

Material Preparation and Biocompatibility Characterization of ZnO-SnO₂ Thin film Sensor for Biosensing Application

Submitted in fulfillment of the requirements for the award of
Bachelor of Engineering degree in Mechatronics

by

Raagav S R (39090040)

Rohit S (39090044)



**DEPARTMENT OF MECHATRONICS
SCHOOL OF MECHANICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

**Accredited with Grade "A" by NAAC | 12B Status by UGC | Approved by AICTE
JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119**

MAY - 2023

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DEPARTMENT OF MECHATRONICS

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Raagav S R (39090040)**, **Rohit S (39090044)** who have done the project work entitled **"Material preparation and Biocompatibility Characterization of ZnO-SnO₂ thin film sensor for biosensing application"** under our supervision from June 2022 to May 2023.

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Submitted for Viva Voce examination held on ____ May 2023

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DECLARATION

We **Raagav S R (39090040)** and **Rohit S (39090044)** hereby declare that the Project Report entitled “**Material preparation and Biocompatibility Characterization of ZnO-SnO₂ thin film sensor for biosensing application**” done by us under the guidance of **Dr. T. CHINNADURAI, M.E., Ph.D.** is submitted in fulfillment of the requirements for the award of Bachelor of Engineering degree in Mechatronics.

1.



2.



DATE: ____ May 2023

PLACE: Chennai, Tamil Nadu

SIGNATURE OF THE CANDIDATE

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I am pleased to acknowledge my sincere thanks to the Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

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ABSTRACT

Thin film sensors are, Robust, reliable, and quantitative detection of biomarkers at ultra-low concentration is of great importance in clinical settings. Biosensor, an analytical device used for sensitive and selective detection of biomarkers offers various advantages over the conventional clinical diagnosis, which is both time consuming and not suitable for point of care/onsite diagnosis. miniaturization of the sensing platform and helping in the commercial success of portable biosensor kits. Transparent Oxide Semiconductor materials sensors are developed to eradicate the problems faced by single-phase materials. Zinc oxide and stannic oxide were chosen as the parent material for the composite. The wide bandgap and non-toxicity of these semiconductors is the reason to go with the above-said materials.

In this work, Zinc oxide and stannic oxide were synthesized through sol-gel method using zinc acetate and tin chloride as a precursor. Composite thin films of ZnO and SnO₂ has been fabricated by spin coating the sol-gel on the substrate. we have conducted *in-vitro* studies on ZnO-SnO₂ sol-gel. KB (HeLa derivative) cancer cells were used for the bio-compatibility assessment. MTT assay, DPPH assay, anti-bacterial activity and immunomodulatory were done. The prepared samples surface morphology was characterized by field emission scanning electron microscope (FESEM). Further, the samples were studied for their impedimetric sensing characteristics. The obtained results confirm ZnO-SnO₂ composite thin film has promising application for impedimetric bio-sensing applications.

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LIST OF ABBREVIATIONS

CPNC	Conducting polymer-based nanocomposites
CNT	Carbon nano tube
PANI	Polyaniline
Ppy	Polypyrrole
CP	Conducting Polymers
MIPs	Molecularly Imprinted Polymers
MEMS	Microelectromechanical systems
SMO	Metal oxide semiconductors
XRD	X-ray diffractometer
FESEM	Field emission scanning electron microscopy
EDS/EDAX	Energy Dispersive X-ray Spectroscopy
AFM	Atomic force microscopy
FTIR	Fourier-transform infrared spectroscopy
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide

CHAPTER 1

INTRODUCTION

In recent decades, the rapid development in the field of biosensor research has seen tremendous growth in transducers and sensors for biosensing applications. Analytical instruments that can perform rapid and accurate analysis are required for monitoring and controlling numerous parameters in industries such as the food industry, medication research, clinical diagnosis, and so on. The primary focus of current biomedical research is on measuring glucose, urea or cholesterol levels by looking for signs of enzymatic reactions in the form of byproducts. Field-effect transistor based, Amperometric, optical, and Impedimetric-based techniques as well as Other transduction mechanisms were utilized to recognize these biomolecules.

The primary nitrogen-containing component of animal urine is urea, It is crucial for the metabolism of nitrogen-containing compounds. Since urea is so prevalent in nature, clinical and agricultural chemistry are quite interested in its investigation. Urea levels in blood serum typically vary from 2.5 to 7.1 mM, and this number is used to assess renal function. However, because urea is influenced by other elements like nutrition and dehydration, it is less reliable than other indications like creatinine. Urinary tract blockage, burns, shock, dehydration, and gastrointestinal bleeding can all result from a rise in urea levels in the blood and urine, whereas nephritic syndrome, cachexia, and hepatic failure can all result from a decrease in urea levels. Many efforts at developing urea electrochemical biosensors have been made in recent decades because to their inherent advantages, such as resilience, flexibility of miniaturisation, and high detection limits.

The evaluation of cholesterol has also been regarded critical. Cardiovascular diseases, obstructive jaundice, diabetes, and nephrosis are all associated with elevated cholesterol levels, whilst low cholesterol levels are brought on by conditions including anaemia, malabsorption wasting syndrome,

hypothyroidism, and others. Biosensing techniques as been suggested recently for cholesterol estimation.

Furthermore, it is now routine and vital to measure blood glucose levels. Many metabolic processes, including glycolysis, require glucose. High blood glucose levels in people are a sign of diabetes mellitus, a metabolic disorder brought on by impaired pancreas function. Along with this, environmental factors like temperature and altitude as well as human factors like poor hand washing and naturally existing interfering compounds may have an impact on accuracy. Most biosensors research concentrate on glucose since it is crucial to monitor blood glucose concentration.

Graphene, polymers, carbon nanotubes, and other composite materials, as well as metal oxide semiconductors (SMO), are frequently used in sensor fabrication. Low cost and simplicity of manufacturing are two benefits of SMO-based sensors. ZnO, ZrO₂, WO₃, TiO₂ and SnO₂ are among the SMOs that have been intensively researched for their sensing properties. The most industrially feasible and adaptable SMOs for bio-sensing applications are ZnO and SnO₂. Furthermore, At temperatures close to room temperature, SnO₂ is conductible. It has previously been noted that SnO₂ composites including TiO₂, ZnO, and Fe have good sensing properties under a variety of circumstances. As a result, SnO₂ doped or combined with other materials has been thoroughly investigated and developed for use in gas or humidity sensors.

ZnO and SnO₂ are both semiconductors with a wideband gap. Direct band gaps for ZnO and SnO₂ are 3.3 and 3.6 eV, respectively. Native anionic vacancies, cationic interstitials, and impurities like hydrogen contribute to the n-type conductivity of these metal oxides. When water molecules are exposed to moisture, they dissociate and get adsorbed at the defect locations. This increases the concentration of surface electrons.

To address the limitations single-phase materials have, composite-based sensors have been created, which are designed for use in biosensing applications to identify chemical and biological substances.

1.1 COMPOSITES

Materials made up of two or more constituent materials, each of which has distinct physical or chemical characteristics, are called composites. In comparison to the sum of its parts, the composite material made from these materials has better mechanical, electrical, and thermal characteristics. Composites are widely used in a variety of industries, including aerospace, architectural, automotive, energy, infrastructure, marine, military, sports, and biomedical. They can be classified based on their reinforcement material, such as carbon fiber, glass fiber, or aramid fiber, and their matrix material, which can be a polymer, metal, or ceramic shown in Figure 1.1. Composites offer many advantages, including excellent corrosion resistance, weight-to-strength ratio, and durability, making them an attractive material for a range of applications.

Composite materials are combined to form a composite with improved properties. These component materials have quite varied chemical and physical properties. These components are combined to create a material that differs from its component parts in specific instances. Composites differ from mixes and solid solutions because individual components stay distinct and separate inside the finished structure.

Composite materials cost less, are lighter, stronger, and last longer than regular materials. Recently, scientists have started to actively incorporate sensing, actuation, computing, and communication into composite materials for robots. The many kinds of composite materials are as follows:

1. Ceramics Matrix Composite
2. Polymer Matrix Composite
3. Metal Matrix Composite
4. High-Performance Composite

Due to their endurance, high strength, exceptional quality, low maintenance, and low weight, composites are employed in a broad range of sectors, including construction, aerospace, automotive, and sports

A number of significant and recent advancements in biomedical applications are also addressed, including wound dressing, dental resin-based composites, and tissue engineering which comprises the fabrication of bone, oral tissues, and skin.

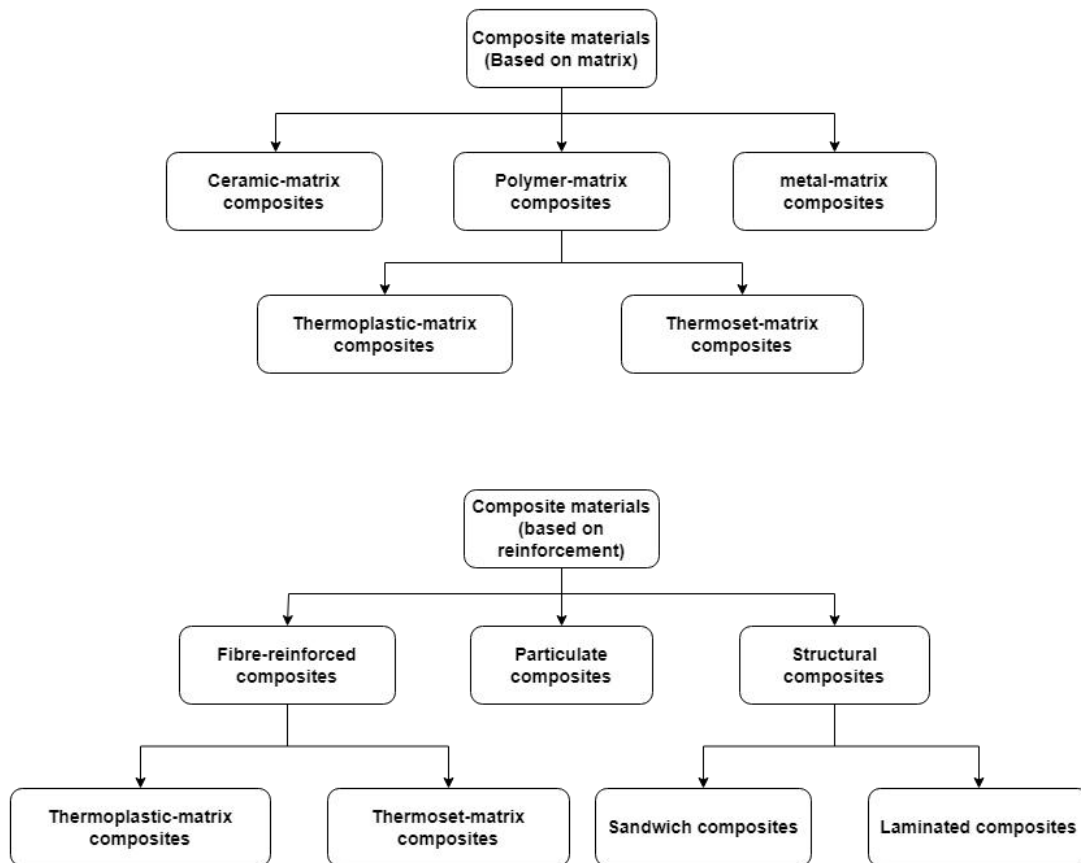


Fig:1.1 : Classification of Composites

1.1.1 Ceramic Matrix Composites

Ceramic Matrix Composites are composite materials made of a ceramic matrix and reinforcing fibres. In contrast to the fibres, which add strength and hardness to the composite, the ceramic matrix gives structure and stability. When conventional metallic materials are insufficient for high-temperature or high-stress applications, CMCs are frequently employed.

Numerous substances, including carbon, silicon carbide, and ceramic oxide fibres like alumina and zirconia, can be used to make the reinforcing fibres in

CMCs. In order to strengthen the link between the fibres and the matrix, these fibres are frequently coated with a thin coating of a substance like boron or silicon carbide.

Alumina, zirconia, and silicon dioxide are examples of oxides found in ceramic matrix materials (CMCs), as well as non-oxide ceramics like silicon carbide and boron carbide. The particular application and required qualities of the composite are taken into consideration while choosing the matrix material.

Producing fibre, preparing the matrix, and constructing the composite are only a few of the processes involved in making CMCs. Usually, a melt infiltration method or a chemical vapour deposition procedure is used to create the fibres. Techniques like chemical synthesis or sol-gel processing are used to create the matrix material. The final composite material is subsequently created by pressing hot the fibres and matrix together or by infusing chemical vapour.

In comparison to conventional metallic materials, CMCs have a number of benefits, such as great heat resistance, high strength and stiffness, and outstanding chemical and corrosion resistance. Additionally, they are less dense than many metallic materials, giving them a high strength-to-weight ratio.

Applications of CMCs include automotive, energy, aerospace, and defence sectors. They are often utilised in high-temperature and high-stress parts of rockets and spacecraft as well as turbine components like combustors and turbine blades.

In conclusion, CMCs are composite materials with great strength and stiffness, exceptional resilience to high temperatures, and outstanding chemical and corrosion resistance consisting of a ceramic matrix and reinforcing fibres. In many high-temperature and high-stress situations where conventional metallic materials are insufficient, they are employed.

Due to its endurance, high strength, excellent quality, low maintenance requirements, and low weight, composites are employed in a broad range of

sectors, including aerospace, construction, automotive, power plants, infrastructure, marine, military, sports, and biomedical industries.

Additionally, major and recent developments in biomedical applications are highlighted. These include wound dressing, dental resin-based composites, and tissue engineering (which includes bone, blood vessels, mouth tissues, and skin).

1.1.2 Polymer Matrix Composites

A polymer matrix that has been reinforced with a fibre or particulate reinforcing material makes up Polymer Matrix Composites (PMCs), a specific kind of composite material. Typically, the reinforcing material consists of ceramic particles, glass, carbon, or aramid fibres. In comparison to the polymer matrix alone, the resultant material has better mechanical qualities, including high stiffness, strength, and toughness.

Both thermoplastic and thermoset polymer matrixes can be employed in PMC. The thermoset polymers are hardened by a chemical process and cannot be remoulded, but the thermoplastic polymers may be melted and remoulded. Depending on the individual application needs, such as temperature resistance, chemical resistance, and durability, a PMC's polymer matrix will either be a particular kind or a mixture of different types.

Weaved textiles, random fibre mats, or unidirectional fibres can all be employed as reinforcing materials in PMCs. The fibres or particles are arranged in a certain orientation and direction to optimise the mechanical properties of the composite material. The size, kind, and orientation of the fibre or particle has an impact on the PMC's characteristics as well.

PMCs are manufactured using a multi-step process that includes material selection, fibre or particle orientation and layup, curing or consolidation, and other stages. An automated manufacturing method, a manual one, or a hybrid of the two are all possible. Despite the pricey production method for PMCs, the material's exceptional mechanical qualities make it a practical choice for many uses.

In conclusion, Polymer Matrix Composites are a popular choice in a variety of sectors because they offer an appealing mix of lightweight, high strength, and durability. In comparison to conventional materials like metal alloys and ceramics, PMCs have a number of benefits, and the manufacturing method is constantly being improved to enhance material characteristics and lower production costs.

1.1.3 Metal oxide Composites

Metal oxide composites are substances that have a metal oxide matrix that has been strengthened by a second substance, such as ceramic materials, carbon nanotubes, or graphene. Due to their special mechanical, electrical, and thermal characteristics, these composites have significant benefits over conventional materials like metals and polymers.

Metal oxide composites have superior mechanical qualities over the metal oxide matrix independently. In order to make the material appropriate for high-performance applications, reinforcing materials provide strength, stiffness, and toughness to it. For instance, metal oxide composites with ceramic particle reinforcement have been utilised to create cutting tools and coatings that are resistant to wear.

Metal oxide composites have been employed in the aerospace sector to create high-strength, lightweight components including engine parts and turbine blades. Metal oxide composites are utilised to create high-performance semiconductors and conductive coatings in the electronics sector. In the biomedical industry, metal oxide composites are used to manufacture implantable devices, such as joint replacements, dental implants, and pacemakers. Metal oxide composites are used as transparent conductive films, electrodes, and sensors in electronics and optoelectronics. They are used in lithium-ion batteries, supercapacitors, and fuel cells for storing and converting energy. Metal oxide composites are effective catalysts for several different chemical reactions while catalysing.

The development of metal oxide composites faces obstacles despite its many benefits, such as ensuring uniform secondary material dispersion, managing

the interface between the matrix and secondary materials, and streamlining the manufacturing process for mass production.

As a result of their many benefits over conventional materials, metal oxide composites are suitable for many applications. They are a desirable material for high-performance applications due to their distinctive mechanical, electrical, and thermal qualities, and the production method is constantly changing to enhance material features and lower prices.

1.1.4 Metal oxide composites for bioMEMS

Metal oxide composites for bioMEMS (biomedical microelectromechanical systems) applications have become an increasingly popular area of research in recent decade. Due to their distinct mechanical, electrical, and biocompatibility characteristics, these composites are superior to conventional materials like polymers and metals in a number of ways.

The metal oxide matrix employed in these composites can be any metal oxide, including zinc oxide, aluminium oxide, or titanium dioxide (TiO₂). Depending on the specific application needs, such as strength, stiffness, and thermal stability, the secondary material used for reinforcement will vary.

Metal oxide composites must be biocompatible in order to be used in bioMEMS. It has been demonstrated that metal oxide composites have high biocompatibility characteristics, making them appropriate for application in biosensors, drug delivery systems, and medical implants. The biocompatibility of metal oxide composites is due to their capacity to provide a biologically active surface layer that fosters cell growth and adhesion while reducing inflammatory and immunological reactions.

The conductivity, dielectric constant, and piezoelectricity of metal oxide composites have also demonstrated good electrical qualities, making them appropriate for application in microfluidics, microactuators, and biosensors. Excellent piezoelectric characteristics of ZnO-based composites make them appropriate for application in sensors and actuators. ZnO-based composites have

been utilised to create flexible piezoelectric sensors for detecting biomolecules in biological fluids, such as glucose and proteins.

Metal oxide composites are commonly produced using methods including sputtering, chemical vapour deposition, and sol-gel. These operations may be carried out at high temperatures, resulting in homogenous reinforcing material distribution throughout the metal oxide matrix.

1.1.5 Conducting Polymer Composite

Conductive polymers are organic materials that have the ability to conduct electricity when appropriately doped or oxidized. Examples of commonly used conductive polymers include polypyrrole, polythiophene, and polyaniline. These materials are known for their high electrical conductivity, lightweight, flexibility, and ease of processing.

The secondary materials used in conducting polymer composites can vary, but they are typically chosen for their ability to improve the composite's processability, mechanical strength, or electrical conductivity. To increase electrical conductivity, carbon-based compounds like graphene or carbon nanotubes are frequently utilised. Ceramic particles, such as alumina or silica, are used to improve mechanical strength, wear resistance, and stability. Polymer matrices, such as polyethylene or polypropylene, can be added to enhance the processability of the composite.

The fabrication of conducting polymer composites typically involves solution-based techniques, such as electrochemical polymerization, chemical polymerization, or in situ polymerization. These techniques allow for the uniform dispersion of additives within the conductive polymer matrix, ensuring the desired properties are achieved.

The unique properties of conducting polymer composites make them highly versatile and suitable for various applications. In electronic devices, they find use as conductive coatings, electrodes, and sensors. They are used in fuel cells, batteries, and supercapacitors for energy conversion and storage. In

biomedical applications, they are used for drug delivery systems, tissue engineering, and bioelectronics.

Conducting polymer composites also offer advantages in terms of environmental sustainability. For instance, conducting polymer-based composites can replace traditional metal-based coatings and electrodes, reducing the use of non-renewable resources and lowering the environmental impact.

Conducting polymers are widely used in biosensors. They may generate quantifiable signals by altering their electrical characteristics in response to various inputs. This phenomenon can be observed due to their molecular and macroscopic structure. Sensors can thus benefit from physical changes that occur when a conducting polymer is subjected to various chemicals.

1.1.6 Electrochromic Conducting Polymers

Electrochromic conducting polymers (ECPs) are a type of conducting polymer that can change color in response to an applied electric potential. Due to their distinct optical and electrical characteristics, which have drawn a lot of research, they are intriguing materials for a variety of applications, including smart windows, displays, and optical storage devices.

ECPs are a subclass of conducting polymers that can undergo reversible electrochemical oxidation and reduction reactions, causing variations to their electrical and optical characteristics. The electrochromic properties of ECPs arise from the formation of different oxidation states, which results in changes in the absorption and reflection of light. The applied potential can influence the colour of the polymer, allowing for the creation of tunable colours.

The most widely studied ECPs are based on three main types of conducting polymers: polyaniline, polypyrrole, and polythiophene. These materials have unique properties such as good environmental stability, high conductivity, and excellent electrochromic behavior. This makes them excellent for use in electrochromic applications.

The electrochromic behavior of ECPs is a result of the movement of electrons and ions in and out of the polymer during electrochemical reactions. The electrochromic properties of ECPs can be improved greater by the incorporation of various additives such as inorganic nanoparticles, electrolytes, and other organic materials. The incorporation of these additives improves the mechanical stability, electrical conductivity, and overall performance of the ECPs.

It should be noted that there may be more than two oxidation states in some circumstances. Materials that may display a variety of colours at various oxidation states are referred to as multielectrochromic materials. Their optical transmittance, reflectance, absorbance, or light emission can be modulated by integrating them into optical/electrochemical devices. The great visual contrast between its extreme states, rapid reaction time, and high stability is predicted in a perfect electrochromic material.

1.1.7 Molecularly Imprinted Polymers (MIPs)

Molecularly Imprinted Polymers (MIPs) are a form of synthetic polymer that can recognise and attach to a specific target molecule, such as a protein, a medication, or a chemical substance. In the presence of a template molecule that acts as a mould for the polymer structure, functional monomers are polymerized to create MIPs. The generated MIPs feature certain cavities that are complementary to the template molecule in terms of size, shape, and functional groups.

MIPs have attracted significant attention in various fields, including analytical chemistry, biotechnology, and biomedical applications, due to their high selectivity, specificity, and stability. They can be used as sensing materials for chemical and biological sensors, separation materials for chromatography and solid-phase extraction, and drug delivery systems.

MIPs are versatile and can be made for a variety of purposes. by choosing suitable functional monomers and template molecules. The polymerization process can be carried out using various techniques such as bulk polymerization, precipitation polymerization, and surface imprinting. After the

template molecule is removed from the MIPs, specific binding sites are left behind that can selectively bind to the target molecule with high affinity.

Compared to other molecular recognition methods like antibodies and aptamers, MIPs have a number of benefits, including as low cost, simplicity of synthesis, and stability under a variety of environments. They also offer a wide range of selectivity, as the polymer structure can be fine-tuned to optimize the binding interactions between the MIP and the target molecule.

Despite their potential, there are still challenges to be addressed in the development of MIPs, including their limited binding capacity, stability under harsh conditions, and optimization of the imprinting process. However, continued research and development in this field are expected to lead to further advances and expanded applications of MIPs in the future.

Conventional bioanalytical methods based on the enzyme-linked immunosorbent assay (ELISA) are suited for the precise detection of target molecules, but the analysis process itself is time-consuming and labour-intensive due to the use of biological materials in ELISA kit development. As a result, the use of affinity sensors is a highly interesting option. MIP-based sensors are particularly promising among other affinity sensors since they are primarily based on the polymeric matrix and do not require biological recognition components, which are often quite costly. MIPs are polymers with unique molecular recognition sites that are complementary to the imprinted target molecule. As a result, MIPs behave similarly to receptors and antibodies.

1.2 THIN FILM SENSORS

A thin film is a layer of material that can range in thickness from a monolayer, which is fractions of a nanometre, to several micrometres. The controlled production of these materials as thin films is achieved through a process called deposition. Techniques such as sputtering are commonly used to deposit metal layers onto surfaces. Recent advancements in thin film deposition techniques have led to breakthroughs in various technologies, including magnetic recording media, electronic semiconductor devices, integrated passive devices,

optical coatings, and energy generation and storage. Thin-film drug delivery is also being utilized in the pharmaceutical industry.

Thin film sensors are devices that utilize a thin layer of material to detect or measure various physical or chemical parameters. The fabrication of thin film sensors involves deposition techniques such as physical vapor deposition (PVD), chemical vapor deposition (CVD), or solution-based methods like spin coating or inkjet printing. These techniques allow for the accurate manipulation of the film thickness and uniformity, ensuring the sensor's optimal performance. The thin film layer is usually only a few nanometers to micrometers thick.

Applications for thin film sensors include monitoring of industrial processes, biomedical sensing, food quality control, and environmental conditions. They are capable of measuring a wide range of variables, including temperature, pressure, humidity, gas concentration, and chemical composition.

The act of depositing a thin layer of material on a substrate or on top of already-deposited layers is known as thin-film deposition. Molecular beam epitaxy, the Langmuir-Blodgett method, atomic layer deposition, and molecular layer deposition are just a few of the methods that may be used to deposit a single layer of atoms or molecules at a time. Deposition methods can be categorized into two types based on whether they rely more on chemical or physical processes.

Thin film sensors have several advantages over traditional sensors, including their rapid response times, high sensitivity, and excellent signal-to-noise ratios. They can also be integrated with microelectronics to create miniaturized sensing systems, which are particularly useful for wearable devices and Internet of Things (IoT) applications. Additionally, thin film sensors can be easily mass-produced, making them cost-effective for large-scale applications.

Despite their many advantages, thin film sensors also have some limitations, including their relatively high cost and the need for specialized fabrication techniques. However, advances in nanotechnology and materials

science are continuously improving the performance and reducing the cost of thin film sensors, making them an increasingly important tool for many applications.

1.3 SINGLE-PHASE MATERIAL SENSORS

Single-phase material sensors are devices that utilize a single material to detect and measure a specific parameter or stimulus. These sensors work by taking advantage of modifications in the physical or chemical characteristics of the detecting substance in response to the target parameter or stimulus. Common types of single-phase material sensors include resistive sensors, capacitive sensors, and piezoelectric sensors. Resistive sensors change their resistance in response to the target parameter, while capacitive sensors change their capacitance. Piezoelectric sensors generate an electrical charge when subjected to mechanical stress.

Single-phase material sensors offer several advantages over other types of sensors, such as ease of fabrication, low cost, and fast response times. They also exhibit high sensitivity and selectivity when optimized for a specific parameter or stimulus. Single-phase material sensors find application in numerous fields, including healthcare, environmental monitoring, and industrial process control. Examples of single-phase material sensors include temperature sensors based on thermistors, pressure sensors based on piezoelectric materials, and gas sensors based on metal oxide materials.

Despite their advantages, single-phase material sensors also face certain limitations. They may exhibit poor selectivity if there are multiple interfering parameters or stimuli present. In addition, single-phase material sensors may exhibit drift or hysteresis over time, which can affect their accuracy and reliability.

The single-phase based sensors have significant issues with their weak hysteresis and lengthy response-recovery times. Significant effort has been dedicated to doping and surface functionality modifications of the single-phase thin films in order to address these disadvantages. Couple two separate semiconductors to create a composite, which is a powerful technique that has

recently attracted a lot of interest. The hysteresis of composite Semiconductor metal oxide based sensors is superior to that of single-phase ones.

1.4 COMPOSITE-BASED SENSORS

Composite-based sensors are a type of sensor that utilizes composite materials as the sensing element. These sensors detect changes in physical or chemical properties of the composite material in response to a particular stimulus or parameter. The composite material is typically made up of two or more components, with at least one component acting as the active sensing element. The other components may act as a support structure or means of enhancing the sensitivity, selectivity, or stability of the sensor.

In composite-based sensors, the interaction between the sensing element and the target analyte serves as the basis for the sensing process. The analyte can be a physical quantity, such as temperature, pressure, or strain, or a chemical species, such as gases or liquids. The composite material undergoes a change in its physical or chemical properties in response to the target analyte, which is then detected and measured by the sensor.

Composite-based sensors offer several advantages over traditional single-phase sensors. One of the primary advantages of composite-based sensors is that they can exhibit improved sensitivity, selectivity, and stability because of the synergistic interactions between the various composite material's components. The use of composite materials also allows for the design of sensors that can operate under a variety of physicochemical conditions.

Carbon nanotube-polymer sensor is a composite-based sensor. This type of sensor utilizes the unique properties of carbon nanotubes to detect changes in strain or pressure. The carbon nanotubes are embedded in a polymer matrix and act as a piezoresistive material. When the sensor is subjected to mechanical stress, the resistance of the carbon nanotube-polymer composite changes, allowing for the detection of changes in strain or pressure. For the development of high-performance composite-based sensors, metal oxide-polymer composite sensors are also a potential approach. This type of sensor is used for

the detection of gases and volatile organic compounds. The metal oxide acts as the active sensing element, while the polymer matrix provides a means of improving the sensitivity, selectivity, and stability of the sensor.

1.5 TRANSPARENT OXIDE SEMICONDUCTORS

Over the past decade, there has been an increase in interest in transparent oxide semiconductors. They possess special qualities including great transparency in the electromagnetic spectrum's visible area and semiconducting behaviour. Compared to crystalline Transparent Oxide Semiconductors films, amorphous Transparent Oxide Semiconductors thin films exhibit better surface morphology and cleaner etched profiles. Bio-sensors made on transparent oxide semiconductors offer several benefits, such as low cost and ease of production. These materials are typically composed of metal oxides, such as indium oxide, zinc oxide, and tin oxide, and are used in many different electronic devices, including sensors, solar cells, and screens.

Binary compounds like In_2O_3 , ZnO , and SnO_2 has been the focus of research in recent decades. However, the scarcity of indium and the high cost of production have led to the development of alternative materials, such as zinc oxide and tin oxide. ZnO 's wide gap, chemical-thermal stability, strong oxidation dependency (60 mV), and non-toxicity have all led to a surge in its utilization in recent years. As a result, Zinc oxide based oxide semiconductors have been considered as extremely potential alternatives for replacing conventional Si-based materials.

Transparent Oxide Semiconductor materials have several advantages over traditional semiconductors. One of the primary advantages is their high transparency, which allows for the production of transparent electronic devices. This transparency is due to the bandgap of the material being in the ultraviolet region of the electromagnetic spectrum, which allows visible light to pass through.

Researchers concentrated on employing multicomponent oxide systems with heavy-metal cations, such as In-Zn-O , In-Ga-Zn-O , and Zn-Sn-O , to produce new and improved active materials in order to improve the characteristics of ZnO

thin films. Amorphous composite oxides, such as Zn-Sn-O and In-Zn-O systems, have lately attracted interest as potential Transparent Oxide Semiconductor materials. ZnO and SnO₂ are semiconductors with a wide bandgap. At room temperature, the wide bandgap energy of ZnO, an II-IV, the n-type semiconductor is about 3.36 eV.

Furthermore, The wide bandgap energy of SnO₂ is 3.6 eV. With a high Isoelectric Point of 9.5, ZnO is a biocompatible material suitable for protein absorption with low Isoelectric Point, where the immobilization of proteins is primarily influenced by electrostatic interactions. These unique properties that make them attractive for use in a variety of electronic devices for bio-sensors. ZnO and SnO₂ are industrially feasible and robust Transparent Oxide Semiconductors for bio-sensing applications.

1.6 BIOCOMPATIBILITY TESTS

Biocompatibility tests are a series of tests conducted to determine the biological response of a material when it comes into contact with living tissues or organisms. These tests are essential for ensuring that medical devices, implants, and other biomedical materials are safe for use in the human body. Biocompatibility tests evaluate the interaction of the material with living tissues at various levels, including cellular, tissue, and systemic levels. The tests typically involve in vitro and in vivo experiments to assess the material's cytotoxicity, immunogenicity, hemocompatibility, and other important properties. The goal of biocompatibility is to provide maximized benefits and minimised biological risks

Cytotoxicity tests evaluate the effect of the material on cell viability and proliferation. In vitro tests such as the MTT assay and LDH assay are commonly used to assess the material's impact on cells. Immunogenicity tests evaluate the material's potential to induce an immune response. In vivo tests such as the sensitization test and the lymphocyte transformation test are used to assess the material's immunogenic potential.

The biocompatibility assessment is primarily done in In-vitro conditions. MTT cytotoxicity test was performed by following the procedure in ISO 10993-

5:2009. MTT assay is a colorimetric method for accessing cell viability, cell proliferation, and cytotoxicity. It assesses mitochondrial succinate dehydrogenase's reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to an insoluble, formazan product by metabolically viable cells which is purple. The colour changes from yellow to purple assures the viability of cells. The formazan product from the mitochondrial reduction is evaluated spectrophotometrically after the cells have been solubilized with an organic solvent (e.g. DMSO). The viability of the cells is measured by the level of activity, since the decrease of MTT can only occur in metabolically active cells. Based on the production of reducing equivalents in metabolic active cells, the MTT assay evaluates cell viability. The greater the absorbance, the greater the cell viability. The percentage of cell viability is determined.

Biocompatibility is also used to characterize a material's capacity to initiate a biological response and complete clearance without causing unacceptable toxicity. Size, morphology, surface characteristics, pore geometry, porosity, and other factors all contribute to nanoparticle biocompatibility. The substance has reached the latter stage of biocompatibility when it does not induce significant toxic, carcinogenic, or immunogenic responses. To analyse a material's biocompatibility in biological applications, the physical characteristics of the composite material should be carefully studied in order to identify its potential interactions with blood and cell organelles. In this study, The potential Transparent Oxide Semiconductor materials ZnO and SnO₂ are analysed in the sol-gel form to evaluate the biocompatibility of those materials. Which are developed for biosensing applications to identify chemical and biological agents.

Zinc oxide and stannic oxide were chosen as the parent material for the composite, and then the sol-gel of zinc oxide and tin oxide was prepared. Above sol-gel is tested with the bio-compatibility assessments such as Cytotoxicity, antioxidant activity, antibacterial activity and immunomodulatory assay. MTT assay is a quantitative testing method to assess the cytotoxicity of the composite material developed. The MTT test is used to measure the viability and rate of cell growth. It is based on the enzyme succinate dehydrogenase in the cell's mitochondria reducing insoluble crystals of MTT formazan. DPPH assay is an anti-

oxidant activity assessment method that is performed based on the principle of the rate of the scavenging capacity of antioxidants.

Biocompatibility tests play a crucial role in the development and evaluation of medical devices and other biomedical materials. By assessing the biological response of materials, biocompatibility tests help ensure that these materials are safe and effective for use in the human body.

1.7 BIOSENSING APPLICATION

Biosensing applications involve the use of biological molecules or systems to detect and quantify a target analyte. The target analyte can be a biological molecule, such as a protein or DNA, or a chemical species, such as a drug or toxin. Biosensors offer a rapid, sensitive, and specific method for detecting and measuring analytes, making them a popular tool in many industries, including healthcare, environmental monitoring, and food safety.

Biosensing applications rely on the interaction between the target analyte and a biological recognition element, such as an antibody or enzyme. The recognition element is immobilized on a surface, such as a sensor chip, and interacts with the target analyte, producing a measurable signal. The signal can be detected and quantified using a variety of techniques, including fluorescence, electrochemistry, or surface plasmon resonance.

In recent decades, the rapid development in the field of biosensor research has seen tremendous growth in transducers and sensors for biosensing applications. Analytical instruments that can perform rapid and accurate analysis are required for monitoring and controlling numerous parameters in industries such as the food industry, medication research, clinical diagnosis, and so on. The primary focus of current biomedical research is on measuring glucose, urea or cholesterol levels by looking for signs of enzymatic reactions in the form of byproducts. Field-effect transistor based, Amperometric, optical, and Impedimetric-based techniques as well as other transduction mechanisms have been used to identify these biomolecules.

The principal nitrogen-containing component of animal urine is urea, which is important in the metabolism of nitrogen-containing compounds. Because urea is so common in nature, clinical and agricultural chemistry are both interested in researching it. While blood urea levels are impacted by other variables such as diet and dehydration, the normal amount of urea in blood serum is between 2.5 and 7.1 mM and is used as an indication of renal function.

An increase in the amount of urea in the blood and urine can cause urinary tract obstruction, dehydration, shock, burns, and gastrointestinal bleeding, whereas a reduction in the level of urea can cause nephritic syndrome, cachexia, and hepatic failure. Several attempts have been undertaken in recent decades to build urea electrochemical biosensors due to inherent benefits such as resilience, simplicity of miniaturisation, and high detection limits.

Similarly, cholesterol estimate has been deemed critical. Increased cholesterol levels are connected with heart disease, obstructive jaundice, diabetes, and nephrosis, whereas reduced cholesterol levels are associated with anaemia, malabsorption wasting syndrome, and hypothyroidism, among other things. Recently, biosensing approaches for cholesterol measurement have been proposed.

Furthermore, measuring blood glucose levels has become vital and common. Glucose is required for a variety of metabolic activities, including glycolysis. A high glucose level in human blood implies diabetes mellitus, a metabolic disorder caused by impaired pancreatic function. Aside from that, environmental factors like temperature and altitude, as well as human factors like improper hand washing and naturally occurring interfering substances, can all have an impact on accuracy. The majority of biosensor research focuses on glucose since it is critical to monitor blood glucose concentrations.

CHAPTER 2

LITERATURE SURVEY

Velumani, M., Meher, S. R., & Alex, Z. C. (2019). Composite metal oxide thin film based impedometric humidity sensors: the sensing capabilities of metal oxides composites and their distinguished characteristics. Zinc oxide and tin oxide composite-based thin film sensor at macro scale with humidity sensing.

Siddiqi, K.S., ur Rahman, A., Tajuddin et al. Properties of Zinc Oxide Nanoparticles and Their Activity Against Microbes. Zinc oxide nanoparticles disintegrate the cell membrane and accumulate in the cytoplasm where they are intact with biomolecules causing cell apoptosis and leading to cell death.

Pal, N. (2020). Nanoporous metal oxide composite materials: A journey from the past, present to future: application of metal oxides in sensors, biosensing capabilities of mesoporous oxides and transition metal oxides, chemical, atomic, conductive properties are reviewed.

Jang, M., Kim, S. & Lee, J. Cancer cell metabolism: implications for therapeutic targets: a study of cancer cell metabolism, glycolysis pathway, defining parameters to detect tumours by means of biochemical sensing methods, composition level breakdown of biochemical elements in cancer, properties of malignant were pitched.

Jiang, J., Pi, J., & Cai, J. (2018). The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications.: the anti-cancer property of zinc oxide results were discussed along with targetted drug delivery applications, and anti-bacterial properties. The usage of ZnO for various clinical diseases was discussed. The role of ZnO in bio-imaging for the characterization of specimens was mentioned

Gupta, R., & Chaudhury, N. K. (2007). Entrapment of biomolecules in sol-gel matrix for applications in biosensors: Problems and future prospects.: various methods of preparing sol-gel and characterization of sol-gels encapsulated with enzymes were tabulated, and factors affecting the quality of the sol-gel derived

thin films were discussed. Strategies and modification suggestions were provided for optimizing the sol-gel derived thin films.

Arce-Molina, R., Cortés-Molina, F., Sandoval, P. Y., Galaz, A., Alegría, K., Schirmeier, S., Barros, L. F., & San Martín, A. (2020). A highly responsive pyruvate sensor reveals the pathway-regulatory role of the mitochondrial pyruvate carrier MPC.: based on the glycolysis pathway, for the sensing of pyruvate the parameters were defined also existing benchtop pyruvate sensor are discussed and flaws on it were pointed, the proposal for micro level pyruvate sensor for invivo applications has been suggested. Mitochondrial functions were studied, and the proposed sensor was said to be highly responsive.

Li, B., Zhou, Q., Peng, S., & Liao, Y. (2020). Recent Advances of SnO₂-Based Sensors for Detecting Volatile Organic Compounds: the sensing ability for volatile organic compounds of tin oxide is compared with similar metal oxides, review of the advancements of tin oxide-based sensors over the past, optimization strategies were mentioned for efficient sensitivity from the nanomaterials and nanotechnology perspective.

Das, S., & Jayaraman, V. (2014). SnO₂: A comprehensive review on structures and gas sensors: the crystalline lattice structure is reviewed along with chemical properties, conductive properties, electron band gaps and mechanical properties. The preparation methodologies for efficient tin oxide were suggested, and the nanoparticle synthesis and characteristics of tin oxide are studied. Also, the self-assembly of materials was discussed.

Zak et al. (2013) conducted research on the starch-stabilized synthesis of ZnO nanopowders at low temperature and studied their optical properties. The study employed a simple, eco-friendly, and low-temperature method to synthesize ZnO nanopowders stabilized with starch. The synthesized ZnO nanopowders were characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy, and UV-visible spectroscopy. The results showed that the ZnO nanopowders synthesized at low temperature had a hexagonal wurtzite structure

and were of high purity. The synthesized ZnO nanopowders exhibited good optical properties, with a bandgap energy of 3.19 eV and high transmittance in the visible region. The study concluded that the low-temperature starch-stabilized synthesis method was effective in producing high-quality ZnO nanopowders with potential applications in optoelectronic devices.

Singh (2010) synthesized and characterized ZnO nanoparticles and studied their electrical and sensing properties. The average particle size was found to be around 28 nm, and the nanoparticles showed high sensitivity towards ethanol vapor. The study concluded that ZnO nanoparticles can be used as an efficient sensing material for gas sensing applications.

Zak et al. (2011) synthesized and characterized zinc oxide (ZnO) nanoparticles using gelatin media. The preparation involved mixing zinc acetate and gelatin in distilled water, followed by heating and stirring until a gel-like substance formed. The gel was then annealed at 300°C to obtain the ZnO nanoparticles. The morphology, size, and structure of the nanoparticles were studied using transmission electron microscopy (TEM) and X-ray diffraction (XRD). The results revealed the formation of crystalline ZnO nanoparticles with an average size of 15 nm. The study suggests that gelatin can be used as an effective medium for synthesizing ZnO nanoparticles with promising applications in nanotechnology.

Ahmadpour Kermani et al. (2021) conducted an in vitro study to compare the antifungal and cytotoxicity activities of titanium dioxide and zinc oxide nanoparticles with amphotericin B against various *Candida* species. The researchers found that both titanium dioxide and zinc oxide nanoparticles had antifungal activity against *Candida* species, with zinc oxide nanoparticles showing greater efficacy than titanium dioxide. However, both types of nanoparticles also exhibited cytotoxicity against human cells, indicating the need for further research to determine their safety and efficacy as antifungal agents.

Baruah et al. (2017) provide a comprehensive review of zinc oxide (ZnO) as a novel material for biosensors. The article covers various aspects such as the properties and synthesis of ZnO, its unique advantages over other materials, and

its applications in different biosensing technologies. The authors highlight the importance of ZnO in developing high-performance biosensors due to its biocompatibility, stability, and sensitivity to various biological analytes. The review concludes by discussing future directions and challenges in the field of ZnO-based biosensors. Overall, the article provides valuable insights into the potential of ZnO as a promising material for biosensing applications.

Giuntini et al. describes the sol-gel preparation of tin oxide thin films and the study of their transport properties. The tin oxide thin films were prepared using a sol-gel method, and the transport properties were measured using a four-point probe technique. The study found that the electrical conductivity of the tin oxide thin films increased with temperature and was affected by the atmosphere in which the measurements were made. The researchers concluded that the sol-gel preparation method was suitable for producing thin films of tin oxide with good transport properties.

Khan et al. (2008) developed a zinc oxide nanoparticles-chitosan composite film for cholesterol biosensing. The composite was prepared using a sol-gel technique and characterized by scanning electron microscopy and Fourier transform infrared spectroscopy. The composite film exhibited good electrochemical performance and excellent cholesterol detection sensitivity.

Zhang et al. (2019) synthesized an urchin-like $\text{SnO}_2/\text{NaNbO}_3$ nanocomposite for humidity sensing at room temperature. The stable humidity-sensing properties were investigated, and the results were published in *Sensors and Actuators B: Chemical*. The nanocomposite's morphology and structure were characterized, and its sensing mechanism was discussed. The study concluded that the $\text{SnO}_2/\text{NaNbO}_3$ nanocomposite exhibited good sensing stability and repeatability, making it a promising candidate for humidity sensing applications.

Sayed Ahmad Mozaffari et al. (2014) developed an impedimetric biosensor using reactive RF magnetron sputtered zinc oxide nanoporous transducer for urea detection. The biosensor exhibited a linear response to urea concentration within the range of 0.1 to 10 mM, and its detection limit was found to be 0.1 mM.

Arya et al. (2012) provide a comprehensive review of recent developments in ZnO nanostructures and thin films for biosensor applications. The authors discuss the properties and synthesis methods of ZnO nanostructures and thin films, as well as their performance in various biosensing applications such as glucose, DNA, and protein sensing. The article also covers the challenges and future prospects of using ZnO nanostructures and thin films in biosensors.

Arya et al. (2012) review recent developments in ZnO nanostructures and thin films for biosensor applications, including DNA, protein sensing, and the challenges associated with their use. They also discuss the future prospects of using ZnO nanostructures and thin films in biosensors.

Jacobs et al. (2014) presented a research article in *Biosensors and Bioelectronics*, describing the development of ultra-sensitive electrical immunoassay biosensors using nanotextured zinc oxide thin films on printed circuit board platforms. The study showed that the biosensors could detect target molecules at extremely low concentrations, with potential applications in healthcare and environmental monitoring.

Kumar and Chen (2008) explored the application of nanostructured zinc oxide particles in biosensors by modifying the electrodes chemically. The article reviews the different methods of preparing zinc oxide nanoparticles and their characterization. The study concludes that the modified electrode with zinc oxide nanoparticles has improved sensing properties for glucose, cholesterol, and lactate.

Naderi et al. (2020) developed a sensitive glucose biosensor using a glucose oxidase/nano-ZnO/thin film deposited on FTO. The innovative clinical transducer demonstrated excellent sensing performance with a linear range of 1-10 mM and a detection limit of 0.5 μ M. The biosensor also showed good selectivity, reproducibility, and stability, making it a promising tool for glucose monitoring in clinical applications.

Wahab et al. (2018) reported the fabrication of a glucose biosensor device using zinc oxide nano-rods. The study demonstrated the performance of the biosensor in terms of its sensitivity, selectivity, and linearity. The results showed that the biosensor had a linear response over a wide glucose concentration range and good selectivity towards glucose. The use of zinc oxide nano-rods as a sensing material holds promise for the development of high-performance biosensors.

F. Muhammad et al. (2011). Acid degradable ZnO quantum dots for targeted delivery of an anticancer drug. The study highlights the fabrication of ZnO quantum dots and their potential as a delivery platform for anticancer drugs. The authors demonstrated the degradation of ZnO in the acidic environment of cancer cells, leading to the release of the drug payload. The results suggest that ZnO quantum dots can be used for targeted drug delivery in cancer therapy.

Singh et al. (2006) developed a cholesterol biosensor by covalently immobilizing cholesterol esterase and cholesterol oxidase on polyaniline films. The immobilization process was optimized, and the biosensor was characterized using electrochemical techniques. The biosensor showed high sensitivity and selectivity towards cholesterol detection, making it a promising tool for clinical applications.

Wang et al. developed a Zinc oxide nanocomb biosensor for glucose detection. The study focused on the development of a biosensor for glucose detection using a zinc oxide nanocomb structure. The results showed that the biosensor had high sensitivity and selectivity for glucose detection, making it a promising candidate for clinical applications.

CHAPTER 3

AIM AND SCOPE OF PRESENT INVESTIGATION

3.1 AIM

The main objective of this study is to develop ZnO-SnO₂ based composite for biosensing applications to identify chemical and biological agents. Thin films were prepared by spin coating techniques. The cytotoxicity, anti-oxidant activity, anti-bacterial and immunomodulatory of ZnO-SnO₂ sol-gel on KB cancer cells was evaluated. The surface morphology of ZnO-SnO₂ thin films was detected by using Field-Emission Scanning Electron Microscope FESEM and the sensing response characteristics of the developed biosensor have been studied.

3.2 SCOPE OF PRESENT INVESTIGATION

The scope of this project is to address some of the key challenges associated with clinical chemotherapy. One of the primary goals is to reduce the side effects of chemotherapy. This will be achieved through the effective delivery of drugs to tumor cells only, thereby minimizing damage to healthy cells. Another objective is to increase the frequency of chemotherapy by reducing the concentration of drugs, while maintaining the therapeutic efficacy. To achieve this, the project will focus on the development of Thin film sensors that can overcome biological barriers and effectively target cancer cells. The ultimate goal is to make chemotherapy feasible for sanctuary sites, which are typically difficult to access with traditional chemotherapy approaches. By addressing these challenges, the project aims to improve the overall effectiveness of chemotherapy and enhance the quality of life for cancer patients.

CHAPTER 4

MATERIALS AND EXPERIMENTAL METHOD

4.1 MATERIAL SELECTION

In the material selection process, two semiconductors with wide bandgap energies were chosen for the current study. ZnO, At ambient temperature, a type II-IV n-type semiconductor has a bandgap energy of around 3.36 eV. This wide band gap energy is suitable for sensing applications as it allows for the detection of ultraviolet light and high energy photons. Additionally, ZnO thin films have a high exciton binding energy of around 60 meV, which is beneficial for enhancing the sensor response. SnO₂, on the other hand, has a wider bandgap energy of 3.6 eV, making it an excellent choice for sensing applications that require high selectivity and sensitivity.

The n-type conductivity of both ZnO and SnO₂ is attributed to impurities such as hydrogen, as well as native anionic vacancies and cationic interstitials. These defects provide sites for water molecules to dissociate and absorb, resulting in an increase in surface electron concentration. This property is advantageous for biosensing applications as it enhances the interaction between the sensor surface and target analytes.

To develop the ZnO-SnO₂ composite thin films, the sol-gel spin coating method was employed. This technique offers the unique advantage of promoting atomic-scale mixing of the compounds, resulting in the formation of nanocrystalline thin films. The high surface area of these films further amplifies the active surface area, making them highly sensitive and selective for biosensing applications. Overall, the selection of ZnO and SnO₂ as the composite materials, along with the sol-gel spin coating method for film deposition, make the resulting thin films highly suitable for biosensing applications.

4.2 MATERIAL PREPERATION

For the preparation of zinc oxide (ZnO) and tin oxide (SnO₂) sol-gels, zinc acetate dihydrate (Zn(CH₃COO)₂·2H₂O) and tin chloride dihydrate (SnCl₂·2H₂O) were used as the respective metal precursors. The solvents employed for the sol-gel synthesis was 2-methoxyethanol, also known as ethylene glycol monomethyl ether (C₃H₈O₂), and the chelating agent used was monoethanolamine (MEA, CH₂(OH)CH₂NH₂). In the synthesis of the ZnO sol-gel, equimolar concentrations of zinc acetate dihydrate (2.96 g) and monoethanolamine (0.6 ml) were dissolved in 50 ml of 2-methoxy ethanol. A transparent and homogenous solution was obtained by stirring the mixture for 6-8 hours at 500 rpm using a magnetic stirrer. For the preparation of SnO₂ sol-gel, a similar process was followed, but instead of zinc acetate dihydrate, 1.892 g of SnCl₂·2H₂O was used as the metal precursor and 0.8 ml of MEA was added to 50 ml of 2-methoxy ethanol. The stirring process was carried out for 6-8 hours to obtain a transparent and homogenous SnO₂ sol-gel solution.

In this study, thin films of ZnO-SnO₂ composites were synthesized using the sol-gel spin coating method. The sol-gels were first aged for 24 hours to ensure proper gelation before being spin-coated onto soda lime glass substrates using an Apex SCU-2008C spin coater at 1500 rpm for 30 seconds. To improve the adhesion and stability of the films, intermediate heating was performed after each layer of coating by placing the samples in a hot air oven at 150 °C for 10 minutes.

The entire process was repeated four times to obtain the desired film thickness. The resulting films were then annealed in a high-temperature furnace at 400 °C for one hour to improve the crystallization and stability of the metal oxide sol-gels. The spin coating method used in this study provides a simple and effective way to prepare thin films of ZnO-SnO₂ composites, which can be useful for bio-sensing applications.

The study conducted involved the characterization of five thin film samples, namely Sample 1 (A/2), Sample 2 (B/2), Sample 3 (C/2), Sample 4 (D/2), and Sample 5 (E/2). Sample films A/2 and B/2 were pure ZnO and SnO₂ thin films, respectively. On the other hand, samples C/2 and D/2 were composite thin films

consisting of two layers of ZnO and two layers of SnO₂, and vice versa, respectively. Meanwhile, Sample E/2 was coated with a sol-gel mixture of ZnO and SnO₂ sol-gels in a 1:1 ratio. The methodology used in the development of the ZnO-SnO₂ composite thin films is shown in Figure 4.1.

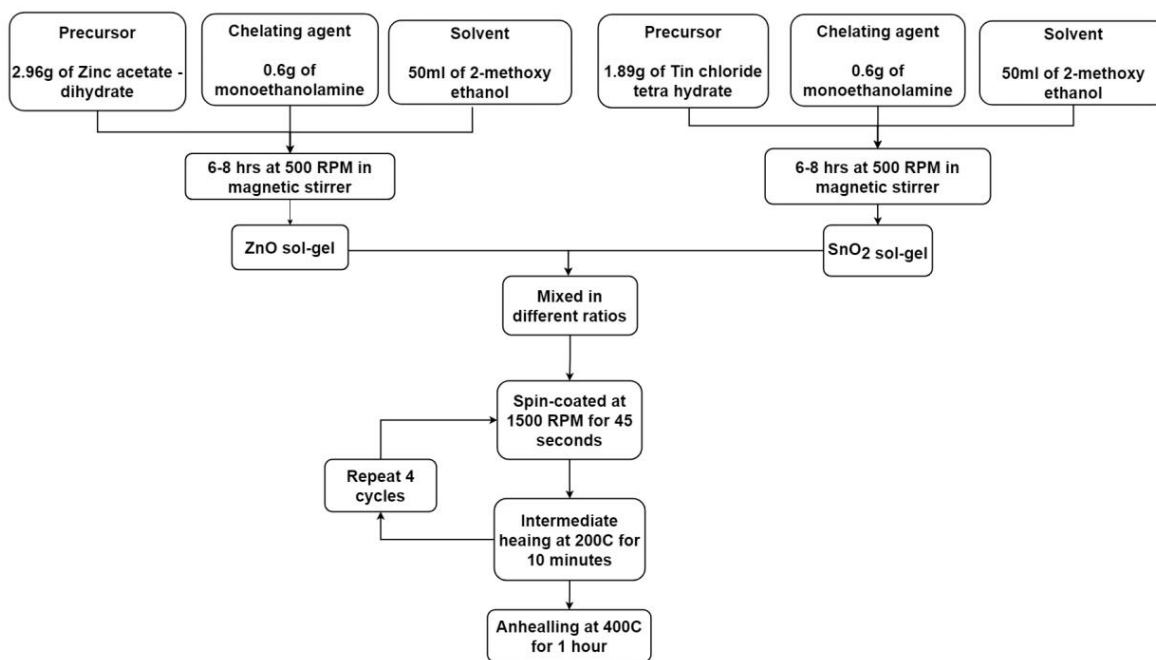


Fig:4.1 : Flowchart for the preparation of Pure and composite ZnO-SnO₂ thin film.

4.3 BIOCOMPATIBILITY ASSAYS

The present investigation aimed to study the various biological activities of ZnO-SnO₂ thin film sensor for biosensing application. In this regard, several assays were performed to assess the cytotoxicity, antioxidant potential, antibacterial activity, and immunomodulatory properties of the sensor. Two different in-vitro denaturation methods were employed to evaluate the immunomodulatory activity of the sensor. This section will discuss the various assays performed in detail.

4.3.1 Cytotoxicity assay

The cytotoxicity of ZnO-SnO₂ sol-gel composite was assessed in an in vitro assay using the MTT assay. To evaluate the cytotoxicity activity on cancer cells, KB cell line was obtained from National Centre for Cell Science, Pune, India (NCSS). The cells were cultured in minimal essential media supplemented with 10% foetal bovine serum, 100 U/ml of penicillin, and 100 µg/ml of streptomycin at 37°C. In 96-well plates, cells were seeded at a density of 5000 cells/well and were incubated for 48 hours. The ZnO-SnO₂ Sol-gel was incubated at various concentrations (0.05 and 0.1 mM) for 24, 48, and 72 hours, respectively. After removing the medium, the sample was rinsed with phosphate-saline solution and then placed in a new medium containing 50 µl of MTT solution (5 mg/ml).

The cells were then incubated for 4 hours, and DMSO was added after the incubation to dissolve the formazan crystals formed by the viable cells. The absorbance at 570 nm was measured using a microplate reader to determine the viable cells. Doxorubicin was used as a control in this study. The MTT assay is a widely used colorimetric assay for measuring cell viability and cytotoxicity and is based on the conversion of the water-soluble MTT reagent to a purple formazan product by the mitochondrial dehydrogenase enzyme of viable cells. The amount of formazan produced is proportional to the number of viable cells in the culture, making it a useful method for assessing the cytotoxicity of materials, such as the ZnO-SnO₂ sol-gel composite in this study.

4.3.2 Antioxidant assay

In accordance with the procedure outlined by Rajakumar Govindasamy et al., the DPPH radical scavenging test was conducted to evaluate the antioxidant activity of the ZnO-SnO₂ sol-gel. Different concentrations of 0.05 and 0.01 mM were employed to screen the sol-gel. For each concentration, 250 µl of a 0.2 mM DPPH methanolic solution was mixed with 1 ml of the respective diluted fraction of the ZnO-SnO₂ sol-gel.

To initiate the reaction, the mixture was thoroughly stirred and placed in a dark environment at room temperature (25 °C) for a duration of 45 minutes. During this incubation period, the interaction between the DPPH radical and the sol-gel

took place. Subsequently, the reduction of the DPPH radical mixture was quantified at a wavelength of 517 nm using a spectrophotometer. A comparative analysis was then performed between the DPPH methanolic solution (control) and the samples.

To determine the percentage of DPPH radical scavenging activity exhibited by the sample and the standard, Equation (1) was employed. This equation allowed for the calculation of the extent to which the sol-gel demonstrated antioxidant properties by neutralizing the DPPH radicals. The assessment of the scavenging activity provided valuable insights into the potential of the ZnO-SnO₂ sol-gel as an effective antioxidant agent.

$$DPPH\ Scavengin\ activity(\%) = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \quad (1)$$

A1 = absorbance of sample; A0 = absorbance of control

4.3.3 Antibacterial activity

The antibacterial activity of the ZnO-SnO₂ Sol-gel composite was evaluated using the agar well diffusion method. To conduct the experiment, Petri dishes were prepared by evenly distributing nutritional agar in different concentrations (0.05 and 0.1 mM). The target pathogenic bacterial strains, *Vibrio cholerae* and *E. coli* (*Escherichia coli*), both gram-negative strains, were selected to assess the antibacterial efficacy of the composite material. Streptomycin, a well-known antibacterial agent, was included as a positive control.

Vibrio cholerae and *E. coli* were chosen due to their widespread presence as virulent contaminants. Following the preparation of the Petri dishes, they were incubated at a temperature of 37 °C for a period of 24 hours to allow for bacterial growth and interaction with the ZnO-SnO₂ Sol-gel composite.

After the incubation period, the diameter of the zone of inhibition surrounding each well was measured. The zone of inhibition indicates the area

where bacterial growth was inhibited by the antibacterial activity of the composite material. A larger diameter of the zone of inhibition suggests a stronger antibacterial effect.

By measuring the zone of inhibition, the effectiveness of the ZnO-SnO₂ Sol-gel composite against *Vibrio cholerae* and *E. coli* could be determined. The results of the experiment provide insights into the composite material's potential as a means of inhibiting the growth of pathogenic bacteria, thus demonstrating its potential utility in various biomedical and antimicrobial applications.

4.3.4 Immunomodulatory

In-vitro egg albumin denaturation method

The test sample consisted of 0.2 ml of egg albumin, 2.8 ml of Phosphate Buffer Saline (pH 6.4), and 2 ml of ZnO-SnO₂ sol-gel in various concentrations ranging from 10 to 100 µg/ml. To serve as a reference, the positive control contained the same mixture with Diclofenac sodium, maintaining the same concentration. The effectiveness of the compound in renaturing the protein was determined by evaluating its ability to inhibit denaturation. To establish the denaturation baseline, the negative control was prepared using an equivalent volume of double-distilled water, resulting in 100% denaturation.

After incubating the aforementioned combinations at 37 °C for 15 minutes, they were preheated at 70 °C for 5 minutes in a BOD incubator. Subsequently, the solution was cooled, and the absorbance was measured at 660 nm using a multi-mode microplate reader (Synergy Biotech, USA). Each concentration was tested in triplicate to ensure accuracy and reliability of the results. This test aimed to evaluate the anti-inflammatory activity of ZnO-SnO₂ sol-gel.

The concentration at which 50% inhibition (IC₅₀) occurred was determined through probit analysis, providing valuable information about the potency and effectiveness of the ZnO-SnO₂ sol-gel in inhibiting the denaturation process and potentially displaying anti-inflammatory properties.

In-vitro BSA denaturation method

In the experimental setup, the reference sample used was aspirin, while the test sample consisted of ZnO-SnO₂ sol-gel at various concentrations ranging from 10 µg/ml to 100 µg/ml. To ensure accuracy and reliability, three separate wells were allocated for each sample. The absorbance rates of the samples were measured at a wavelength of 255 nm using a multi-mode microplate reader, specifically the Synergy Biotech instrument from the USA.

To evaluate the efficiency of the test compound in renaturing proteins, the control represented 100% protein denaturation. The ability of the compound to inhibit protein denaturation was then determined by comparing the outcomes with the reference medication. The percentage of inhibition was calculated using Equation (2), which provided a quantitative measure of the compound's effectiveness in preventing protein denaturation.

$$\text{Percentage of Inhibition} = 100 \times \left(\frac{V_T}{V_C - 1} \right) \quad (2)$$

Where, V_t = absorbance of the test sample; V_c = absorbance of the control

4.4 FIELD-EMISSION SCANNING ELECTRON MICROSCOPY

Field-emission scanning electron microscopy (FESEM) is a powerful imaging and analysis tool that is widely used in the characterization of bio-microelectromechanical systems (Bio-MEMS) sensors. Bio-MEMS sensors are devices that integrate biological and mechanical components for the detection of biological or chemical analytes. The characterization of these devices is essential for the optimization of their performance, and FESEM plays a crucial role in this process.

FESEM uses a beam of electrons to generate high-resolution images of the surface of a sample. The electrons are emitted from a source and accelerated through a high voltage to create a beam that is focused onto the sample using

magnetic lenses. The electrons interact with the atoms on the surface of the sample, generating signals that can be used to create an image.

In the case of bio-MEMS sensors, FESEM is used to visualize the structure of the device, including the surface features and the integrity of the microfabricated components. FESEM can also be used to analyze the surface chemistry of the device by detecting the backscattered electrons or secondary electrons that are generated by the interaction of the electron beam with the sample. This information can be used to determine the composition of the device, including the presence of any contaminants or residues.

FESEM can also be used to analyze the performance of the bio-MEMS sensor by visualizing the response of the device to the analyte of interest. For example, the detection of a specific protein can be visualized by observing the binding of the protein to the surface of the device. This information can be used to optimize the performance of the device by adjusting the design parameters or the surface chemistry of the device.

FESEM is a valuable tool for the characterization of bio-MEMS sensors because it provides high-resolution imaging and analysis of the surface of the device. This information can be used to optimize the performance of the device, including the sensitivity, specificity, and selectivity of the sensor.

By combining FESEM with other techniques such as X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM), a more complete understanding of the device can be obtained. FESEM is, therefore, an essential tool in the development of bio-MEMS sensors for a wide range of applications, including medical diagnostics, environmental monitoring, and food safety testing.

4.5 ELECTROCATALYTIC CHARACTERIZATION

Electrocatalytic characterization is an important aspect of the characterization of bio-MEMS sensors. Bio-MEMS sensors utilize electrochemical principles to detect biological molecules such as glucose, DNA, and proteins, and electrocatalytic characterization provides insight into the performance and behavior of these sensors.

Electrocatalytic characterization involves the measurement of the electrochemical response of the sensor surface to the target analyte. This response is influenced by several factors such as the nature of the electrode material, the immobilization method of the biological molecule on the electrode surface, and the nature of the target analyte. The response is typically measured through a voltage sweep, where the potential applied to the electrode surface is gradually increased or decreased, and the resulting current is measured. The resulting current-voltage (I-V) curves provide information on the electrochemical properties of the electrode surface and the analyte of interest.

One of the key aspects of electrocatalytic characterization is the determination of the kinetics of the electrochemical reaction occurring at the electrode surface. This is typically done by measuring the current response of the electrode surface to varying concentrations of the analyte. The resulting current-concentration curves provide information on the reaction rate, the sensitivity of the sensor, and the limit of detection.

Another important aspect of electrocatalytic characterization is the determination of the selectivity of the sensor. This is done by measuring the response of the sensor to other interfering species that may be present in the sample. Selectivity is critical to the accuracy of the sensor since biological samples are complex and contain many different species that may interfere with the measurement of the target analyte.

Electrocatalytic characterization is typically carried out using techniques such as cyclic voltammetry, chronoamperometry, and impedance spectroscopy. These

techniques provide information on the surface properties of the sensor and the kinetics of the electrochemical reaction, allowing for optimization of the sensor performance.

In conclusion, electrocatalytic characterization is an important aspect of the characterization of bio-MEMS sensors. By providing insight into the electrochemical properties of the sensor surface and the target analyte, electrocatalytic characterization allows for the optimization of the sensor performance, selectivity, and sensitivity.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 CYTOTOXICITY ASSAY

The study conducted to investigate the cell viability rate of KB, Cancer cell line exposed to ZnO-SnO₂ sol-gel is presented in Table 5.1. Figure 5.1 shows the inhibition of KB cancer cell line treated with ZnO-SnO₂ Sol-gel at intervals of 24, 48, and 72 hours. The IC₅₀ value of the control (Doxorubicin) was found to be 42.45 µg/mL at 24hrs.

The results show that the percentage of live cells at the interval of 24 hours, 48 hours, and 72 hours gradually decreases with increasing concentration of the ZnO-SnO₂ sol-gel composite. For the concentration of 0.1 mM, the percentage of live cells was 75.77%, 59.23%, and 47.88% at 24 hours, 48 hours, and 72 hours, respectively. Similarly, for the concentration of 0.05 mM, the percentage of live cells was 64.83%, 53.92%, and 43.05% at 24 hours, 48 hours, and 72 hours, respectively.

These results suggest that the death rate of cancer cells is high in the initial stage of exposure to ZnO-SnO₂ sol-gel composite and gradually decreases over time, possibly due to environmental interactions. However, it is noteworthy that a lower concentration of the ZnO-SnO₂ sol-gel composite showed an adverse effect on the KB, cancer cell line, indicating that the concentration of the composite should be optimized to achieve maximum efficacy. The findings of this study suggest that ZnO-SnO₂ sol-gel composite has potential as an effective anticancer agent, and further research is needed to explore its full potential in cancer treatment.

Time (h)	Concentration (mM)	% of cell death			Mean	SD	SEM	% Live cell
72h	0.05	56.98	58.00	55.86	56.95	1.07	0.62	43.05
	0.1	50.36	52.40	53.62	52.12	1.65	0.95	47.88
48h	0.05	45.77	47.81	44.65	46.08	1.60	0.92	53.92
	0.1	40.06	41.08	41.18	40.77	0.62	0.36	59.23
24h	0.05	36.70	35.58	33.23	35.17	1.77	1.02	64.83
	0.1	24.26	26.40	22.02	24.23	2.19	1.27	75.77

Table 5.1 : Percent Cytotoxicity of the compound has been represented

Time (h)	Conc. (μM)	Cell death (%)
72h	50	56.95
	100	52.13
48h	50	46.08
	100	40.77
24h	50	35.17
	100	24.23

Table 5.2 : Time and activity relation

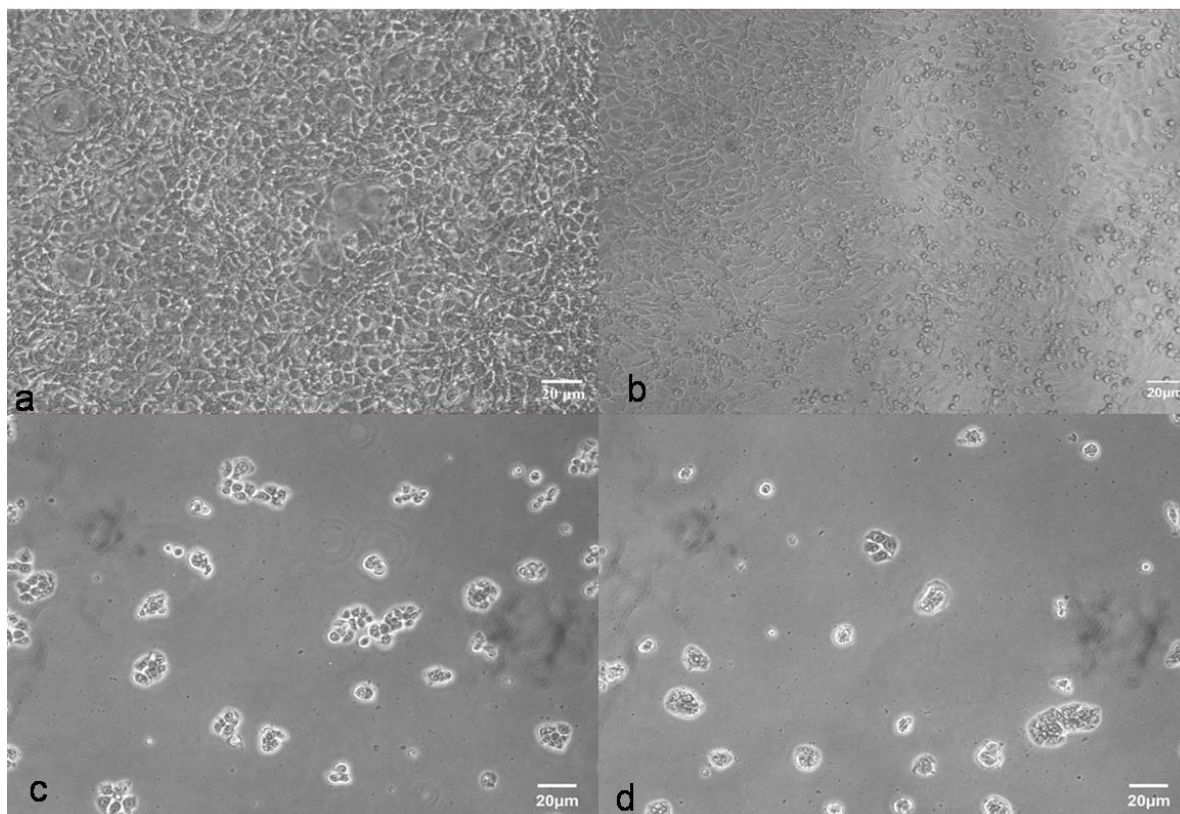


Fig:5.1 : Inhibition of KB cells treated with ZnO-SnO₂ Sol-gel using MTT assay, a = control, cells without treatment, b, c and d = cells treated with 0.05mM at 24h, 48h and 72h (Canon Inverted Microscope; 40× magnification).

5.2 ANTIOXIDANT ASSAY

The ZnO-SnO₂ sol-gels have been found to exhibit potent antioxidant activity, effectively protecting cells from oxidative stress induced by free radicals. Free radical scavenging activities play a crucial role in preventing the detrimental effects of various disorders, including cancer. In a study, the ZnO-SnO₂ sol-gel was added to a violet DPPH solution, and depending on the concentration used, the solution turned yellow. The results of the study indicated the ZnO-SnO₂ sol-gel's ability to scavenge free radicals, which is demonstrated in Figure 5.2. To evaluate the antioxidant activity of the ZnO-SnO₂ sol-gel, the natural anti-oxidant, alpha-tocopherol, was taken as the reference compound and compared with the sol-gel using the DPPH assay.

The study was conducted at concentrations of 0.5 mM and 0.01 mM, and the IC₅₀ values obtained for alpha-tocopherol and ZnO-SnO₂ sol-gel were found to be 18±3 µM and 27±8 µM, respectively, as shown in Table 5.3. The results of the study indicate that a concentration of 27±8 µM of ZnO-SnO₂ sol-gel is required to scavenge 50% of the DPPH radical. These findings highlight the potential use of ZnO-SnO₂ sol-gels in the development of antioxidant-based therapies for various disorders, including cancer, and further research is warranted to explore their full potential.

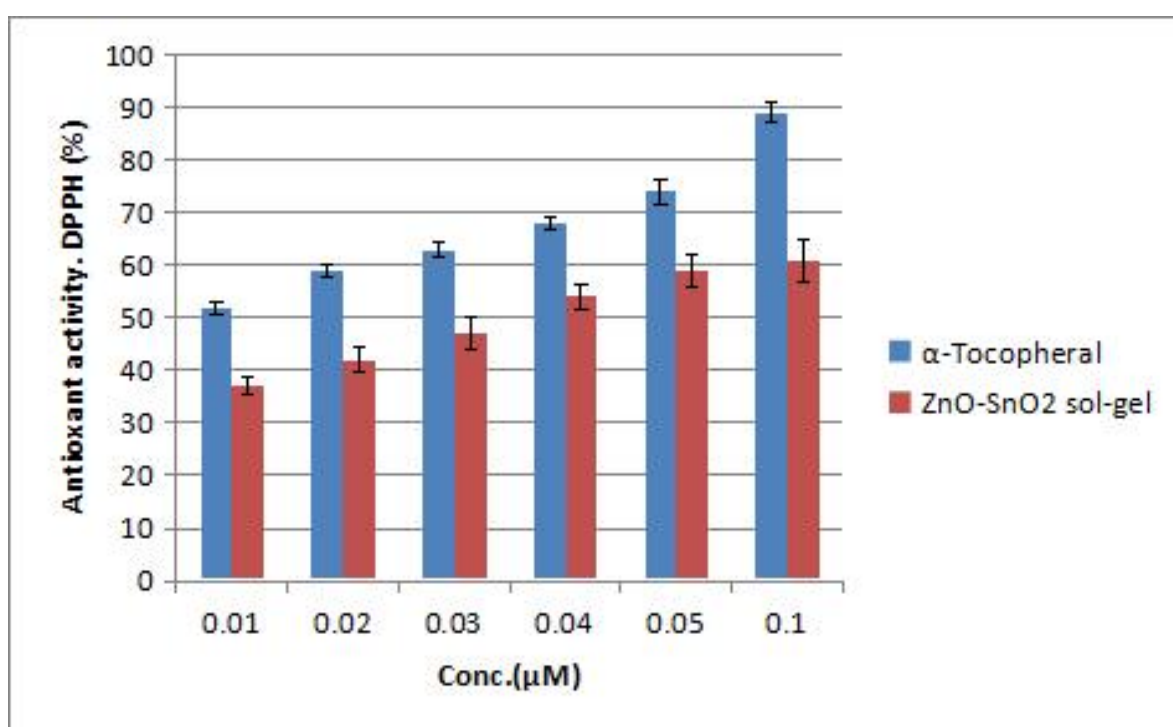


Fig:5.2 : Antioxidant Activity of ZnO-SnO₂ Sol-gel

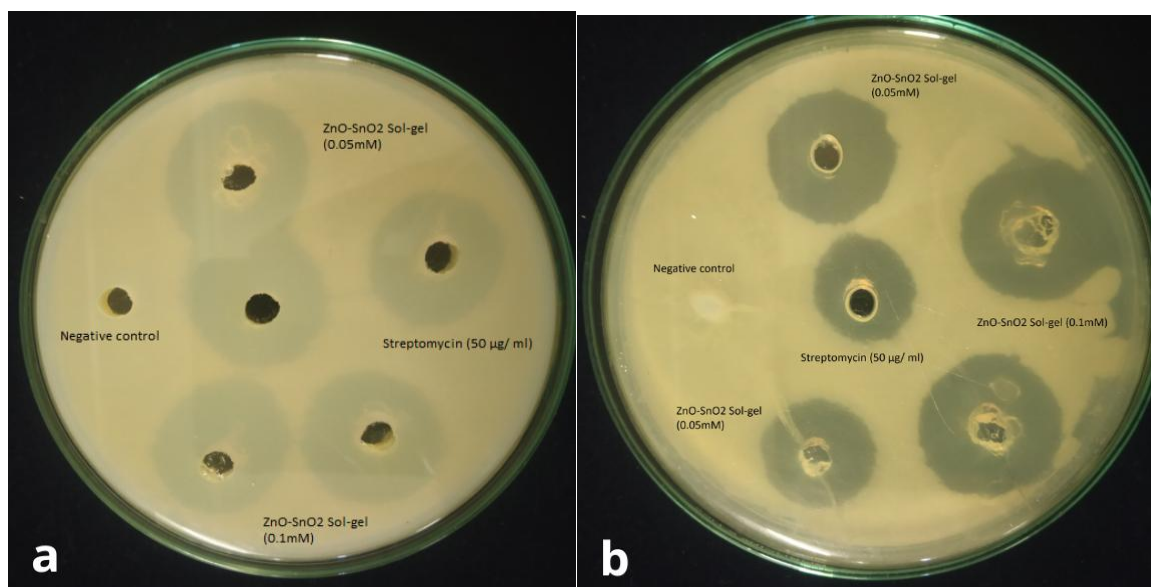
	A- tocopherol	ZnO-SnO ₂ Sol-gel IC ₅₀
IC ₅₀	18±3 µM	27±8 µM

Table 5.3 : IC₅₀ values of A-tocopherol and sol-gel

5.3 ANTIBACTERIAL ACTIVITY

The antibacterial properties of ZnO-SnO₂ sol-gel composite were evaluated using the Agar well diffusion method against two bacterial strains, *V. cholerae* and *E. coli*, with streptomycin used as a reference control agent. Different concentrations of ZnO-SnO₂ sol-gel, namely 0.05 mM and 0.1 mM, were tested, and the results indicated that the composite exhibited superior antibacterial activity against *E. coli* and *V. cholerae* compared to the standard reference streptomycin. Specifically, the zone of inhibition for the composite was observed to be 21 and 33 mm for *E. coli*, and 24 and 35 mm for *V. cholerae*, respectively. Furthermore, even at a concentration of 50 µM, the antibacterial activity was only slightly lower than that of streptomycin, with an inhibition zone of 21 mm for *E. coli* and 24 mm for *V. cholerae*.

In addition to its antibacterial activity, the ZnO-SnO₂ sol-gel composite also displayed dose-dependent anticancer activity against KB cancer cell lines. These findings indicate that the composite has potential for use in both antibacterial and anticancer applications. Overall, the results suggest that ZnO-SnO₂ sol-gel composite is a promising material for biomedical applications and further studies are warranted to investigate its potential in these fields.



**Fig:5.3 : (a) Anti-bacterial activity of ZnO-SnO₂ sol-gel against *V. cholera*,
(b). Anti-bacterial activity of ZnO-SnO₂ sol-gel against *E.coli***

Dimension of the well plate: 90 mm

Dimension of the well: 6 mm

Organism	Standard antibiotic (Streptomycin, 50µM)	Zone of Inhibition (mm)	
		ZnO-SnO₂ (50µM)	ZnO-SnO₂ (100µM)
<i>Escherichia coli</i>	25mm	21mm	33mm
<i>Vibrio cholera</i>	28mm	24mm	35mm

Table 5.4 : Antibacterial activity

5.4 IMMUNOMODULATORY

5.4.1 *In-vitro* egg albumin denaturation method

The anti-inflammatory properties of ZnO-SnO₂ sol-gel were further evaluated using the denaturation of egg albumin method. The results in Figure 5.4 illustrate that the percentage of protein denaturation is directly proportional to the concentration of the sol-gel composite. ZnO-SnO₂ sol-gel demonstrated a significant ability to prevent denaturation of egg albumin, with a 52% reduction at the highest concentration of 100 µg/mL. This performance was found to be comparable to Diclofenac sodium, which exhibited a 71% blockade of pathological modification of egg albumin.

The IC₅₀ value of Diclofenac sodium was found to be 65 µg/mL, while that of ZnO-SnO₂ sol-gel was 235 µg/mL, as presented in Table 5.5. The data clearly suggests that the sol-gel composite exhibits a significant anti-inflammatory activity, which could be beneficial in various biomedical applications. Further investigations are required to explore the underlying mechanisms of this activity and optimize the formulation of ZnO-SnO₂ sol-gel for various anti-inflammatory applications.

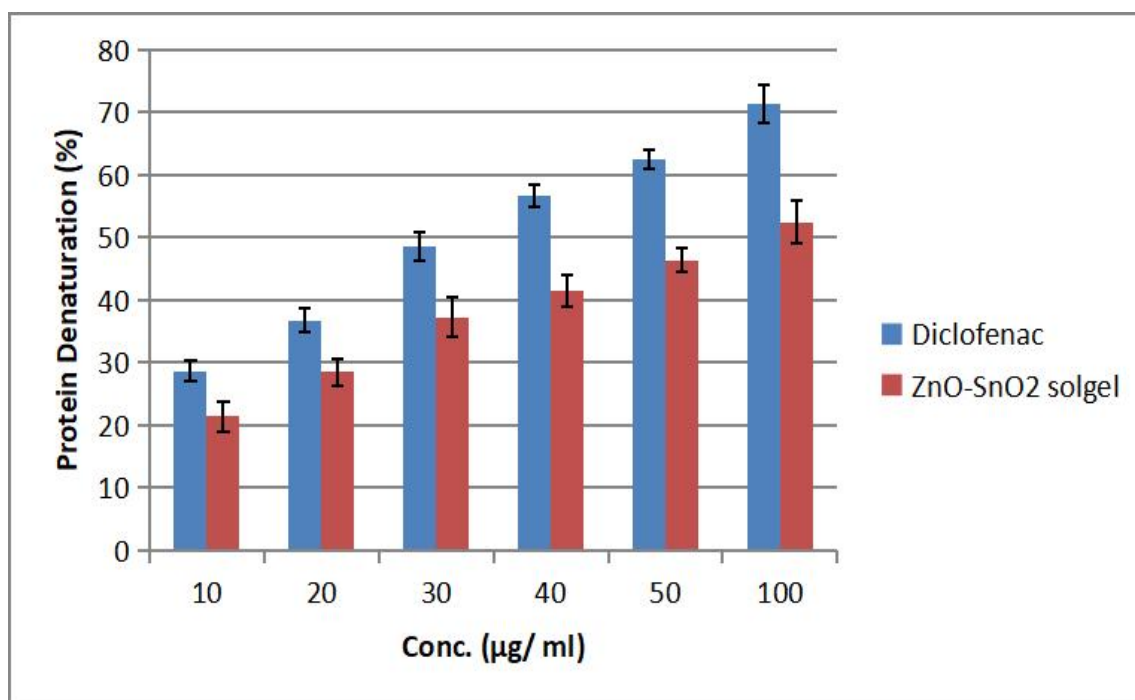


Fig:5.4 : In-vitro egg albumin denaturation

Compound	IC ₅₀ (µg/ ml)
Diclofenac sodium	65
ZnO-SnO ₂	235

Table 5.5 : IC₅₀ value of Diclofenac sodium and sol-gel

5.4.2 In-vitro Bovine Serum Albumin denaturation method

The results of the investigation into the effects of ZnO-SnO₂ sol-gel on protein denaturation are shown in Table 5.6. As can be seen from the data, the concentration of sol-gel has a direct impact on the percentage of protein denaturation. The higher the concentration, the higher the percentage of protein denaturation inhibition. For instance, at a concentration of 100 µg/mL, ZnO-SnO₂ sol-gel demonstrated a 68% inhibition of protein denaturation for bovine serum albumin.

In comparison, aspirin showed a much stronger inhibitory effect on heat-induced structural changes in protein, exhibiting an 86% inhibition for bovine

serum albumin. The IC₅₀ value, which is the concentration of the compound that inhibits 50% of protein denaturation, was determined to be 75 µg/mL for aspirin and 164 µg/mL for ZnO-SnO₂ sol-gel. These results suggest that the sol-gel composite possesses significant anti-inflammatory activity, albeit not as strong as aspirin.

Overall, the findings indicate that ZnO-SnO₂ sol-gel has the potential to be used as an anti-inflammatory agent. Further research is needed to explore its effectiveness *in vivo* and to investigate its safety profile before it can be used in clinical applications.

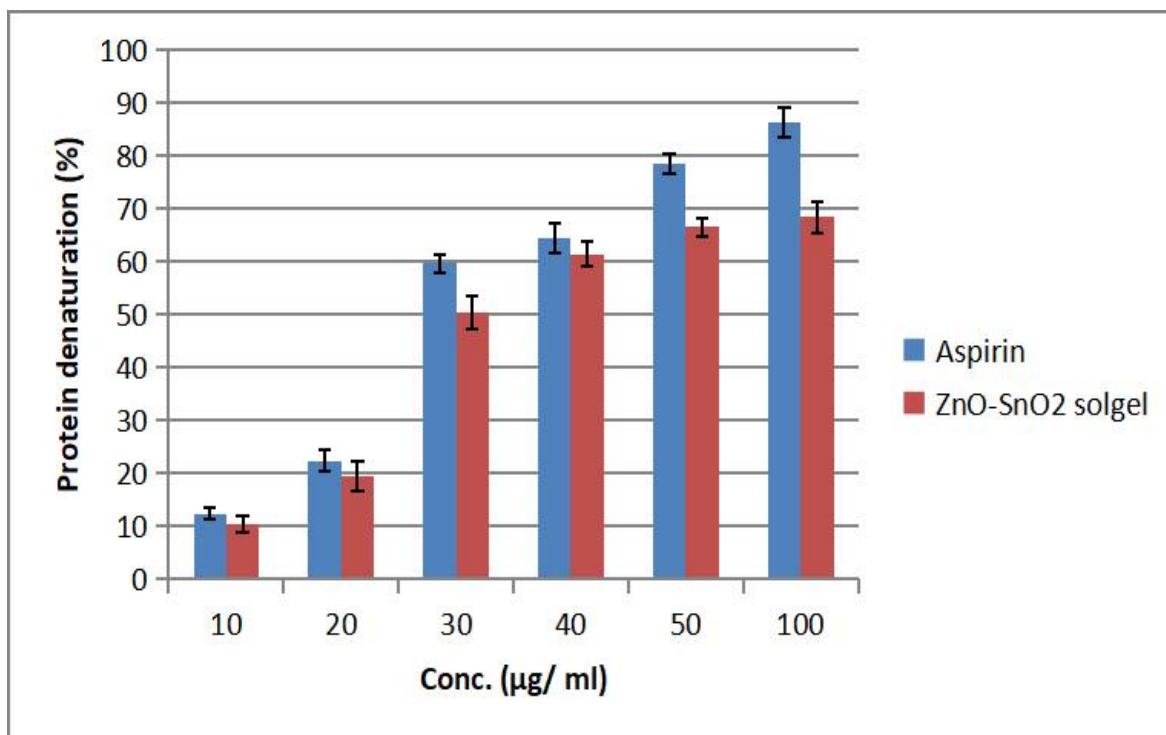


Fig:5.5 : In vitro BSA denaturation

Compound	IC ₅₀ (µg/ ml)
Aspirin	75
ZnO-SnO ₂	164

Table 5.6 : IC₅₀ value of Aspirin and sol-gel

5.5 MORPHOLOGICAL CHARACTERIZATION

The surface morphology of ZnO-SnO₂ thin films was analyzed using a Carl Zeiss NTS GMBH, Germany, SUPRA 55 Field Emission Scanning Electron Microscope (FESEM). Figure 5.6 displays the FESEM images of ZnO and SnO₂ particles at X25,000 and X50,000 magnifications. The images reveal that the ZnO and SnO₂ particles have a spherical shape with a smooth surface, and their sizes range between 50-100 nm.

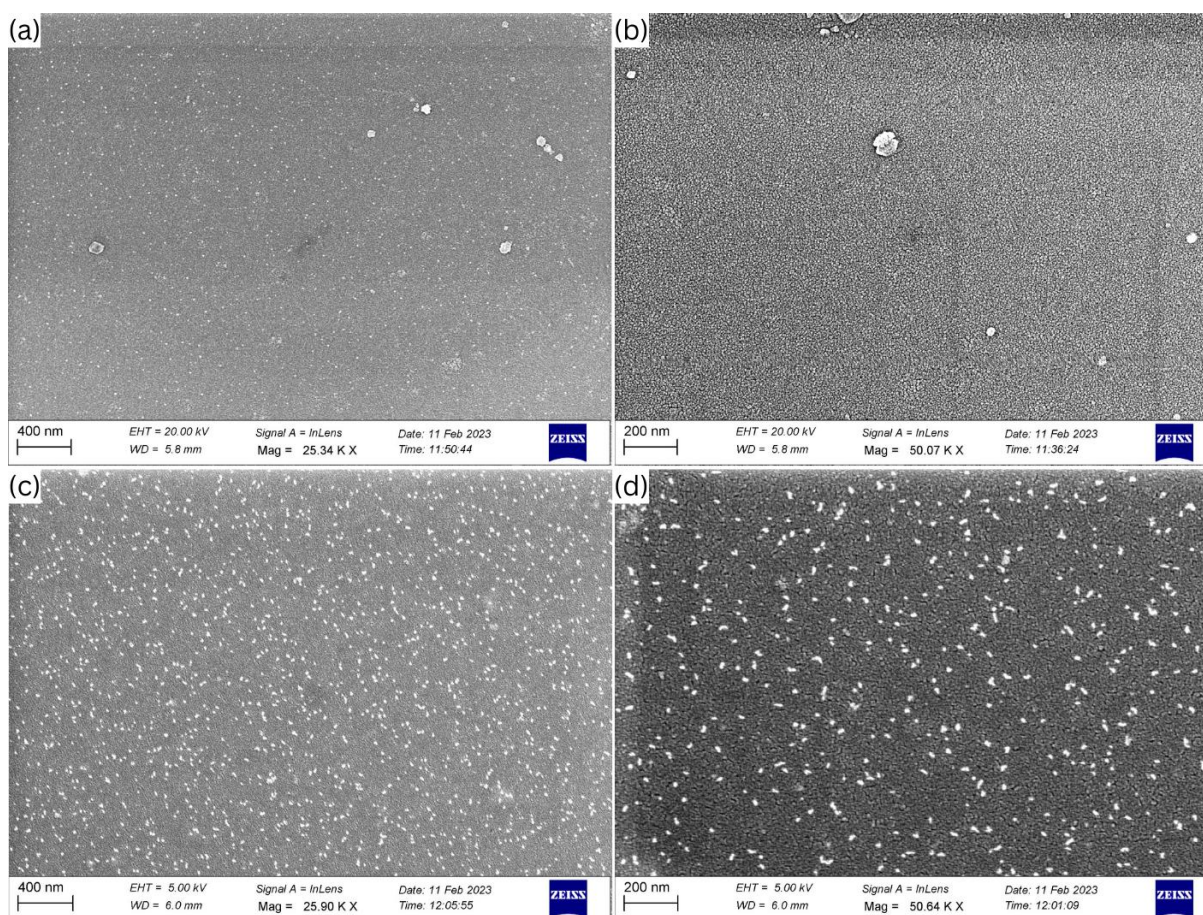


Fig:5.6 : Field emission scanning electron microscopy images of (a,b) ZnO thin films and (c,d) SnO₂ thin film.

The results of the thin films' morphology analysis confirm that ZnO and SnO₂ were uniformly distributed as a film on the substrate's surface, as shown in Figure 2.

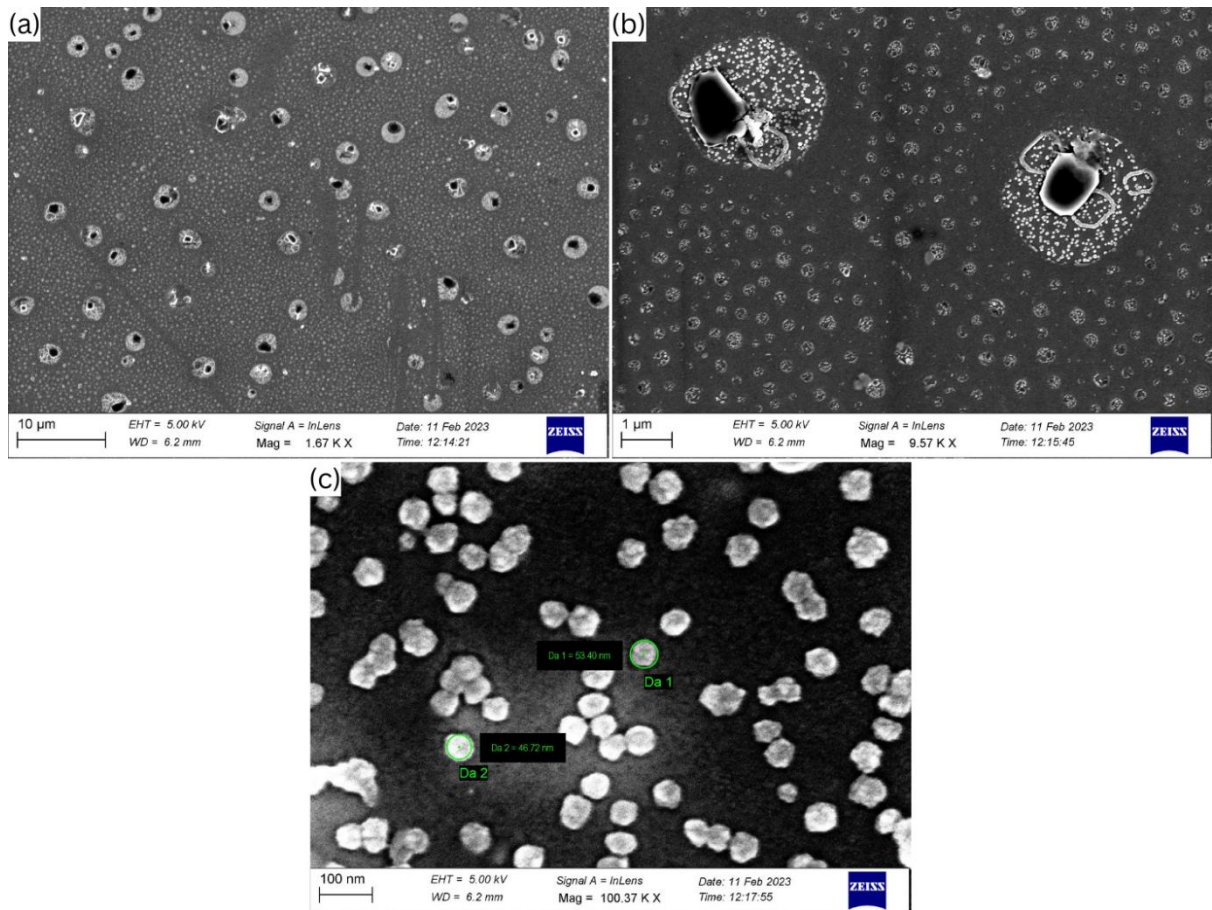


Fig:5.7 : FESEM images of ZnO-SnO₂ thin film in (a)low, (b)medium and (c)high magnification.

Further investigation was conducted on the ZnO-SnO₂ composite thin film with a 1:1 ratio, and the FESEM images in Figure 5.7 were taken at X25,000 and X50,000 magnifications. The analysis revealed that the ZnO and SnO₂ particles in the thin film were also spherical with a smooth surface. However, the size of the particles was found to be slightly smaller, ranging between 40-70 nm. These results demonstrate that the ZnO-SnO₂ thin film composite with a 1:1 ratio exhibits desirable surface morphology properties for potential applications in biosensing.

5.6 ELECTROCATALYTIC CHARACTERIZATION

The electrochemical properties of ZnO, SnO₂ and ZnO-SnO₂ thin film sensors were further investigated using cyclic voltammetry. This study was conducted in the absence of any test medium, and the results are presented in Figure 5.8. The cyclic potential range was set to 0-5 V with a scan rate of 100 mV/s. The peak oxidation current values recorded were 2.35×10^{-8} for ZnO thin film, 3.89×10^{-8} for SnO₂ thin film, and 9.24×10^{-8} for ZnO-SnO₂ composite thin film. The results indicate that the ZnO-SnO₂ composite thin film sensor exhibits the highest oxidation peak current value among the three sensors tested.

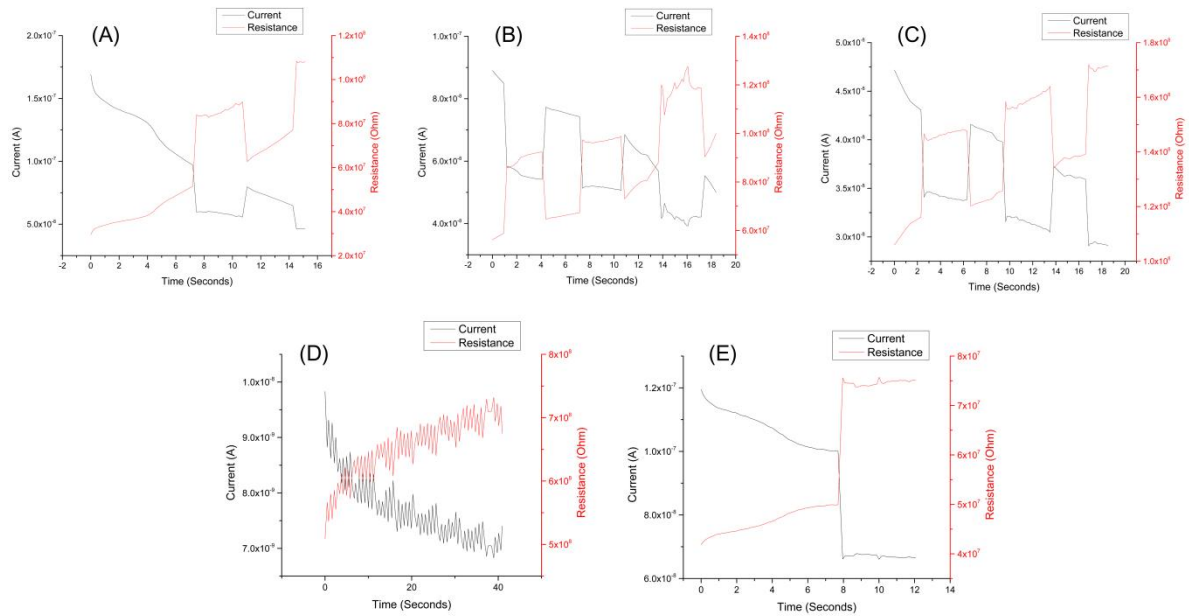


Fig:5.8 : Bias voltage of ZnO-SnO₂ thin film bio-sensors

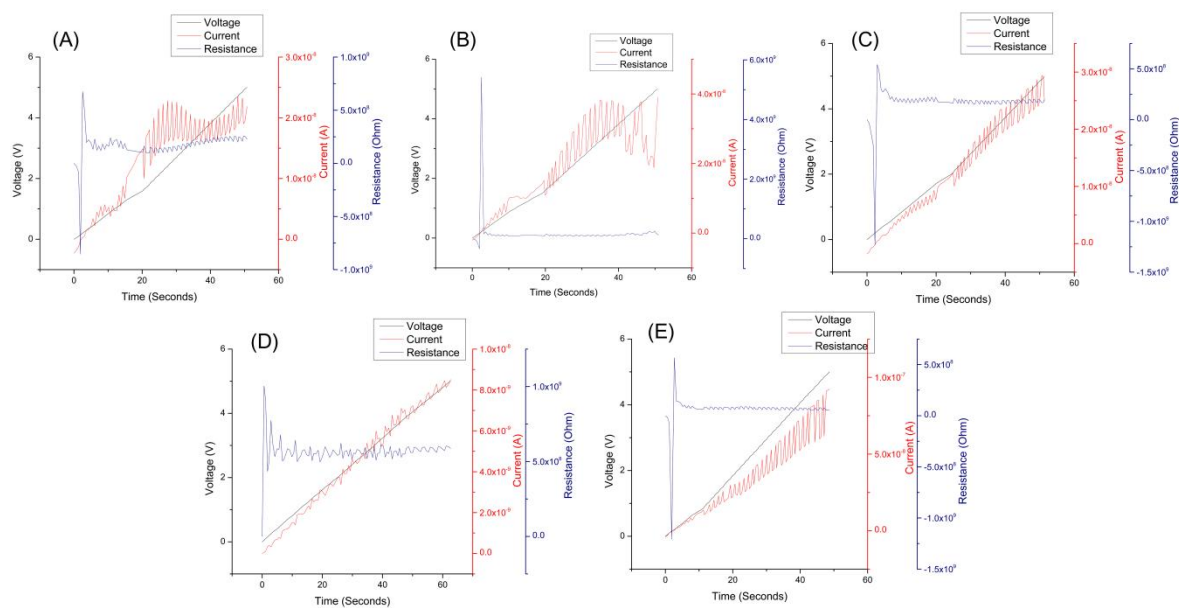


Fig:5.9 : Voltammetry curve of ZnO-SnO₂ thin film bio-sensors

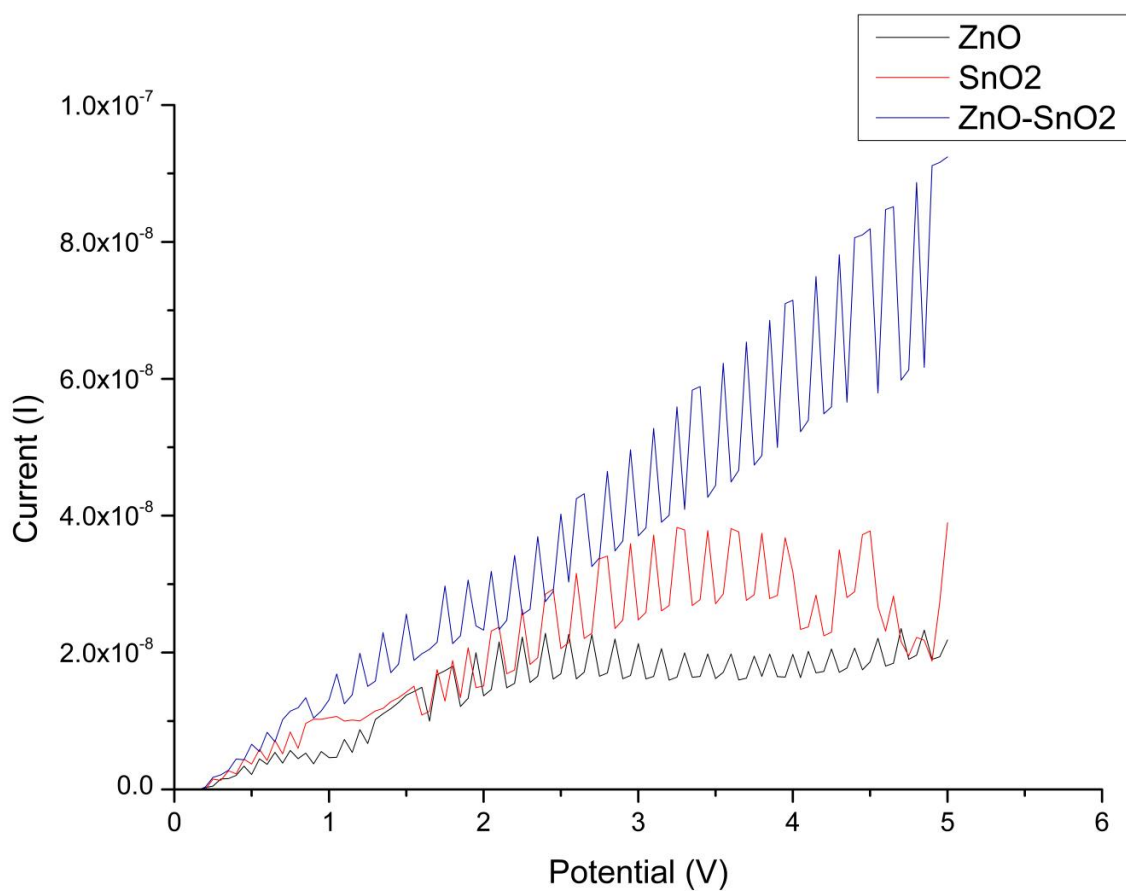


Fig:5.10 : Cyclic voltammogram curve of ZnO(black), SnO₂(red) and ZnO-SnO₂(blue) thin film bio-sensors at scan rate of 100 mV/ s

Moreover, the peak oxidation potential shifts towards a more positive potential with an increase in scan rate, indicating that the electrochemical reaction on the surface of ZnO, SnO₂, and Zn-SnO₂ thin film sensors is a surface-controlled process. This relationship is confirmed by the linear relationship between the peak currents and scan rate. These findings suggest that the ZnO-SnO₂ composite thin film sensor has excellent electrochemical properties and could potentially be used in biosensing applications. Further investigations are needed to optimize the sensing parameters and enhance the sensitivity and selectivity of the sensor for specific target analytes.

CHAPTER 6

SUMMARY AND CONCLUSION

The potential of Transparent Oxide Semiconductor materials, Zinc Oxide (ZnO) and Stannic Oxide (SnO₂), in the sol-gel form has been investigated to evaluate their biocompatibility. These materials have been developed for biosensing applications to identify chemical and biological agents due to their wide bandgap. ZnO and SnO₂ were chosen as the parent materials for the composite. The sol-gels of both materials were prepared in parallel, and the obtained gels were mixed in a 1:1 ratio. To evaluate the biocompatibility, several assays were performed, including MTT assay, DPPH assay, antibacterial activity assay, and immunomodulatory assay. The results showed that the ZnO-SnO₂ composite thin film sensor is non-toxic and possesses anti-inflammatory properties.

ZnO-SnO₂ thin films have been successfully prepared by the spin-coating method, which is a simple and effective technique for depositing thin films. The prepared ZnO-SnO₂ particles were characterized using FESEM techniques, and the average particle size was found to be 50 - 100 nm. Cyclic voltammetry analysis was also performed, which revealed that the ZnO-SnO₂ thin film sensor is suitable for biosensing applications.

The biocompatibility characterization of ZnO-SnO₂ thin film sensors is an important step in developing biosensors for various applications, including disease diagnosis and environmental monitoring. The non-toxic and anti-inflammatory properties of the ZnO-SnO₂ composite thin film sensor make it an ideal candidate for biosensing applications. Further research and development in this area will continue to improve the performance of biosensing devices and expand their applications.

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ANNEXURE:

- Research paper on the title of “ Bio-Compatibility, Characterization of ZnO - SnO₂ Composite Based Thin-Film Sensor for Biosensing Application” has been presented in International Conference on Biotechnology for Sustainable Bioresources and Bioeconomy (BSBB-2022) by Indian Institute of Technology Guwahati, Guwahati on December 9th, 2022.
- Research paper on the title of "Bio-Compatibility assessment of ZnO - SnO₂ Composite Based Thin-Film Sensor for Biosensing Application" by Raagav S R, Rohit S, Chinnadurai T, L. Inbathamizh has been submitted in Journal of Sol-Gel Science and Technology.
- Research paper on the title of "Development of ZnO-SnO₂ based thin-film bio-mems sensor for bio-sensing applications" by Raagav S R, Rohit S, Chinnadurai T has been submitted in Thin Solid Films.



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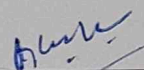
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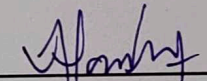
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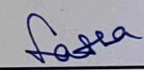
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applications


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Date: 10th December 2022

Bio-Compatibility assessment of ZnO - SnO₂ Composite Based Thin-Film Sensor for Biosensing Application

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Abstract

Transparent Oxide Semiconductor materials sensors are developed to eradicate the problems faced by single-phase materials. Which are developed for biosensing applications to identify chemical and biological agents. Zinc oxide and stannic oxide were chosen as the parent material for the composite. The wide bandgap and non-toxicity of these semiconductors is the reason to go with the above-said materials. The sol-gel of zinc oxide and tin oxide is prepared. In this paper, we have conducted *in-vitro* studies on ZnO-SnO₂ sol-gel. KB (HeLa derivative) cancer cells were used for the bio-compatibility assessment. MTT assay, DPPH assay, anti-bacterial activity and immunomodulatory were done. MTT assay is a quantitative testing method to assess the cytotoxicity of the composite material developed, where the assay works on the basis of the ability of metabolically active cells to transform an aqua soluble dye into an insoluble formazan. DPPH assay is an anti-oxidant activity assessment method that is performed based on the principle of the rate of the scavenging capacity of antioxidants.

Keywords: Biosensors, Semiconductors, Sol-gel, Composite, Biocompatibility

1. Introduction

Biocompatibility testing is a critical part to approve the subject to be used in medical and biological environments. It is the risk assessment of the interaction between the subject and the biological compounds. The goal of biocompatibility is to provide maximized benefits and minimised biological risks[1]. The biocompatibility assessment is primarily done in In-vitro conditions. MTT cytotoxicity test was performed by following the procedure in ISO 10993-5:2009.

MTT assay is a colorimetric method for accessing cell viability, cell proliferation, and cytotoxicity. It assesses mitochondrial succinate dehydrogenase's reduction of 3-(4,5-

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In the introduction, it would be helpful to add some contents about the potential of ZnO - SnO₂ Composite Based Thin-Film Sensor use in Biosensing Application. Additionally, it would be beneficial to mention other similar research works about ZnO - SnO₂ Composite Based Thin-Film Sensor for Biosensing Application.

Development of ZnO-SnO₂ based thin-film bio-mems sensor for bio-sensing applications

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Abstract

In clinical settings, the accurate, quantitative, and robust identification of biomarkers at extremely low concentrations is crucial. The employment of a biosensor, an analytical tool for the sensitive and focused detection of biomarkers, has a number of benefits over traditional clinical diagnosis, which is both time consuming and not suitable for point of care/onsite diagnosis. miniaturization of the sensing platform and helping in the commercial success of portable biosensor kits. In this work, Zinc oxide and stannic oxide were synthesized through sol-gel method using zinc acetate and tin chloride as a precursor. Spin coating the sol-gel on the substrate was a technique employed to produce composite thin films of ZnO and SnO₂. The surface morphology of the produced samples was evaluated using a field emission scanning electron microscope (FESEM). In addition, the impedimetric sensing properties of the material were investigated. The obtained results confirm ZnO -SnO₂ composite thin film has promising application for impedimetric bio-sensing applications.

Keywords: thin film sensors, composite, biosensors, bio-mems

Introduction

In recent decades, the rapid development in the field of biosensor research has seen tremendous growth in transducers and sensors for biosensing applications.[1, 2] Analytical instruments that can perform rapid and accurate analysis are required for monitoring and controlling numerous parameters in industries such as the food industry, medication research, clinical diagnosis, and so on.[3,4] The primary focus of current biomedical research is on measuring glucose, urea or cholesterol levels by looking for signs of enzymatic reactions in the form of byproducts. Field-effect transistor based, Amperometric, optical, and Impedimetric-based techniques as well as Other transduction mechanisms were utilized to recognize these biomolecules.[5] The primary nitrogen-containing component of animal urine is urea, which plays a significant role in the metabolism of substances containing nitrogen. Since urea is so prevalent in nature, clinical and agricultural chemistry are quite interested in

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