 7.0) after the incubation. For five minutes, vortexing was used to move germs from the surface to the solution. 100 L of bacteria were put to the Nutrient solid medium and serially diluted with 0.875 % NaCl before utilizing the spreading plate method and leaving the mixture in a 37 °C oven for 24 hours. The colonies that developed in the petri dishes were counted at the conclusion of the waiting period, and the number of live bacteria was determined using the formula below. Number of Colonies x Dilution Factor / Amount of Bacteria Planted on Petri Dish = Number of Viable Bacteria (CFU/mL) (mL) Equation (4.1).

$$\text{Dilution factor} = \frac{1}{\text{Dilution ratio}} \tag{4.1}$$

4.5 Antimicrobial Activity

According to the results of the analysis, while no zone formation was observed at low Nicel concentrations, it was determined that both the zone formation and the size of the zone diameter generally increased depending on the increasing concentration.

Table 4.1 The zone diameters (mm) of the bacteria

Bacteria	Undoped Glass (Control)	FTO-Ni (Undoped)	FTO-Ni 1%	FTO-Ni 2%	FTO-Ni 3%
E. coli ATCC 35218	-	3.7	9.5	11.6	4.9
S. aureus ATCC 25923	-	-	-	-	-

Antibacterial activities of metal samples, which were integrated into the glass surface FTO-Ni (Undoped), FTO-Ni (1, 2, 3%), FTO ZnO, were investigated using Gram-positive (*Staphylococcus aureus* ATCC 25923) and Gram-negative (*Escherichia coli* ATCC 35218) pathogens (Table 4.1). Among the samples used, only undoped Glass, FTO-Ni (Undoped), and doped FTO-Ni (1, 2, 3%) showed antimicrobial activity against *E. coli* ATCC 35218. The highest zone formation was observed in 2 % doped FTO-Ni (11.6 mm), while the lowest zone diameter was found in undoped FTO-Ni (3.7 mm). No antimicrobial activity was found against *S. aureus* ATCC 25923 in all samples used.

An adhesion test was performed to determine whether *E. coli* ATCC 35218 and *S. aureus* ATCC 25923 pathogenic microorganisms adhered to glass surfaces on which different metals were integrated (Figure 4.4). The number of survival organisms after adhesion and adhesion rates were given in Table 4.2 and Table 4.3.

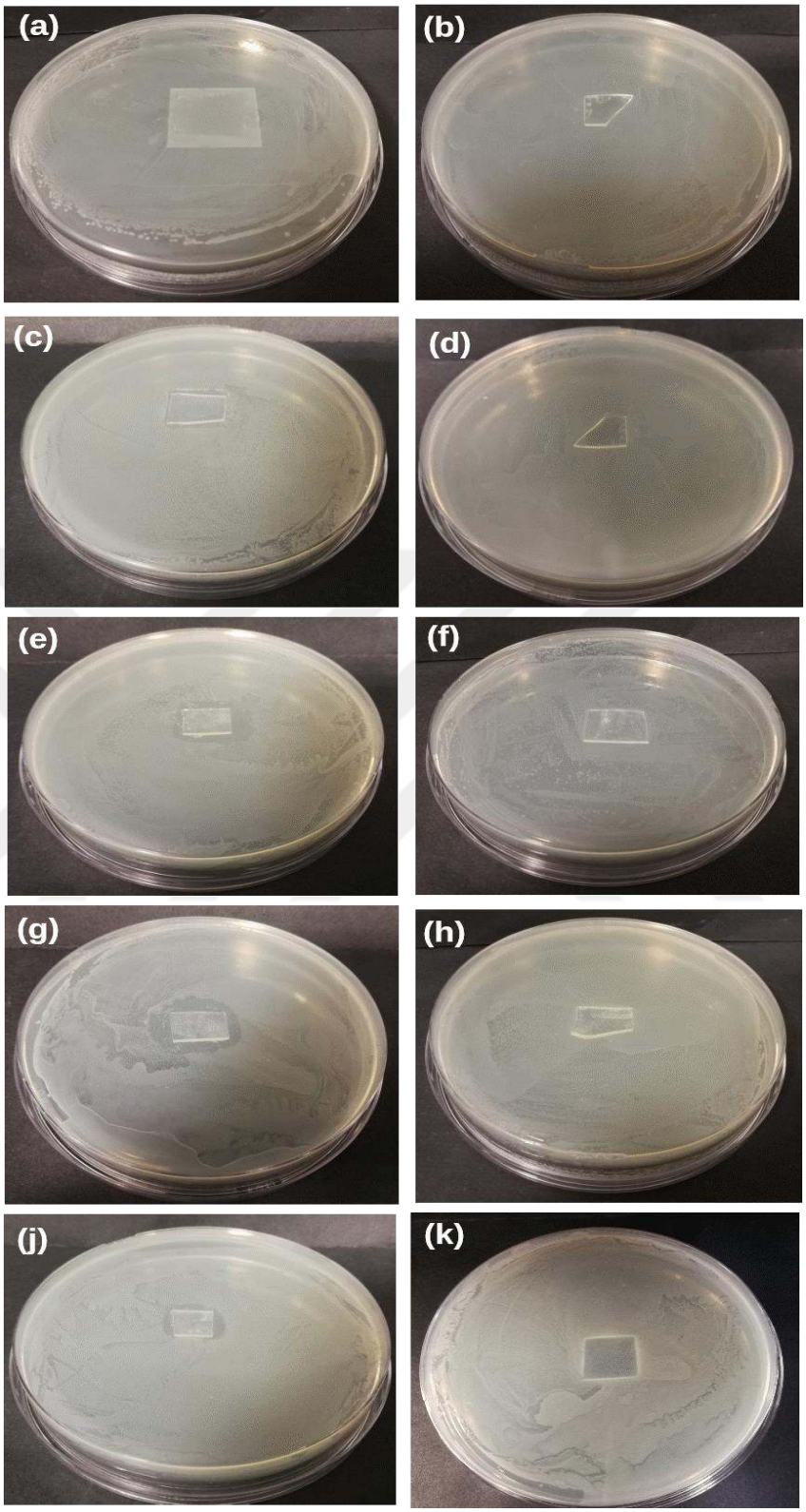

Figure 4.4 Zone formations of (a-E.coli, b-S.aureus) Undoped Glass, (*c-E.coli, d-S.aureus*) FTO-ZnO, (*e-E.coli, f-S.aureus*) FTO-ZnO Ni 1%, (*g-E.coli, h-S.aureus*) FTO-ZnO Ni 2%, and (*j-E.coli, k-S.aureus*) FTO-ZnO Ni 3% metal at different concentrations

Table 4.2 Number of survival organisms after adhesion

NUMBER OF SURVIVAL ORGANISMS (log_{10})						
Bacteria	Un-doped Glass (Control)	FTO-Ni (Undoped)	FTO-Ni %1	FTO-Ni %2	FTO-Ni %3	Bacteria (Control)
E. coli ATCC 35218	5.39	-	4.18	4.46	-	7.00
S. aureus ATCC 25923	4.10	3.89	3.99	4.04	4.08	7.36

Table 4.3 Bacterial adhesion on different Ni-doped (1, 2, 3%) ZnO surfaces and un-doped surface

BACTERIAL ADHESION (%)		
Metal	*E. coli* ATCC 35218	*S. aureus* ATCC 25923
Un-doped Glass (Control)	77	55.7
FTO-Ni (Undoped)	-	52.9
FTO-Ni %1	59.7	54.2
FTO-Ni %2	63.7	54.9
FTO-Ni %3	-	55.4

According to analyse results, for *S. aureus* ATCC 25923, no bacterial adhesion was detected on the surfaces of undoped FTO-Ni, FTO ZnO, and doped FTO-Ni (1, 2, 3%). The highest adhesion for the same bacteria was observed in the undoped class. In *S. aureus* ATCC 25923 bacteria, it was determined that as the metal additive percentage increased in the doped FTO-Ni samples, there was some enhancement in the adhesion percentages of the bacteria. When *E. coli* ATCC 35218 was examined, it was determined that the adhesion percentages varied between 58.4 and 77. In *E. coli* ATCC 35218, no significant decrease or increase was observed in the doped FTO-Ni samples depending on the increase in the percentage of Ni-doped additives.

Also, pathogenic microorganisms were kept on the Ni-doped samples integrated on the glass surface for 24 hours and viability counts were made at the end of the incubation period. No viability was observed in any of the glass samples.

5. CONCLUSION AND RECOMMENDATIONS

Recently, there have been new developments in the field of nanotechnology, which encompasses the design, production, and functional use of nanostructured materials and devices. One of the most important steps in these developments is the production of thin films, as their use can enhance the performance of devices and reduce production costs.

In this study, thin films of undoped ZnO and FTO substrate (FTO/ZnO, FTO/ZnO/Ni 1%, FTO/ZnO/Ni 2%, and FTO/ZnO/Ni 3%) were successfully grown at room temperature using the SILAR method. The morphological, and antibacterial properties of the thin film samples produced by the SILAR method were examined.

SEM analysis:

SEM analysis revealed that the surface morphology of the undoped ZnO/FTO thin film samples showed the formation of nano-flower structures on the surface. These nano-flower structures merged into larger structures in small clusters. When examining the surface morphology of Ni-doped ZnO/FTO thin films, it was observed that the size of the nano-flower structures decreased.

Antimicrobial Activity:

Antibacterial activities of undoped FTO, FTO/ZnO, FTO/ZnO/Ni 1 %, FTO/ZnO/Ni 2 %, and FTO/ZnO/Ni 3 %thin films were investigated using Gram positive (*S.aureus* ATCC 25923) and Gram negative (*E. coli* ATCC 35218) pathogens.

As a result of the analysis, the samples were tested against the two pathogens to determine their ability to inhibit bacterial growth. The results showed that the FTO-Ni 2% sample had the highest antimicrobial activity against E. coli ATCC 35218, while no activity was observed against S. aureus ATCC 25923.

For the bacterial adhesion experiments, the samples were tested to determine whether the pathogens could adhere to their surfaces. The results showed that all samples had bacterial adhesion for S. aureus ATCC 25923, with the highest adhesion rate observed for the FTO-Ni 2% sample. For E. coli ATCC 35218, bacterial adhesion was not observed for the pure FTO and FTO-Ni 3% samples.

Finally, the samples were tested for their ability to support the growth of the pathogens over a 24-hour period. The results showed that all samples except for undoped FTO had live bacteria after incubation, with the highest growth observed for E. coli ATCC 35218 on the FTO-Ni

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