

SYNTHESIS OF TWO-DIMENSIONAL NANOMATERIALS FOR ELECTROCHEMICAL SENSING APPLICATION

A Thesis submitted
In Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

By

Sandeep Sarpal
(2K18/PHDAP/24)

Under the Supervision of

PROF. VINOD SINGH

Department of Applied Physics
Delhi Technological University
Delhi
(Supervisor)

PROF. NITIN K. PURI

Department of Applied Physics
Delhi Technological University
Delhi
(Joint Supervisor)

DR. PARTIMA SOLANKI

Special Centre for Nanoscience
Jawaharlal Nehru University
Delhi
(Joint Supervisor)



**DEPARTMENT OF APPLIED PHYSICS
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042, India
OCTOBER 2026**

©DELHI TECHNOLOGICAL UNIVERSITY-2026

ALL RIGHTS RESERVED.

*With deep love & gratitude,
I dedicate this work to my
mother*

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to everyone whose support, guidance, and encouragement have been instrumental in shaping my Ph.D. journey and contributing to the successful completion of this work.

First and foremost, I express my deepest and most sincere gratitude to my esteemed supervisor, the Head of the Department of Applied Physics, and the Chairperson of the Departmental Research Committee (DRC), **Prof. Vinod Singh**, for his exceptional mentorship, constant encouragement, and profound scientific guidance. His patience, clarity of vision, and unwavering faith in my capabilities have been instrumental in shaping both this research and my growth as an independent researcher. I am profoundly thankful for his continuous support throughout every phase of my doctoral journey.

I am equally grateful to my joint-supervisor, **Prof. Nitin K Puri**, Department of Applied Physics, for his valuable insights, constructive suggestions, and academic rigor, which have greatly enhanced the quality of this research. I am deeply grateful to my joint-supervisor, **Dr. Partima Solanki**, Assistant Professor, Special Centre for Nanoscience, Jawaharlal Nehru University, for her guidance and for illuminating the path throughout my PhD journey.

I express my sincere gratitude to **Dr. Avinash Kumar Singh**, IIT Jodhpur for his continuous support, invaluable guidance, and encouragement throughout my PhD journey. I express my sincere gratitude to **Dr. Hema Bhardwaj**, Special Centre for Nanoscience, Jawaharlal Nehru University, for her continuous support, invaluable guidance, and encouragement throughout my PhD journey.

I also express my sincere respect and gratitude to **Prof. Prateek Sharma**, Vice-Chancellor of DTU, for his encouragement and support. I am deeply thankful to the experts and members of the SRC and DRC for their valuable time, meticulous evaluation, and constructive feedback, which significantly enriched the quality, direction, and depth of my research.

I am deeply indebted to my mother, **Smt. Mina Sirpal**, for her unconditional love, unwavering support, and constant encouragement throughout my Ph.D. journey. Her faith in me and her continuous motivation inspired me to complete this research. Her blessings have always been my greatest strength and have shaped me into the person I am today.

I gratefully acknowledge Delhi Technological University (DTU) for providing a conducive academic environment that enabled me to carry out this research successfully. I also extend my sincere gratitude to all the faculty and staff members of the Department for their cooperation, encouragement, and unwavering support throughout the course of my research.

I owe a deep debt of gratitude to my laboratory colleagues in the Department of Applied Physics at Delhi Technological University (DTU) **Dr. Ritika Khatri, Dr. Nikita Jain, Dr. Kanika Sharma, Mr. Anurag Kumar, Mr. Hemant Kumar Arora, and Mr. Sunil Kumar** for their unwavering support during both the highs and lows of my research journey. I sincerely cherish the camaraderie, friendship, and mutual understanding we developed over the years. Their encouragement, cooperation, and companionship have made this journey both memorable and rewarding, leaving a lasting impact on my personal and professional growth.

I also express my heartfelt gratitude to my seniors **Dr. Vikash Sharma, Dr. Savita Sharma, Dr. Deepika Sandil, and Dr. Kamal Arora** for guiding me on the path of research. Their valuable discussions, insightful suggestions, and willingness to share their knowledge whenever needed have been a constant source of inspiration and support throughout my doctoral journey.

I would like to express my sincere gratitude to my seniors **Dr. Priya Pardeep Kumar, Dr. Umang, Dr. Kailash Garg, and Dr. Naima Tyagi** for their constant support, insightful scientific discussions, and valuable guidance. Their encouragement has played a significant role in shaping my academic journey.

I extend my heartfelt thanks to my current lab group **Dr. G. B. V. S. Lakshmi, Dr. Reena Sajwan, Dr. Archana, Dr. Amit Ahlawat, Jayendra Himanshu** for their

cooperation, shared responsibilities, and for fostering a positive environment that made the laboratory a space of learning and camaraderie.

Furthermore, I wish to express my gratitude to my friends and colleagues at AIRF, JNU including **Dr. Saroj K Jha, Dr. Manoj Pratap Singh, Dr. Ajay Kumar, Dr. Neetu Singh, Dr. Ruchita Pal, Mr. Plabon Borah, Mr. Shanker Dutt and Mr. Parveen Kumar** for their unwavering technical guidance, valuable suggestions, and support throughout this journey. I sincerely appreciate the insightful conversations and priceless advice they gave us during our research endeavors. I would also like to acknowledge all my teachers, mentors, and well-wishers who have directly or indirectly contributed to my academic journey. Their knowledge, advice, and encouragement have been invaluable.

Finally, I am thankful to everyone who has been a part of this journey, whether through support, guidance, or encouragement. This achievement would not have been possible without all of you.

Above all, I extend my heartfelt gratitude to the Almighty for bestowing upon me the strength, wisdom, and patience to persevere through the challenges of this Ph.D. journey and to successfully bring this work to completion.

Sandeep Sarpal

2K18/PHDAP/24

Date:



DELHI TECHNOLOGICAL UNIVERSITY
(Govt. of National Capital Territory of Delhi)
Shahbad Daulatpur, Bawana Road, Delhi-110042

CANDIDATE'S DECLARATION

I Sandeep Sarpal hereby certify that the work which is being presented in the thesis entitled **SYNTHESIS OF TWO-DIMENSIONAL NANOMATERIALS FOR ELECTROCHEMICAL SENSING APPLICATION** in partial fulfilment of the requirements for the award of the Degree of Doctor of Philosophy, submitted in the Department of Applied Physics, Delhi Technological University is an authentic record of my own work carried out during the period from 2018 to 2026 under the supervision of **Prof. Vinod Singh and Prof. Nitin K Puri, Department of Applied Physics, Delhi Technological University, Delhi and Dr. Partima Solanki, Special Centre for Nanoscience, Jawaharlal Nehru University, Delhi**. The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

Candidate's Signature
(SANDEEP SARPAL)

This is to certify that the student has incorporated all the corrections suggested by the examiners in the thesis and the statement made by the candidate is correct to the best of our knowledge.

PROF. VINOD SINGH
(Supervisor)

PROF. NITIN K. PURI
(Joint-Supervisor)

DR. PARTIMA SOLANKI
(Joint-Supervisor)

.....
(EXTERNAL EXAMINER)



DELHI TECHNOLOGICAL UNIVERSITY
(Govt. of National Capital Territory of Delhi)
Shahbad Daultapur, Bawana Road, Delhi-110042

CERTIFICATE BY THE SUPERVISORS

Certified that **Mr. Sandeep Sarpal (2K18/PHDAP/24)** has carried out his research work presented in this thesis entitled “*Synthesis of Two-dimensional Nanomaterials for Electrochemical Sensing Application*” for the award of Doctor of Philosophy from Department of Applied Physics, Delhi Technological University (DTU), Delhi, India under our supervision. The thesis embodies result of original work, studies are carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

PROF. VINOD SINGH

(Supervisor)
Professor
Department of Applied Physics
Delhi Technological University
Delhi-110042, INDIA

PROF. NITIN K PURI

(Joint-Supervisor)
Professor
Department of Applied Physics
Delhi Technological University
Delhi-110042, INDIA

Dr. PARTIMA SOLANKI

(Joint-Supervisor)
Asstt. Professor
Special Centre for Nanoscience
Jawaharlal Nehru University
New Delhi-110067, INDIA

Date:

ABSTRACT

Synthesis of two-dimensional nanomaterials for electrochemical sensing application

Unique surface features, chemical, optical, and electronic properties of 2 dimensional (2D) nanomaterials have made them attract tremendous recognition for a vast range of applications. 2D nanomaterials like hexagonal boron nitride (h-BN), transition metal dichalcogenides (TMDs), black phosphorus, and layered double hydroxides (LDHs) etc. are being widely explored in the field of sensing. The large surface area makes 2D nanomaterials ideal platforms for loading more and more entities for sensitive detection of the target analyte. Amongst various 2D nanomaterials, graphene has displayed a substantial potential for further exploration due to its vast range of interesting properties. Other 2D nanomaterials (Mn_3O_4 and $\alpha\text{-Fe}_2\text{O}_3$) are used for sensing due to their interesting properties viz. high charge carrier ability, good biocompatibility, and chemical stability etc. In this work, Mn_3O_4 and $\alpha\text{-Fe}_2\text{O}_3$ nanoparticles were synthesized using simple co-precipitation and hydrothermal methods, respectively. The integration of these nanomaterials with graphene oxide (GO) for the detection of *fumonisin B₁* demonstrated excellent performance, with a low limit of detection (LOD) and high recovery rates. It provides high specificity, and affinity toward target antigens. The sensing method uses the electrochemical transduction method. It is a cost-effective method as compared to optical methods. It has very high sensitivity, often reaching pico/nanomolar detection limits, suitable for miniaturization and point-of-care applications, and also compatible with label-free detection formats. It uses covalent immobilization over physical adsorption. The nanostructure surface has reactive groups ($-\text{COOH}$, and $-\text{NH}_2$) for antibody conjugation via standard chemistries e.g. EDC/NHS coupling. It targets Fc region to maximize antigen-binding site accessibility. It provides sufficient surface area for antibody loading. The blocking agents like BSA is used post-immobilization to reduce background signal. The covalent binding which is a chemical method is more favorable when testings are done in buffer solutions and repeated measurements are to be recorded.

Therefore, the nano composites (GOMn_3O_4 , and $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}$) are used for covalent

attachment of antibodies on the immobilization matrix. The growth of mycotoxins is a major global challenge in food contamination to both humans and animal health. Amongst various types of mycotoxins, *fumonisin B1* is very important mycotoxin, and is designated as a carcinogen by the International Agency for Research on Cancer (IARC), placed it in class 2B group of human carcinogens. Conventional techniques used for detecting the mycotoxins are time-consuming, expensive, cumbersome, and require trained personnel for operation.

The findings of the research work conducted for achieving the above-mentioned objectives have been divided into 5 chapters. **Chapter 1** begins with a brief introduction to nanomaterials, and nanotechnology, gradually followed by intriguing properties of 2D nanomaterials, a discussion about biosensors and their components, choice of nanomaterials (GOMn_3O_4 , and $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}$) as an immobilization matrix, based on a comprehensive literature review. The chapter then elucidates the motivation to pursue this work and the objectives of the present thesis work. Chapter 2 describes in detail the methods chosen for the synthesis of nanomaterials, and the technique used for thin film formation. This is succeeded by a discussion on all the characterization techniques which have been used in the present thesis work for confirmation of the formation of desired nanostructures and testing the biosensing performance of the fabricated platform. **Chapter 3** presents the study on detection of *FBI* using synthesized $\text{Mn}_3\text{O}_4/\text{GO}$ nanostructures. **Chapter 4** presents the study on detection of *FBI* using synthesized $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}$ nanostructures. **Chapter 5** summarizes the conclusions, future of sensors. This chapter winds up with prospects of this research work.

LIST OF PUBLICATIONS

List of Publications included in the thesis:

- [1] **Sandeep Sarpal**, Avinash Kumar Singh, Hema Bhardwaj, Nitin Kumar Puri, and Pratima R Solanki, “Graphene oxide-Mn₃O₄ nanocomposites for advanced electrochemical biosensor for *fumonisin B1* detection” *Nanotechnology*, 2023, 34(46), p.465708.

Impact Factor – 2.9

- [2] **Sandeep Sarpal**, Hema Bhardwaj, Nitin Kumar Puri, Vinod Singh, and Pratima R Solanki, “Hematite-reduced graphene oxide Nanocomposite-Based Electrochemical Biosensing Platform for Rapid Detection of *Fumonisin B1* in Corn Samples” *Chemistry Select*, 2026, 11(23), p.e06376

Impact Factor – 2.0

CONFERENCES CONTRIBUTIONS-POSTER AND ORAL PRESENTATIONS

1. **Sandeep Sarpal**, A.S.Rao, Nitin K. Puri, Hema Bhardwaj, Pratima Solanki, “Mn₃O₄/GO Nanocomposites for Advanced Electrochemical Biosensor for detection of *Fumonisin B1*”, Recent Trends in Applied Physics & Material Science (RAM 2024) at Bikaner Technical University, Bikaner, 15th – 16th Nov 2024. **(POSTER)**
2. **Sandeep Sarpal**, A.S.Rao, Nitin K. Puri, Hema Bhardwaj, Pratima Solanki, “Ferric Oxide EOGO nanocomposite for mycotoxin detection”, International Conference on Materials & Applied Science for Society (MASS 2024), Govt Engineering College, Bikaner, 30th – 31st Dec 2024. **(POSTER)**

Workshops and Webinars Attended:

1. Attended a one-day national seminar on “Sensors for Society” organized by The Electrochemical Society at Jawaharlal Nehru University, New Delhi, on 27th April 2022.

2. Participated in a six-day course on “Radiation Biology & Its Clinical Applications” in Global Initiative of Academic Networks (GIAN) from 26th March to 31st March **2022**.
3. Participated in a six-day course on “Nanotechnology in Water and Wastewater Treatment” in Global Initiative of Academic Networks (GIAN) from 04th April to 09th April **2022**.

TABLE OF CONTENTS

<i>Title</i>	<i>Page No.</i>
Acknowledgments	i
Candidate's Declaration	iv
Certificate by the Supervisors	v
Abstract	vi
List of Publications and conferences	viii
Table of Contents	x
List of Tables	xv
List of Figures	xvi
List of Schematics	xx
List of Symbols and Acronyms	xxi
Chapter 1: Introduction to 2D Nanomaterials and Scientific Motivation	1-33
1.1 Introduction	2-4
1.1.1 Nanomaterials	2
1.1.2 Importance of 2D Nanomaterials	3-4
1.2 Biosensors and their important components	5-10
1.2.1 Electrochemical Biosensors	5
1.2.2 Amperometric Biosensors	6
1.2.3 Potentiometric Biosensors	7
1.2.4 Conductometric Biosensors	8
1.2.5 Impedimetric Biosensors	9
1.2.6 Optical Biosensors	9
1.2.7 Thermal Biosensors	10
1.3 Classification of biosensors based upon the biological element	11-16
1.3.1 Enzyme-based biosensors	11
1.3.2 Antibody-based biosensors	11-15
1.3.3 Aptamer-based biosensors	15-16
1.3.4 Whole cells biosensors	16
1.4 Characteristics of Biosensors	16-18
1.5 Mycotoxins	18-21
1.6 Importance of <i>Fumonisin B1</i>	22-23

<i>Title</i>	<i>Page No.</i>
1.7 Measurement techniques for detection of <i>FBI</i>	23-26
1.8 Limitations of Conventional Techniques	26
1.9 Materials explored for the detection of <i>FBI</i>	26-33
1.9.1 Graphene Oxide	26-29
1.9.2 Trimanganese tetraoxide	29-31
1.9.3 Iron Oxide	31-32
1.10 Objectives of the present work	32-33
1.11 Plan of Thesis	32-33
Chapter 2: Methodologies: Synthesis, Thin Film Fabrication, and Characterization Techniques	34-68
2.1 Synthesis of GO, rGO, Mn ₃ O ₄ , α -Fe ₂ O ₃ nanomaterials, and its nanocomposites	35-39
2.1.1 Hydrothermal Method of Synthesis	36-38
2.1.2 Co-precipitation Method	38-39
2.1.3 Solid-state Method	39
2.2 Method of thin film fabrication for Mn ₃ O ₄ /GO, α -Fe ₂ O ₃ /rGO nanocomposites	39-40
2.2.1 Electrophoretic deposition (EPD) technique	39-40
2.3 Characterization techniques used for structural, morphological, topographical, and spectroscopic investigation of GO, rGO, Mn ₃ O ₄ , α -Fe ₂ O ₃ nanomaterials and its nanocomposites	40-61
2.3.1 X-ray diffraction (XRD) for structural investigations	41-44
2.3.2 Scanning electron microscopy (SEM) for morphological investigations	44-47
2.3.3 Field Emission Scanning Electron Microscope (FESEM) for morphological investigations	47-48
2.3.4 Energy dispersive X-ray analysis (EDAX) for elemental analysis	48-50
2.3.5 Transmission Electron Microscopy (TEM) for morphological, and crystallographic investigations	51-53
2.3.6 Raman spectroscopy	53-56
2.3.7 X-ray Photoelectron Spectroscopy (XPS)	56-58
2.3.8 Fourier transform infrared spectroscopy (FTIR) for identification of functional groups	58-60

<i>Title</i>	<i>Page No.</i>
2.3.9 Ultrapformance liquid chromatography tandem mass spectrometer (UPLC-MS/MS)	60-61
2.4 Electrochemical (EC) Measurement techniques	62-68
2.4.1 Cyclic Voltammetry (CV)	64-66
2.4.2 Differential pulse voltammetry (DPV)	66
2.4.3 Electrochemical Impedance Spectroscopy (EIS)	66-67
Chapter 3: Graphene Oxide/Mn₃O₄ nanocomposites for Advanced Electrochemical Biosensor for <i>Fumonisin B1</i> detection	68-94
3.1 Introduction	69-72
3.2 Experimental Section	73-74
3.2.1 Material used	73
3.2.2 Synthesis of Graphene Oxide (GO)	73-74
3.2.3 Synthesis of Mn ₃ O ₄ nanoparticles	74
3.2.4 Synthesis of GOMn ₃ O ₄ nanocomposite	74
3.3 Characterization Techniques	74-75
3.4 Thin film fabrication of GOMn ₃ O ₄ /ITO electrode using electrophoretic deposition Technique	75
3.5 The fabrication process of Immuno-electrode	76
3.6 Results and discussions	77-83
3.6.1 Structural analysis of GOMn ₃ O ₄ nanocomposite	77-80
3.6.2 FTIR study of GO, Mn ₃ O ₄ , GOMn ₃ O ₄	80-81
3.6.3 Elemental and morphological study of GOMn ₃ O ₄ nanocomposite	81-82
3.6.4 FTIR study of ab- <i>FBI</i> onto GOMn ₃ O ₄ /ITO	82-83
3.7 Electrochemical Study	83-92
3.7.1 Optimization conditions	83-84
3.7.2 An electro-kinetic rate study	84-87
3.7.3 Response study	88
3.7.4 Repeatability, reproducibility, control, and interferent study	89-91
3.7.5 Spiked study	91-92
3.8 Conclusions	92-93
3.9 Acknowledgments	93

<i>Title</i>	<i>Page No.</i>
Chapter 4: Hematite-reduced graphene oxide Nanocomposite-Based Electrochemical Biosensing Platform for rapid Detection of <i>Fumonisin B1</i> in corn samples	94-126
4.1 Introduction	95-98
4.2 Experimental Section	98-102
4.2.1 Chemicals	98
4.2.2 Synthesis of α -Fe ₂ O ₃ /rGO Nanocomposite	98-99
4.2.3 Characterization Techniques	99
4.2.4 Fabrication of α -Fe ₂ O ₃ /rGO Thin Film Using EPD Technique	100
4.2.5 Thin Film Fabrication Process of Immuno-electrode	100-101
4.2.6 Preparation of Real Corn Samples for Biosensor and UPLC-MS/MS Study	101-102
4.3 Results and Discussion	102-110
4.3.1 XRD Studies	102-103
4.3.2 Raman Study	104
4.3.3 FTIR Study of prepared nanomaterials	104-105
4.3.4 FTIR study of Modified ITO Substrate	105-106
4.3.5 Elemental and Morphological study	106-108
4.3.6 XPS Study of α -Fe ₂ O ₃ /rGO nanocomposite	108-110
4.3.7 Electrochemical study	110-125
4.3.7.1 Cyclic Voltammetry study	110-111
4.3.7.2 Differential pulse voltammetry study	111-112
4.3.7.3 Electrochemical impedance spectroscopy study	112-113
4.3.7.4 A study of Electrochemical scan rate	113-116
4.3.7.5 pH Optimization study	116-117
4.3.7.6 Response study	117-121
4.3.7.7 Reproducibility, repeatability, interferent, and stability study	121-122
4.3.7.8 Real sample study	122-124
4.3.7.9 Control study	124-125
4.4 Conclusions	125
4.5 Acknowledgments	125-126

<i>Title</i>	<i>Page No.</i>
Chapter 5: Conclusion, Future Scope, and Social Impact	127-131
5.1 Conclusion	128
5.1.1 Key Findings	128
5.1.2 Structural and Morphological Analysis	128
5.1.3 <i>FBI</i> Interaction Studies (In-situ XRD)	128
5.1.4 <i>FBI</i> Sensing Performance	128
5.1.5 Performance Limitations	129
5.1.6 Overall Conclusion	129
5.2 Future Scope of Work	129-130
5.2.1 Improvement in Selectivity	129
5.2.2 Room Temperature <i>FBI</i> Sensing	130
5.2.3 Environmental Stability Studies	130
5.2.4 Device Miniaturization and Integration	130
5.3 Social Impact	130-131
5.3.1 Environmental Monitoring	130
5.3.2 Public Health Benefits	130
5.3.3 Smart Cities and IoT Integration	130
5.3.4 Economic and Technological Impact	131
5.3.5 Scientific Contribution	131
References	132-159
Curriculum Vitae	160-161

LIST OF TABLES

Table No.	Title of Table	Page No.
Table 1.1	Variety of mycotoxins and their sources	21
Table 3.1	Comparison of present work with other reported methods for the detection of <i>FBI</i>	72
Table 4.1	Electrochemical characteristics of the BSA/ab- <i>FBI</i> /α-Fe ₂ O ₃ /rGO/ITO immunoelectrode for <i>FBI</i> detection	116
Table 4.2	Evaluation of the present study findings with earlier published research articles for <i>FBI</i> detection	120
Table 4.3	Tested fabricated biosensor BSA/ab- <i>FBI</i> /α-Fe ₂ O ₃ /rGO/ITO with spiked sweet corn samples using DPV technique	123
Table 4.4	Comparative recovery analysis of <i>FBI</i> in spiked samples using UPLC-MS/MS and fabricated DPV based electrochemical biosensor BSA/ab- <i>FBI</i> /α-Fe ₂ O ₃ /rGO/ITO	123

LIST OF FIGURES

<i>Figure No.</i>	<i>Title of Schematics</i>	<i>Page No.</i>
Fig. 1.1	Classification of nanomaterials based on dimensions	2
Fig. 1.2	Features of 2D nanomaterials	4
Fig. 1.3	Schematic illustration of 2D nanomaterials	4
Fig. 1.4	Basic principle of biosensor	5
Fig. 1.5	Schematic illustration of amperometric biosensor	6
Fig. 1.6	Schematic diagram of an ion-sensitive field-effect transistor	7
Fig. 1.7	Schematic diagram of a conductometric sensor	8
Fig. 1.8	Schematic diagram of an SPR biosensor	10
Fig. 1.9	Schematic diagram of enzyme-based biosensors	11
Fig. 1.10	Schematic diagram of an antibody	13
Fig. 1.11	Chemistry of EDC-NHS with the antibodies	15
Fig. 1.12	Schematic diagram of antibody-based biosensors	15
Fig. 1.13	Schematic diagram of DNA-based biosensors	16
Fig. 1.14	Characteristics of biosensors	17
Fig. 1.15	Different groups of mycotoxins	21
Fig. 1.16	Comparison of fresh fruits and <i>FBI</i> -contaminated fruits	23
Fig. 1.17	Chemical structure of <i>FB1</i>	23
Fig. 1.18	Different techniques of detection of <i>FB1</i>	24
Fig. 1.19	Schematic diagram of graphite	27
Fig. 1.20	Exfoliation of graphite	29
Fig. 1.21	Schematic diagram of Mn_3O_4	30
Fig. 1.22	Structure of Fe_2O_3	32
Fig. 2.1	Schematic diagram of various synthesis procedures	35
Fig. 2.2	Schematic diagram of (a) autoclave and (b) muffle furnace	37
Fig. 2.3	Schematic diagram of Mortar and pestle	39
Fig. 2.4	Schematic diagram of EPD	40
Fig. 2.5	Schematic diagram of X-Ray Diffraction	42
Fig. 2.6	Picture of PANalytical X-Ray Diffractometer	43
Fig. 2.7	Schematic diagram of SEM	45

<i>Figure No.</i>	<i>Title of Schematics</i>	<i>Page No.</i>
Fig. 2.8	Schematic diagram of interaction of electron beam with sample	46
Fig. 2.9	Scanning Electron Microscope Carl Zeiss EVO 40	47
Fig. 2.10	Picture of Tescan Lyra3 FESEM	48
Fig. 2.11	Irradiation of an atom	49
Fig. 2.12	Overview of the most important lines with their transitions	50
Fig. 2.13	Schematic diagram of TEM	52
Fig. 2.14	Picture of JEOL 2100 HRTEM	53
Fig. 2.15	Schematic diagram of Raman lines	55
Fig. 2.16	Picture of Witec Raman spectrometer	55
Fig. 2.17	Components of the XPS instrument	57
Fig. 2.18	Picture of Thermo Fisher Scientific X-ray photoelectron spectrometer	58
Fig. 2.19	Schematic diagram of FTIR Instrument	59
Fig. 2.20	Picture of Perkin Elmer FTIR Instrument	60
Fig. 2.21	Schematic diagram of UPLC-MS/MS	61
Fig. 2.22	Picture of Waters SYNAPT G2 with 2D nano ACQUITY System	61
Fig. 2.23	Schematic diagram of the electrochemical cell	63
Fig. 2.24	Picture of Autolab galvanostat potentiostat	63
Fig. 2.25	Cyclic voltammetry (a) applied ramp potential, and (b) a typical voltammogram	65
Fig. 2.26	Formation of staircase potential for DPV, and a typical DPV plot	66
Fig. 2.27	Randels circuit, and Semicircle in Nyquist plot	67
Fig. 3.1	XRD spectrum of (a) GO, (b) Mn_3O_4 , and (c) GOMn_3O_4 nanocomposite	78
Fig. 3.2	Raman spectrum of (a) GO, (b) Mn_3O_4 , and (c) GOMn_3O_4 nanocomposite	79
Fig. 3.3	Picture of FESEM Images of synthesized GO	80
Fig. 3.4	FTIR spectrum of (a) GO, (b) Mn_3O_4 , and (c) GOMn_3O_4 nanocomposite	81
Fig. 3.5	HR-TEM images of GOMn_3O_4 nanocomposite, EDX image of GOMn_3O_4 nanocomposite.	82

Figure No.	Title of Schematics	Page No.
Fig. 3.6	FTIR spectrum of (i) GOMn ₃ O ₄ /ITO, (ii) ab-FBI/GOMn ₃ O ₄ /ITO, and (iii) BSA/ab-FBI/GOMn ₃ O ₄ /ITO immunoelectrode	83
Fig. 3.7	The effect of different pH from 6.0 to 8.0 in PBS containing [Fe(CN) ₆] ^{3-/4-} , and incubation plot for BSA/ab-FBI/GOMn ₃ O ₄ /ITO immunoelectrode, in PBS containing [Fe(CN) ₆] ^{3-/4-} .	84
Fig. 3.8	Scan rate study from 20 to 100 mV/s and linear plot between the square root of scan rate vs peak current of (a) GOMn ₃ O ₄ /ITO, (b) ab-FBI/GOMn ₃ O ₄ /ITO, and (c) BSA/ab-FBI/GOMn ₃ O ₄ /ITO immunoelectrode	86
Fig. 3.9	DPV response study of the (i) GOMn ₃ O ₄ /ITO, (ii) ab-FBI/GOMn ₃ O ₄ /ITO, (iii) BSA/ab-FBI/GOMn ₃ O ₄ /ITO electrode surface	87
Fig. 3.10	DPV biosensing response, and a linear plot of the BSA/ab-FBI/GOMn ₃ O ₄ /ITO immunoelectrode, respectively against FBI in the concentration range from 1 pg mL ⁻¹ to 800 ng mL ⁻¹	89
Fig. 3.11	Reproducibility plot for five different immunoelectrodes, repeatability study measured for five different consecutive readings with 1.06 % RSD value, interferent study plot with various interfering analytes, and control measurement plot GOMn ₃ O ₄ /ITO with different concentrations range from 1 pg mL ⁻¹ to 800 ng mL ⁻¹	91
Fig. 3.12	Linear plot of FBI and spiked sample studies for concentrations of 1 pg mL ⁻¹ , 10 pg mL ⁻¹ , 100 pg mL ⁻¹ , and 1 ng mL ⁻¹ , 100 ng mL ⁻¹ , 300 ng mL ⁻¹ and 500 ng mL ⁻¹ performed in the sweet maize samples using the fabricated BSA/ab-FBI/GOMn ₃ O ₄ /ITO immunoelectrode	92
Fig. 4.1	XRD pattern of (a) GO, (b) α-Fe ₂ O ₃ , and (c) α-Fe ₂ O ₃ /rGO nanocomposite [Inset image: XRD of (b) α-Fe ₂ O ₃ , and (c) α-Fe ₂ O ₃ /rGO], (B) Raman spectra of (a) GO, (b) α-Fe ₂ O ₃ , and (c) α-Fe ₂ O ₃ /rGO nanocomposite	103
Fig. 4.2	FTIR spectra of (a) GO, (b) α-Fe ₂ O ₃ , and (c) α-Fe ₂ O ₃ /rGO nanocomposite	105
Fig. 4.3	FTIR spectra of (a) α-Fe ₂ O ₃ /rGO/ITO, (b) ab-FBI/α-Fe ₂ O ₃ /rGO/ITO, (c) BSA/ab-FBI/α-Fe ₂ O ₃ /rGO/ITO nanocomposite	106
Fig. 4.4	FE-SEM of α-Fe ₂ O ₃ /rGO nanocomposite at (a) 500 μm, (b) 5 μm, (c) 2 μm, (d) mapping, (e) Elemental spectra of α-Fe ₂ O ₃ /rGO nanocomposite	107

Figure No.	Title of Schematics	Page No.
Fig. 4.5	HR-TEM images of α -Fe ₂ O ₃ /rGO nanocomposite and particle size distribution graph, Lattice image of α -Fe ₂ O ₃ /rGO, and SAED pattern of α -Fe ₂ O ₃ /rGO nanocomposite	108
Fig. 4.6	XPS spectra (a) Survey spectrum of α -Fe ₂ O ₃ /rGO nanocomposite, (b) Deconvolution spectra of Fe2p, (c) C1s spectra, and (d) O1s spectra of α -Fe ₂ O ₃ /rGO nanocomposite	109
Fig. 4.7	Survey of XPS spectra of <i>FBI</i> over the α -Fe ₂ O ₃ /rGO/ITO immunoelectrode	109
Fig. 4.8	CV response study of the (i) ITO, (ii) α -Fe ₂ O ₃ /rGO/ITO, (iii) ab- <i>FBI</i> / α -Fe ₂ O ₃ /rGO/ITO, (iv) BSA/ab- <i>FBI</i> / α -Fe ₂ O ₃ /rGO/ITO electrode surfaces, DPV responses obtained from four modified electrode surfaces: (i) ITO (ii) α -Fe ₂ O ₃ /rGO/ITO, (iii) ab- <i>FBI</i> / α -Fe ₂ O ₃ /rGO/ITO, (iv) BSA/ab- <i>FBI</i> / α -Fe ₂ O ₃ /rGO/ITO electrode surface, and EIS response of (i) ITO, (ii) α -Fe ₂ O ₃ /rGO/ITO, (iii) ab- <i>FBI</i> / α -Fe ₂ O ₃ /rGO/ITO, (iv) BSA/ab- <i>FBI</i> / α -Fe ₂ O ₃ /rGO/ITO, (v) <i>FBI</i> /BSA/ab- <i>FBI</i> / α -Fe ₂ O ₃ /rGO/ITO	111
Fig. 4.9	Scan rate study conducted across a range of 10 to 100 mV/s and presents linear plots between the square root of the scan rate and the peak current for three different immunoelectrodes: (a) α -Fe ₂ O ₃ /rGO/ITO, (b) ab- <i>FBI</i> / α -Fe ₂ O ₃ /rGO/ITO, and (c) BSA/ab- <i>FBI</i> / α -Fe ₂ O ₃ /rGO/ITO	114
Fig. 4.10	pH effect on BSA/ab- <i>FBI</i> / α -Fe ₂ O ₃ /rGO/ITO immunoelectrode in PBS containing [Fe(CN) ₆] ^{3-/4-}	117
Fig. 4.11	DPV biosensing response, a linear plot in the concentration range from 1x10 ⁻³ to 400 ng mL ⁻¹ against <i>FBI</i> of the BSA/ab- <i>FBI</i> / α -Fe ₂ O ₃ /rGO/ITO immunoelectrode	119
Fig. 4.12	DPV response of reproducibility study of six different electrode, (b) column graph of reproducibility study, (c) column graph of repeatability study of six different electrode responses with 0.33% RSD value, (d) DPV response of interference study, (e) column graph of interferent study with different interfering species, and (f) column graph of stability study of BSA/ab- <i>FBI</i> / α -Fe ₂ O ₃ /rGO/ITO immunoelectrode.	122
Fig. 4.13	control study of α -Fe ₂ O ₃ /rGO/ITO fabricated electrode with various concentration of <i>FBI</i> without immobilization of anti- <i>FBI</i> using DPV technique	124

LIST OF SCHEMATICS

<i>Scheme No.</i>	<i>Title of Figure</i>	<i>Page No.</i>
Scheme 3.1	Schematic of synthesis of GOMn ₃ O ₄ nanocomposite and fabrication of immunoelectrode on the ITO electrode surface for FB1 detection; (b) illustrates the EPD set-up used for the electrophoretic deposition of GOMn ₃ O ₄ nanocomposite onto the ITO electrode surface under the applied voltage for a period of time, followed by surface activation, immobilization and detection of <i>FB1</i> using the electrochemical set-up of three-electrode system comprised of working, counter and reference electrode	76
Scheme 4.1	Synthesis of α -Fe ₂ O ₃ /rGO nanocomposite and the subsequent fabrication of an immunoelectrode onto an ITO-coated glass surface to detect <i>FB1</i> , EPD set-up used for the EPD of α -Fe ₂ O ₃ /rGO nanocomposite onto the ITO electrode surface	101

LIST OF SYMBOLS AND ACRONYMS

0D	:	Zero Dimension
1D	:	One Dimension
2D	:	Two Dimension
3D	:	Three Dimension
TMDs	:	Transition metal dichalcogenides
hBN	:	Hexagonal boron nitride
g-C ₃ N ₄	:	Graphitic carbon nitride
DNA	:	Deoxyribonucleic Acid
RNA	:	Ribonucleic Acid
nm	:	Nanometer
mm	:	Millimeter
cm	:	Centimeter
mA	:	milli Ampere
mg	:	Milligram
mL	:	Milliliter
kHz	:	Kilohertz
eV	:	Electron volt
KV	:	Kilovolt
NPs	:	Nanoparticles
NS	:	Nanosheet
BP	:	Black phosphorus
CNTs	:	Carbon nanotubes
Fig	:	Figure
AM	:	Antimonene
JCPDS	:	Joint Committee on Powder Diffraction Standards
GO	:	Graphene oxide
rGO	:	reduced Graphene oxide
pH	:	Potential of Hydrogen
SPR	:	Surface plasmons resonance
BSA	:	Bovine Serum Albumin
ISFET	:	Ion-sensitive field effect transistor
FET	:	Field effect transistor
Ag/AgCl	:	Silver-silver chloride electrode
DI	:	Deionized water

Chapter 1

Emerging 2D Nanomaterials and Scientific Motivation

This chapter discusses 2D nanomaterials and their importance in the field of nanoscience and nanotechnology. Special focus is given to 2D nanomaterials due to their most attractive properties, which make them more distinguishable than other nanomaterials, viz., zero-dimensional, one-dimensional, and three-dimensional. Further, this chapter discusses the use of 2D nanomaterials in various applications. Also, this chapter discusses their applications in the field of biosensors and various important components of the biosensors. The choice of nanocomposites of graphene, trimanganese tetraoxide (Mn_3O_4), and iron oxide (Fe_2O_3) as materials for the fabrication of immunoelectrodes is discussed in detail, with special focus on their versatile properties and also the previous literature on these materials. The chapter continues to discuss mycotoxins, their types, and the importance of *fumonisin B1* in detail. The chapter continues to discuss the various conventional methods for the detection of *FB1* and its detection using immunoelectrodes. The chapter continues to discuss the motivation of the work with the realization that the fabricated immunoelectrode has better selectivity, is low-cost, and easy to fabricate. The low-cost immobilization matrix in the immunoelectrode is one of the most important features in biosensors.

1.1. Introduction

1.1.1. Nanomaterials

Nanoscience plays a very important role in the field of nanotechnology, leading to the use of new nanomaterials and nano-size-based components. Nanotechnology provides the ability to customize the materials for new enhanced properties and new types of smart sensors. Nanomaterials are very tiny materials with an external diameter of only up to 100 nm [1]. Nanomaterials possess very different physical and chemical properties in contrast to bulk materials. The size of nanomaterials is too tiny, and they are added to different materials to make them useful in various fields, including electronics and environmental hazards [2, 3]. The size of the nanomaterials can be controlled by various techniques. Nanomaterials can be divided into their various types, viz. zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D). In 0D, all three dimensions are in the nanoscale range, e.g., quantum dots, nanospheres, and nanoclusters. In 1D, two dimensions merge, e.g., nanotubes and nanorods. In two dimensions, two dimensions are outside, and one dimension is at the nanoscale, e.g., nanofilms and nanolayers. In 3D, nanomaterials are bulk. A schematic diagram of classifications of nanomaterials based on dimensions is provided in **Figure 1.1**.

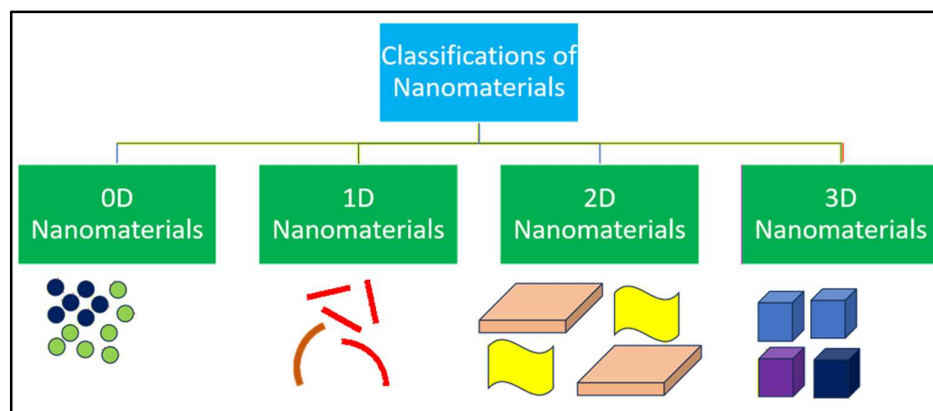


Figure 1.1: Classification of nanomaterials based on dimensions

1.1.2. Importance of 2D Nanomaterials

The 2D nanomaterials such as black phosphorus (BP), Mxenes, silicene, and perovskites etc. possess a sheet-like structure with a thickness of a few atomic layers. They have high anisotropy and electronic confinement in two dimensions only. The chemical, electronic, and optical properties of 2D nanomaterials are of much interest. **Figure 1.2** shows the important features of 2D nanomaterials. For example, graphene is of huge interest due to its very unique properties like large surface area, excellent optical transparency, high Young's modulus, and quantum Hall effect [4]. These properties make 2D nanomaterials suitable for use in various fields such as energy storage applications, catalysis, (opto)electronics, sensors, and biomedicine. In recent years, great research efforts have been made to study the defects and phase engineering of 2D nanomaterials. A large number of 2D nanomaterials have been discovered so far, e.g., hexagonal boron nitride (h-BN) [5], transition metal dichalcogenides (TMDs) [6], black phosphorus (BP) [7], antimonene (AM) [8], layered double hydroxides (LDHs) [9], and graphitic carbon nitride (g-C₃N₄) [10]. **Figure 1.3** illustrates the types of 2D nanomaterials. These nanomaterials have huge applications in various aspects, e.g., optoelectronics [11], energy storage systems [12], biomedicine applications [13], sensors [14], supercapacitors [15], batteries [16], and so on. The chemical composition and crystal structure of all 2D nanomaterials are different. These 2D nanomaterials can be classified into two types, viz. layered and non-layered 2D nanomaterials. In layered nanomaterials, there are strong chemical bonds between atoms and van der Waals interactions between layers, while in non-layered 2D nanomaterials, the nanomaterials crystallize in 3D to form bulk crystals through chemical bonds [17]. Graphene has zero band gap, is chemically inert, and thinnest (monatomic thickness) nanomaterial. Graphene oxide (GO) is synthesized by partial oxidation of graphene, increasing its functionalization and hydrophilicity [18]. Reduced graphene oxide (rGO) is synthesized by removing most of the oxygenated groups from graphene oxide. Research studies have shown that graphene and its derivatives are used in various biomedical applications [19].

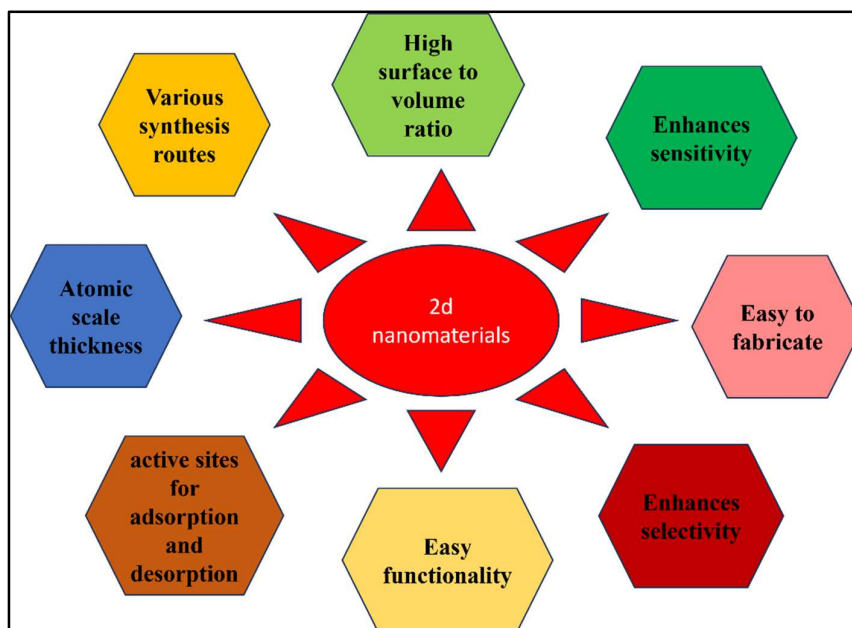


Figure 1.2: Features of 2D nanomaterials

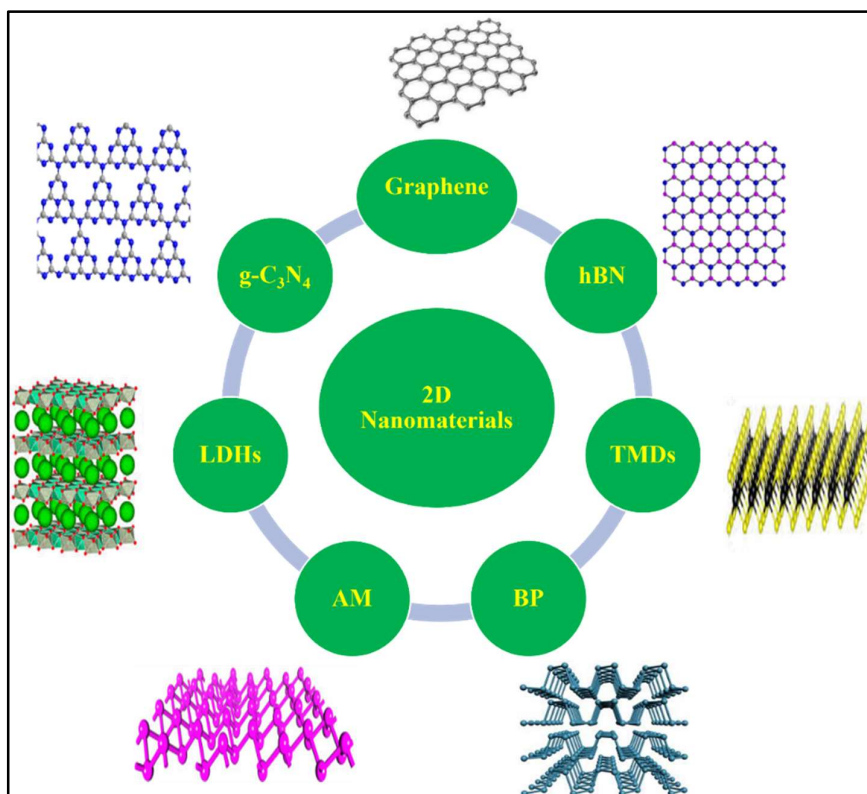


Figure 1.3: Schematic illustration of 2D nanomaterials

1.2. Biosensors and their important components

The term biosensor is a class of chemical sensors that make use of a selective biological reaction to provide an analytical signal. A biosensor is an analytical tool that converts biological signals into electrical signals [20]. A biosensor uses a biological recognition element in direct contact with a transducer element and provides quantitative analytical information. Bio receptors include the biological recognition elements such as nucleic acids, enzymes, enzyme components, antibodies, organic molecules, membranes, and cells. The transducers convert the signal obtained from the biological interaction between the bio receptor and analyte into another form. The classification of biosensors depends on their transfer and bio recognition elements, and the transduction process may be optical, chemical, thermal, mechanical, acoustic, and piezoelectric. Clark and Lyons proposed that enzymes can be immobilized to form the enzyme electrode [21]. According to Turner, a biosensor is a device including the biological sensing element either connected to or integrated within the transducer [22]. Gronow defined the biosensor as an analytical tool for converting a biochemical signal into an electrical signal [23]. In other words, a biosensor is a device with a union of a receptor for biomolecular regulation and a transducer for electrical output. The basic principle of a biosensor is shown in **Figure 1.4**.

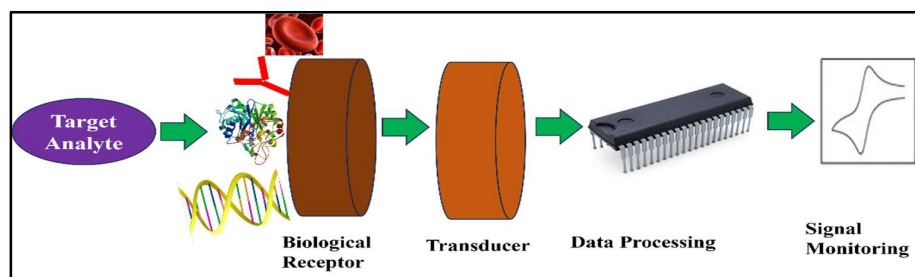


Figure 1.4: Basic principle of biosensor

1.2.1. Electrochemical Biosensors

The electrochemical method is very simple and translates the properties of a reacting system into an electrochemical impulse [24]. An electrochemical biosensor is a very rapid analytical tool for clinical, chemical, pharmaceutical, and environmental studies. The analysis of species through electrochemical biosensors allows great specificity,

sensitivity, good selectivity, and fast detection. An electrochemical biosensor is comparatively cheaper and more cost-effective compared to other biosensors such as pyroelectric, optical, acoustic wave, and other nano-biosensors. This class of biosensor works on the principle of interaction between immobilized bioreceptor and analyte, which produces a signal affecting the electrical properties of the electrolyte solution in terms of electrical current, voltage, impedance, and capacitance. This technique has both qualitative and quantitative significance for analyte measurement. Electrochemical biosensors can be classified into three major subclasses, viz. potentiometry, amperometry, and conductometry or impedance spectroscopy.

1.2.2 Amperometric Biosensors

In amperometry, current is generated by oxidation or reduction of redox-active species at the electrode surface due to the application of a constant potential between the sensing electrode and reference electrode, and is directly proportional to analyte concentration. The measurement depends on the rate of analyte transport to the electrode surface. In the fabrication of biosensors, oxygen and hydrogen peroxide electrodes are mainly used as amperometric electrodes [25]. For O_2 measurement, a platinum or gold electrode is used at -600 mV to -800 mV with reference to an Ag/AgCl electrode. For H_2O_2 measurement, platinum is used as the anode at +800 mV with reference to an Ag/AgCl electrode. Amperometry biosensor is a three-electrode system that includes a working electrode (noble metals/ ITO), a reference electrode (Ag/AgCl), and a counter electrode (Pt). **Figure 1.5** illustrates the schematic of an amperometric biosensor.

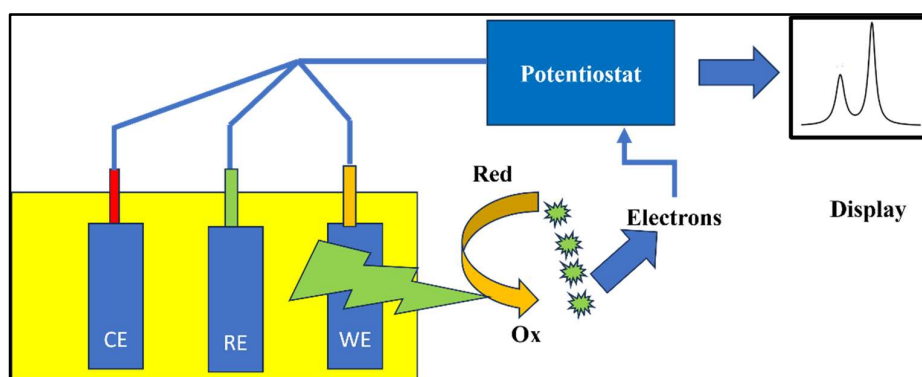


Figure 1.5: Schematic illustration of an amperometric biosensor.

It is used for rapid and real-time chemical measurements. It is used for glucose

monitoring of a diabetic patient at home. It is also used in environmental monitoring to detect heavy metals, pesticides, and pollutants in water samples. It is also used to determine alcohol content in food. It is simple, and low cost, and easy to carry but has interference effects due to high voltage. It is slightly selective.

1.2.3 Potentiometric Biosensors

In potentiometric biosensors, the potential difference between the sensing electrode and the reference electrode is measured [26]. It measures the accumulation of charge at the recognition interface. Potentiometric biosensors provide a logarithmic relationship between analyte species and the measured potential. There are several types of potentiometric biosensors, viz. ion-selective electrodes (ISE), gas sensing electrodes (GSE), and field effect transistors (FETs). Ion-selective electrodes (pH, ammonia, fluoride) and gas-sensing electrodes have been widely used in biosensor fabrication [27]. The integration of ISEs with FETs led to the development of ion-sensitive field effect transistors (ISFETs). **Figure 1.6** shows the schematic diagram of an ion-sensitive field-effect transistor. It has low power consumption and has high sensitivity and temperature sensitivity. It is used to monitor various electrolytes (K^+ , Na^+ , Ca^{2+}) in tracking cancer biomarkers and water contaminants.

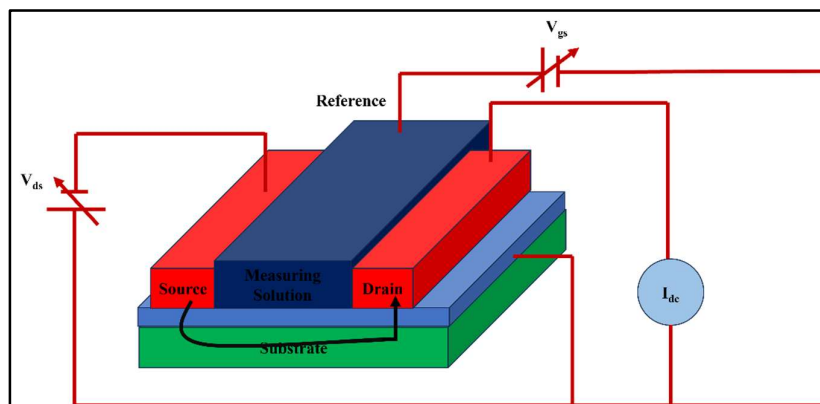


Figure 1.6 Schematic diagram of an ion-sensitive field-effect transistor.

1.2.4 Conductometric Biosensors

These biosensors measure the change in electrical conductivity due to changes in the biochemical reaction. It is based on the principle of applying a low-amplitude alternating current (AC) voltage across electrodes and monitoring how the target analyte changes the ionic composition of the sample. It operates using two electrode systems and does not require a reference electrode. The basic principle of a conductometric biosensor is applicable when a biochemical reaction produces ionic species, and it changes the electrical conductivity of the solution. The resistance of a solution depends on the migration of all ions present in the solution. While in high frequency analysis, the dielectric and/or diamagnetic behavior of the solution plays a major role. **Figure 1.7** shows the schematic diagram of a conductometric sensor.

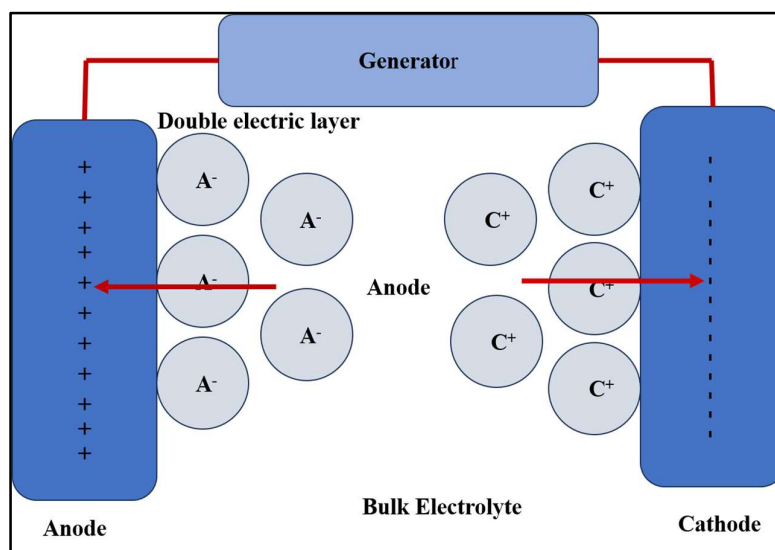


Figure 1.7: Schematic diagram of a conductometric sensor.

The dissociation of an electrolyte converts it into its ionic components, and the liquid becomes conducting. The migration of ions occurs on the application of an electric field. On the application of a potential difference. The electric field is developed within the electrolyte, creating the random movement of ions. The negatively charged ions start moving towards anodes, and the positively charged ions start moving towards cathodes. Therefore, the current in the electrolyte is produced by the movement of ions towards the electrodes [28]. It is used for fast screening of kidney diseases and also environmental monitoring.

1.2.5 Impedimetric Biosensors

It uses the electrochemical impedance spectroscopy (EIS) technique. These measure the change in charge transfer resistance (R_{ct}) and double layer capacitance (C_{dl}) when a target molecule binds to the bioreceptor. These are of two types, viz. Faradaic and non-Faradic. Faradaic biosensors utilize the ferri/ferrocyanide in the solution to enable electron transfer. The measurement of the signal depends upon the interaction of the probe with the electrode. While the non-Faradaic Biosensors do not require a redox reaction in the solution. They directly measure changes in the electrical properties between the two electrodes [29]. The impedimetric biosensor method is a very sensitive technique for the detection of interaction between antigen and antibody. The antibodies are immobilized onto the surface of the electrode, and binding of antigen with antibodies leads to immunocomplex formation, which results in the change in electron transfer kinetics. It is used for the rapid detection of pathogens; it allows researchers to track the changes in protein interactions.

1.2.6 Optical Biosensors

These biosensors use the integrated biological element recognition system (enzymes, antibodies, or DNA/RNA) and an optical transducer [30]. The optical transducer can be an interferometer, waveguide, resonator, grating, or surface plasmon resonance. Surface plasmon resonance (SPR) is the most efficient label-free method as it is desirable and well-suited for real-time detection. It allows the rate of association and dissociation with interacting molecules. SPR is a resonant oscillating phenomenon of conducting electrons that occurs due to the incidence of polarized light at the surface of a metal film with different refractive indices. The surface plasmon is the charge density wave generation that originates at the interface of two media. A thin layer of conducting metal (gold) is deposited onto the surface of glass (prism) in such a way that there is dielectric contact with both the faces of the film; one in contact with the face of the glass while the other is in contact with the air. When the photon of incident light interacts with the metal film, a charge density oscillation takes place at the interface of two media, and a change is observed in angle leading to variation in refractive index [31]. These systems can be excited due to waveguide coupling where dielectric and metal couple, and generate the waveguiding layer, creating an interface

between metal and waveguide. Light propagation in the waveguide generates an evanescent field at the interface and excites the surface plasmon wave. In SPR biosensing, interaction of targeted analyte with the bioreceptor/ antibody is measured by a change in surface plasmon wave due to resonance coupling with incident light. The SPR consists of a functional monolayer of carboxymethylated dextran covered on a metal (gold) surface, which enables self-assembled immobilization. During the experiment, biomolecules (bioreceptor) are permanently attached or immobilized onto the chip, and another component, such as an analyte, flows over the surface and binds with the bioreceptor. **Figure 1.8** shows the schematic diagram of an SPR biosensor.

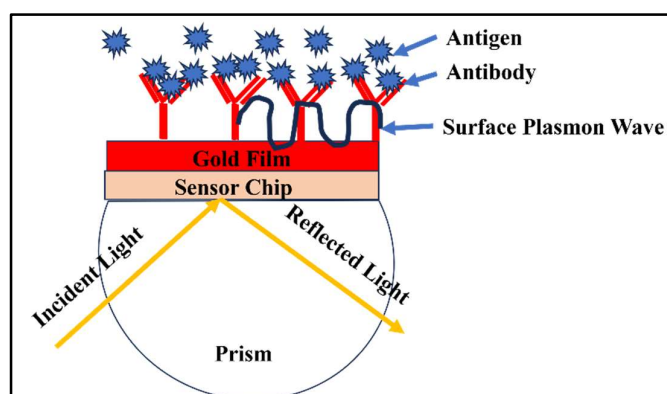


Figure 1.8: Schematic diagram of an SPR biosensor

It is used in remote sensing. It is only applicable to a narrow concentration range.

1.2.7 Thermal Biosensors

Thermal biosensors measure the heat energy absorbed or released during the biochemical reaction. The most common type of biosensor is MEMS (Micro Electro Mechanical Systems) [32]. MEMS technology provides low-cost fabrication, improved thermal isolation, and low sample volume; it provides good linearity, high sensitivity, and low measurement time. Thermal biosensors are of two types, viz. thermistors and thermopiles. A thermistor measures the changes in the electrical resistance with temperature, while thermopiles measure the temperature difference between the two regions. Thermal biosensors can't be used for liquid samples and can only be used for solid or gas samples. These are stable for the long term and are less costly. The major drawback of thermal biosensors is that they suffer from a low

response time, low stability, and accuracy, which limits their utilization.

1.3 Classification of biosensors based upon the biological element

1.3.1 Enzyme-based biosensors

These biosensors measure the changes in metabolite levels in real time. The enzyme-based biosensors can be divided in three different ways. The first is as an offline device involving the collection of biological samples and the measurement of target molecules using analytical equipment, e.g., blood glucose measurement [33]. The second one is the in vivo biosensors, where the in vivo sensors are implanted, and they detect the change in concentration of the analyte. The third one is an online device, where the biosensors are integrated with the sampling device implanted in the body, e.g., a microdialysis probe. The enzyme-based biosensors suffer from the main disadvantage of interference due to chemicals present in the sample matrix. The matrix interferences can be overcome by various methods, viz. pretreatments, extraction, filtration, and derivatization. **Figure 1.9** shows the schematic diagram of enzyme-based biosensors.

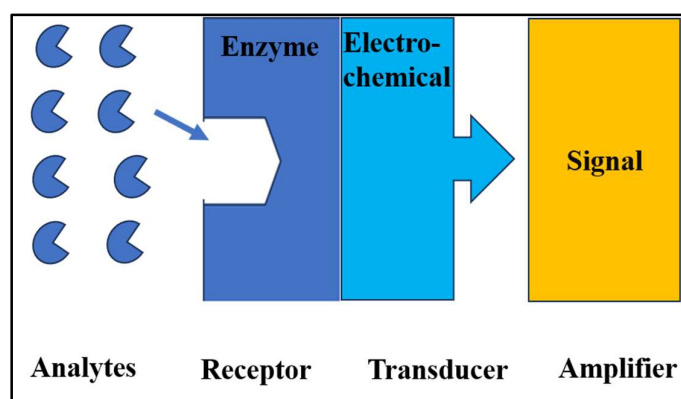


Figure 1.9 Schematic diagram of enzyme-based biosensors.

1.3.2 Antibody-based biosensors

Immunosensors

Immunosensors are a type of analytical device in which an antibody and antigen form a complex (called an immunocomplex), and the event of information is monitored and converted by means of a transducer to an electrical or optical signal. Various transducing systems such as electrical, optical, mass change, and thermal are used in

immunological biosensors in signal generation during the formation of antibody-antigen immunocomplexes. An immunosensor is a kind of biosensor based on the specific interaction between biorecognition (antibody or enzyme) and analyte (antigen). Immunosensors attain properties like high selectivity and sensitivity towards specific interaction between antibody and corresponding antigen, making them suitable platforms for several applications in clinical devices. Immunoassays are immunosensors that work according to the response of antibody-antigen complexes. Immunoassay design is based on the immobilization of the antibody or antigen over a solid supporting surface such as nanomaterials (such as gold, manganese oxide, titanium oxide, and copper oxide, etc) coated chips. Immunoassay can be categorized in the form of competitive, noncompetitive, direct, and indirect immunoassays [34].

a) Competitive Immunoassay

Competitive immunoassay is related to interaction between antibody and antigen with a limited number of binding sites. In this assay format, unlabelled analyte of interest competes with labelled analyte for the limited amount of specific antibody. During the competition between these analytes, two antigens (analytes) compete with the same antibody. This immunoassay is very slow and requires a long incubation time to reach equilibrium [35].

b) Direct Immunoassay

Direct immunoassay reveals the quantification of antigens of high molecular weight. This assay allows the direct immobilization of antibody or antigen on the solid surface for detection and measurement [36].

c) Indirect Immunoassay

Indirect immunoassay relates to the direct immobilization of secondary antibody on the assay system compared to primary antibody. Secondary antibody is responsible for signal amplification instead of primary antibody.

d) Noncompetitive Immunoassay

In this assay system, two antibodies, one labelled and another unlabelled antibody, are used and fixed with solid support. Following the antibody-antigen reaction, antigens

(analyte) are captured by immobilized bound labelled antibodies to generate the detectable signal, and this signal is directly proportional to the concentration of antigen. This is also called a sandwich because the antigen is placed between two antibody entities. A brief introduction of antibody, antigen, and cross-linking agents involved in the formation of an immunosensor is given below:

Antibody

Another name for an antibody is immunoglobulin (Ig), which is a Y-shaped protein, and it responds in the presence of a foreign substance as an antigen. Antibodies are a type of biopolymer, having a molecular mass of 150 kDa. The antibody is divided into two parts, viz. fragments antigen binding (Fab)₂ region and the fragment crystallizable region (F_c). The (Fab) region is linked with the hinge region, while the (Fab)₂ fragment is different from the F_c region. The F_c region is a very important part of an antibody, which provides the orientation of attachment of the substrate. **Figure 1.10** shows the schematic diagram of an antibody.

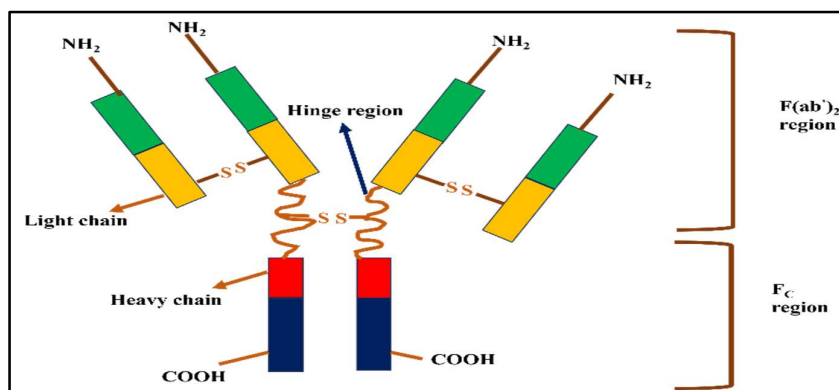


Figure 1.10 Schematic diagram of an antibody

Antigen

An antigen is a substance which activates lymphocytes to produce antibodies against it. It also stimulates the immune system. Usually, there are two types of antigens, viz. foreign antigens (heteroantigens) and self-antigens or autoantigens. The foreign antigens originate from outside the body, e.g., *bacteria*, *viruses*, *pollen*, and *protozoa*, etc., while the autoantigens are produced within the body, such as in autoimmune disorders. In autoimmune disorders, the human body itself forms the antibodies. As the antigen induces the immune system, that is why it is also called an immunogen.

Cross-linker

A crosslinker is a chemical substance that binds covalently with the specific antibodies, which react with the particular antigen. Here in the research, EDC and NHS are used as crosslinkers for antibodies.

EDC-NHS interaction

EDC-NHS chemistry provides a medium for immobilization of antibodies on the bioanalytical platform with covalent bonding [37]. EDC is known as 1-Ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride, and is used as a zero-length cross-linking agent; it helps in the coupling of a carboxylic group with the primary amine to an amine-reactive NHS-ester. NHS stands for N-hydroxysuccinimide, which, in the presence of EDC, converts the carboxylic acid into the amine-reactive NHS-ester. The detailed chemistry of EDC-NHS with the antibodies is described in **Figure 1.11**. EDC reacts with the carboxylic group of antibodies, forming an O-acylisourea intermediate. This intermediate reacts much faster with a primary amine and forms an amide bond, releasing isourea as a by-product, as shown in **Figure 1.11(a)**. The O-acylisourea intermediate is very unstable in aqueous solution, making it nonreactive with the amine and easily hydrolyzed. It regenerates the carboxyl group and releases the N-substituted urea indicates in **Figure 1.11(b)**. So, NHS is required to stabilize the aqueous solution, and it reacts with the O-acylisourea intermediate, forming the semi-stable amine-reactive NHS ester shown in **Figure 1.11(c)**, and it remains stable even at pH 7.4. NHS-activated antibodies show the best crosslinking results with amines or amine-functionalized substrates. **Figure 1.12** shows the schematic diagram of antibody-based biosensors.

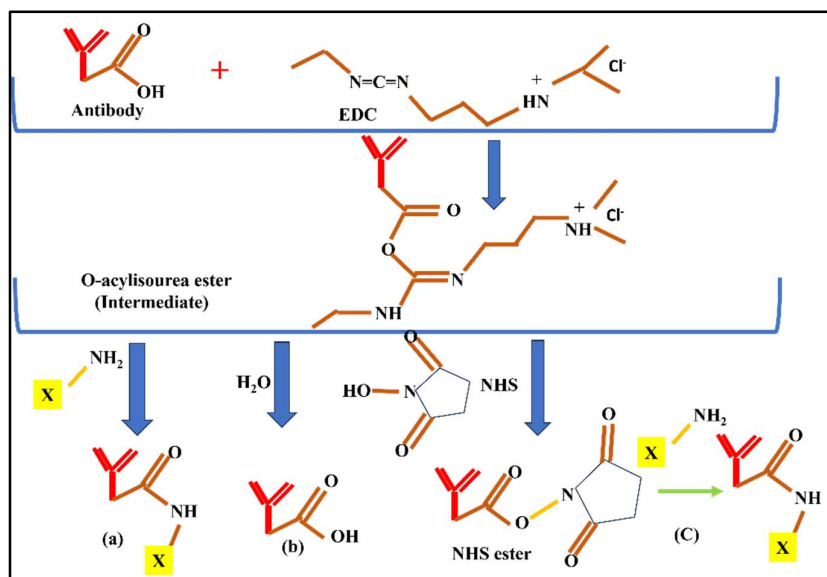


Figure 1.11: Chemistry of EDC-NHS with the antibodies

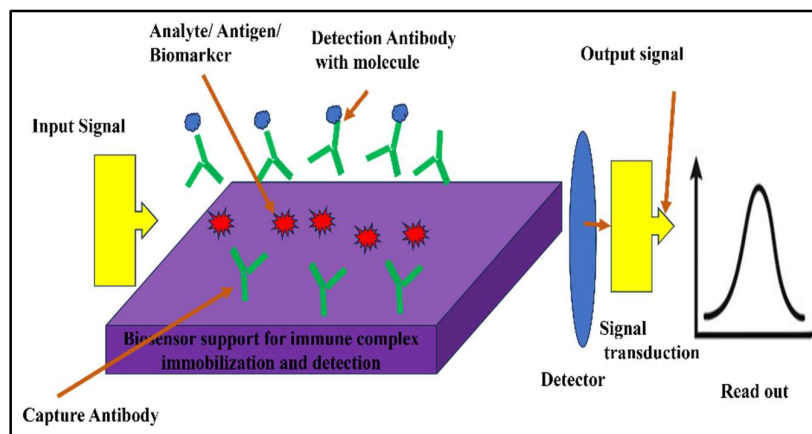


Figure 1.12: Schematic diagram of antibody-based biosensors.

1.3.3 Aptamer-based biosensors

The aptamer-based biosensors are also called DNA-based biosensors. Aptamers are sequences of DNA or RNA that bind to the target molecule. The most attractive features of DNA-based biosensors are very high selectivity for the target molecule [38, 39]. DNA biosensors that use nucleic acids as their biological receptors detect non-molecular compounds and proteins which attract with DNA fragments, called DNA probes. The immobilization of the probe is very important. **Figure 1.13** shows the schematic diagram of DNA-based biosensors.

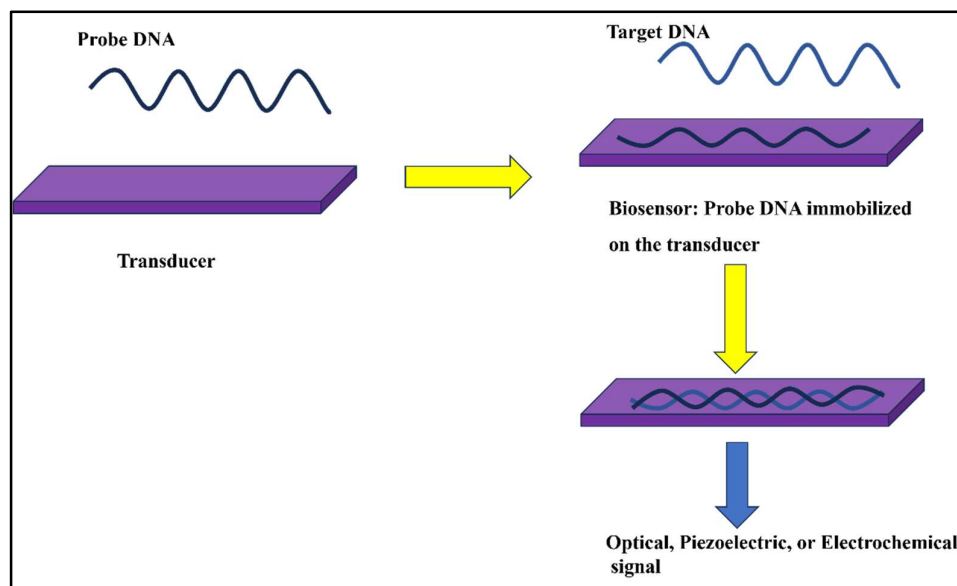


Figure 1.13 Schematic diagram of DNA-based biosensors

1.3.4 Whole Cells biosensors

Cell-based biosensors utilize microorganisms, viz. fungi, bacteria, algae, viruses, etc., that respond to functional biologically active analytes. They offer high selectivity and sensitivity. These are used for environmental monitoring of pollutants and detection of organic contaminants [40].

1.4 Characteristics of Biosensors

The quality of biosensors is determined by the following functional characteristics.

- Selectivity
- Long lifetime
- Minimal or no calibration
- Reproducibility (sample to sample)
- Stability (day to day)
- High sensitivity
- Low detection limit
- Large dynamic range

- Fast response time
- Fast recovery time
- Simple
- Inexpensive
- Rugged
- Miniaturizable

Figure 1.14 shows the characteristics of biosensors.

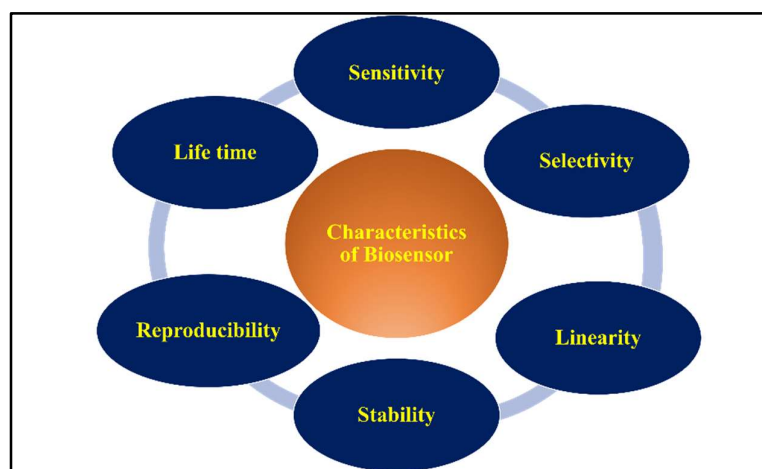


Figure 1.14: Characteristics of biosensors

The following are the explanations of the above terms.

i. Selectivity

The selectivity of a biosensor is its ability to determine its response with the desired analyte, while ignoring interference from other molecules or chemicals in its surroundings. Such interferences can be either positive or negative, depending on the increase or decrease in the electrode output. Positive interferences are produced by various substances, while the negative interferences act as poisons of the enzymes catalyzing the chemical transducers of the sample [41].

ii. Sensitivity

Sensitivity depends on the slopes of the response curve. More slope means more sensitivity. The characteristic of the slope is determined by the limit of detection. The

sensitivity of the biosensor is defined as the concentration of the analyte that gives a response twice the noise level. It is possible to have a biosensor with high sensitivity even if the noise level is too high.

iii. Reproducibility

Reproducibility of biosensors is the ability to produce identical responses for repeated measurements. Reproducibility is expressed by the accuracy and precision of the transducer in a biosensor. Precision is an important feature of sensors that provides similar results, while accuracy of a biosensor is to provide a mean value close to the true value during repeated measurement of a sample.

iv. Repeatability

The repeatability of a biosensor is its ability to produce similar results when subjected to the same analyte concentration, under the same conditions, using the same sensor over a short period. It is calculated by the Relative Standard Deviation (RSD).

v. Response time

It is defined as the time needed for the response of the electrode to reach a stationary value, and it measures the speed of response.

vi. Linearity

Linearity represents the accuracy of the measured response. Linearity of biosensors depends upon the range and analyte concentrations. A little change in analyte concentration affects the response of the biosensor, which is considered the resolution of the biosensor.

1.5 Mycotoxins

Mycotoxins are naturally occurring toxic organic compounds that are the secondary metabolites produced by *fungi*, causing adverse effects in living organisms, including humans and animals, through their exposure [42]. Mycotoxin exposure occurs by ingestion, inhalation, and absorption through the skin, which can cause adverse health conditions that can lead to death of animals and birds as well. Biochemical, physiological, and pathological changes in different species take place through the

mycotoxicosis process, which is indicated by the poisoning of the biological system [43]. The word mycotoxin consists of two words: “myco,” meaning mold or fungi, and toxin, a poison originating from those fungi [44]. Many plant species, such as corn, barley, maize, etc., are basic ingredients of human and animal consumption, and these are responsible for *fungus* metabolite infection [45]. *Fungi* are aerobic organisms, and their interaction with plants is very complex because fungi obtain nutrients from dead organic matter. *Fungi* infect plants before harvesting or in dry conditions or after storage, although some fungi proliferate before harvesting and during storage. Temperature and moisture variations play a very crucial role in the growth rate of such *fungi* and the amount of toxin production. The optimal moisture content for growth is 22-25 % in which *fungi* invade both mature and developing seed grains in the plants. Mostly, *fungi* grow in the temperature range between 20 °C and 30 °C, but many of them are produced above 30 °C and below 20°C. Hence, the optimal temperature range is between 0 °C and 60 °C [46]. Mycotoxicosis is the natural process of poisoning that occurs due to exposure to pathological changes caused by the natural toxins produced by fungi. Mycotoxicosis depends on several factors such as types of mycotoxins; amount and time of exposure; age, health condition, and sex of the individual; *fungus*-contaminated dietary exposure; and inhalation of toxins secreted by *fungus* spores, etc. Hence, the severity of mycotoxin poisoning can be enhanced due to vitamin deficiency, alcohol consumption, calorie deprivation, and the impact of infectious diseases, etc. [47, 48]. Classifications of mycotoxins are very challenging due to their variety of characteristics related to their different chemical structures and biosynthesis sources. However, generally, mycotoxins can be categorized as *hepatotoxins*, *neurotoxins*, *immunotoxins*, and others. Cell biologists classified these mycotoxins into generic groups such as carcinogens, mutagens, and others. Organic chemists have classified mycotoxins based on their chemical structure [49, 50, 51]. Mycotoxins have been known to be toxic for a long time, but the first case of foodborne *fungi* and the cause of animal diseases and death appeared in the 1960s [52]. During recent times, mycotoxins have been recognised as an epidemic in humans and animals. Hundreds and

thousands of people have died in Europe in the last millennium during ergotism. Besides these, between 1942 and 1948 at least 100,000 people died in the USSR due to alimentary toxic aleukia (ATA), a type of mycotoxicosis, which was responsible for killing around one lakh horses in the USSR in the 1930s, and aflatoxicosis was also responsible for the deaths of 100,000 young turkeys in the United Kingdom in the 1960s [53, 54, 55, 56]. The worldwide occurrence of food contamination via mycotoxin production from *fungi* has been classified into four major genera: *Aspergillus*, *Penicillium*, *Fusarium* and *Claviceps*. *Fusarium* species are destructive pathogens associated with cereals and other commodities that cause mycotoxin production before or after immediate harvesting. *Aspergillus flavus* is an exceptional kind of pathogen that grows in plants without affecting them, or a storage fungus that produces mycotoxin in all three conditions. The fourth genus, *Claviceps*, is also a significant pathogenic entity found in a variety of cereals and crops having a resting structure, called ergots. **Figure 1.15** shows the different groups of mycotoxins. The categories of *fungal* mycotoxins and their occurrence are given in **Table 1.1**.

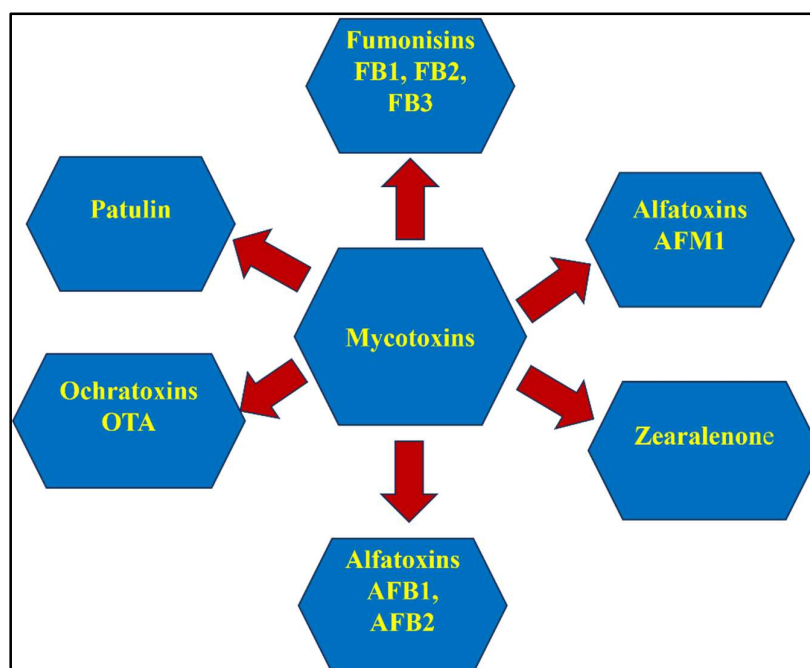


Figure 1.15: Different groups of mycotoxins

The categories of fungal mycotoxins and their occurrence are given in **Table 1.1**.

Table 1.1: Variety of mycotoxins and their sources.

Sr. No.	Fungal species	Mycotoxins	Commodities	Disease
1	<i>Aspergillus flavus</i>	<i>Aflatoxin</i>	Corn, peanut meal, milo, cottonseed, palm meal, soybean meal	Liver Cancer
2	<i>F. graminearum</i>	<i>Deoxynivalenol (DON)</i>	Corn, milo, barley, wheat	Acute disease
3	<i>Claviceps purpurea</i> , <i>Neotyphodium</i>	<i>Ergot alkaloids</i>	Wheat, barley, oats	Ischemic necrosis and dry gangrene
4	<i>Penicillium citrinum</i>	<i>Citrinin</i>	Wheat, rye, barley, beans, fruits, vegetable juices, herbs, and spices	Kidney problem
5	<i>A. ochraceous</i>	<i>Ochratoxin A</i>	Milo, barley, wheat, oats, bran	Anorexia, dehydration and depression
6	<i>F. verticillioides</i> , <i>F. proliferatum</i>	<i>Fumonisin</i>	Corn, milo, barley	Liver necrosis
7	<i>Fusarium sporotrichioides</i>	<i>T-2 toxin</i>	Corn, milo	Leukopenia
8	<i>F. graminearum</i>	<i>Zearalenone</i>	Milo, corn, barley	Vulval swelling

1.6 Importance of *Fumonisin B1*

Fumonisin is one of the dominant toxic compounds among various mycotoxins and is due to fungal species including *Fusarium verticillioides*, *F. proliferatum*, and *F. anthophilum* [57]. *Fumonisin*s were first isolated by Gelderblom and were chemically characterized by Bezuidenhout in 1988. Maize and wheat-based products are contaminated mainly with *fumonisin*s, and these are responsible for the contamination of other food items such as grains and grain products, which play a very crucial role in human health [58, 59, 60]. *Fumonisin*s are divided into four major groups: A, B, C, and P series. Group “B” *fumonisin*s are abundant in nature, such as *FB1*, *FB2*, and *FB3* [61, 62, 63]. Among these, *FB1* is the most toxic form of *fumonisin*s. The International Agency for Research on Cancer (IARC) has suggested cancerous effect of *FB1* in humans, and categorised it in the class 2B group [64]. The optimum growing temperature for the growth of *fumonisin*s is at 25 °C [65]. Corn is one of the major sources of occurrence of *fumonisin*s in various countries like Western Europe, Canada, the United States, and African countries [66]. *FB1* is detected in mg/kg concentrations and found mostly in corn and corn-related products. *FB1* is present in various maize-based food items such as germ, flour, bran, fibre, gluten and steep water [67]. The *fumonisin*s are present in *F. verticillioides* cells (mycelia, spores and conidiophores) [68]. *Fumonisin*s (particularly *FB1*, the most abundant form) have a structural resemblance to sphinganine (also called dihydrosphingosine), the precursor to sphingosine. This similarity allows *fumonisin*s to act as competitive inhibitors of key enzymes in sphingolipid biosynthesis. [69]. The toxicity of *FB1* can lead to several effects such as abnormal cell growth of the liver, heart failure, cell death, fluid in the lungs causing breathing problems, nephrotoxicity [70, 71]. *FB1* also shows genotoxicity, which increases the cancer risk [72]. Higher dose of *FB1* causes leukoencephalomalacia (ELEM) in horses and rabbits [73,74,75]. **Figure 1.16** shows the comparison of fresh fruits and *FB1*-contaminated fruits.

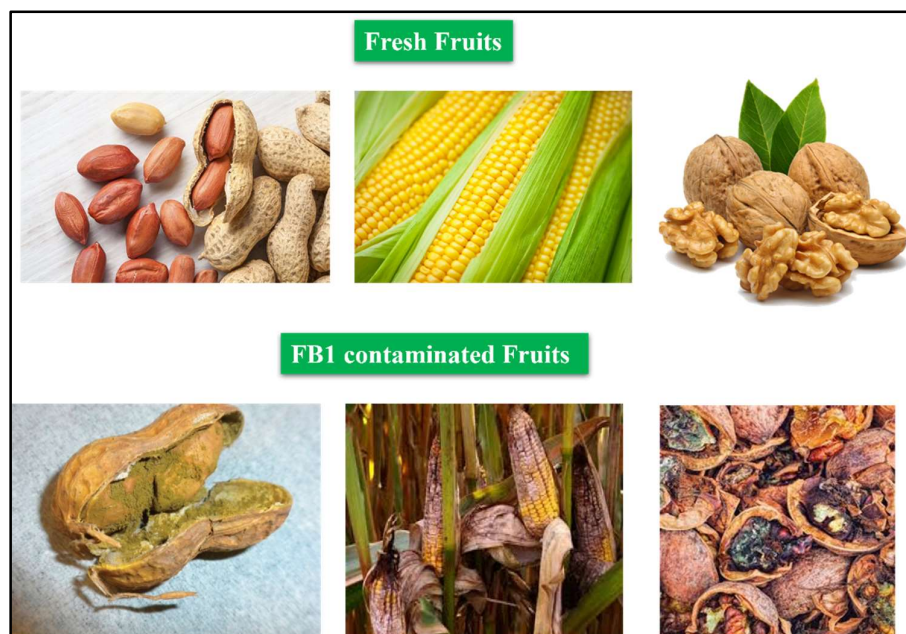


Figure 1.16: Comparison of fresh fruits and *FBI*-contaminated fruits.

The chemical structure of *FBI* is shown in **Figure 1.17**. The molecular structure of *FBI* is $C_{34}H_{59}NO_{15}$; the molecular weight of *FBI* is 721.83 gm/mol.

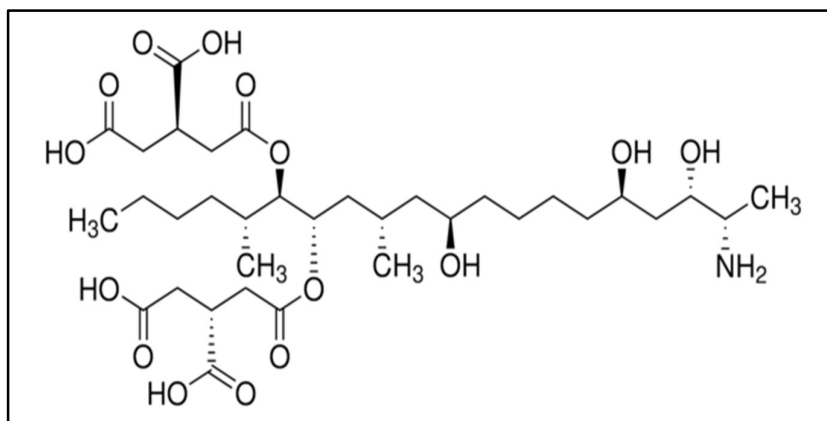


Figure 1.17: Chemical structure of *FBI*

1.7 Measurement techniques for detection of *FBI*

Several techniques are available for the detection of *FBI*. These techniques are shown in **Figure 1.18**.

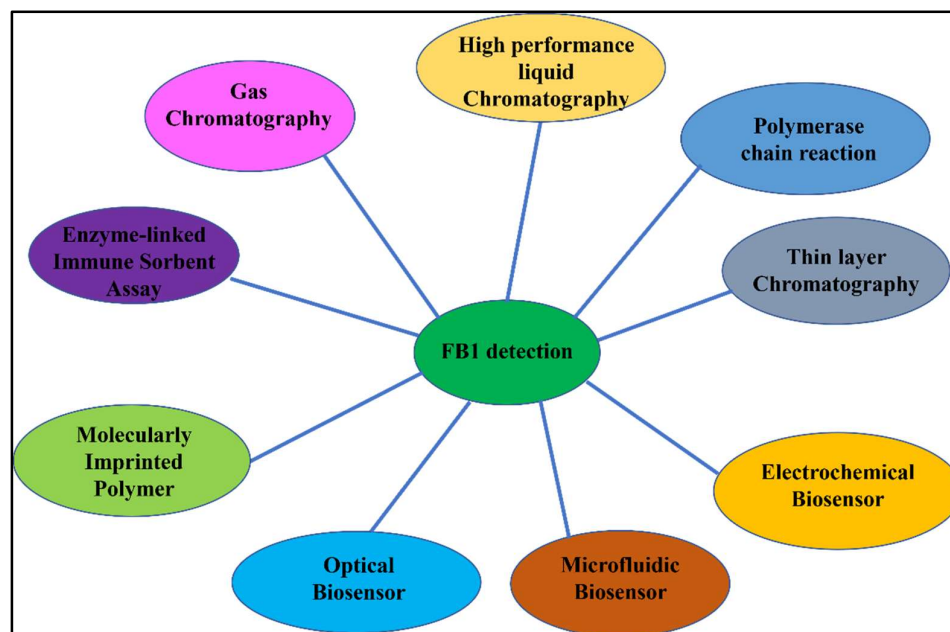


Figure 1.18: Different techniques of detection of FBI

Some are described below:

a) High-performance Liquid Chromatography

High-performance liquid chromatography (HPLC) is a conventional technique and is widely used in terms of sensitivity, selectivity, and analysis time. HPLC is considered a routine tool for the separation of complex mixtures, and it can detect various substances at very low concentrations. HPLC is commonly used in today's time for detection, separation, and quantification of samples, and is also used in biochemistry to separate, identify, and quantify the active compounds [76].

b) Polymerase chain reaction

The polymerase chain reaction (PCR) is a powerful molecular technique for detecting *fumonisin*s. It is an indirect method of detection that identifies the DNA of the mold that produces the toxins. The presence of fungal DNA does not guarantee the accumulation of toxins. It has high contamination risks [77].

c) Thin layer chromatography

It is a versatile analytical method for detecting organic compounds. It uses a post-chromatographic technique for the detection of *FBI* and is a semiquantitative method, but poorer thermal stability is the disadvantage of this technique [78].

d) Microfluidic biosensor

This detection system uses laminar flow, which accelerates the binding and detection systems, and is a class of lab-on-chip devices. The system is a class of "lab-on-a-chip" devices that isolate, process, and measure mycotoxins in food and agricultural samples [79]. This detection system has various disadvantages such as matrix interferences, complex multi-step preparation requirements, and challenges with bioreceptor stability.

e) Optical biosensor

It uses bioreceptors bound to optical transducers to rapidly detect the *FBI* in food samples [80]. This method has disadvantages such as sample pretreatment to avoid matrix interferences, high costs of noble-metal substrates, and temperature sensitivity.

f) Molecularly imprinted polymer

Conducting polymers are used, such as polyaniline (PANI); it is of high interest due to its remarkable properties like environmental friendliness, stability for longer time, inexpensive, excellent conductivity, and biocompatibility [81]. It has various disadvantages like solvent compatibility, cross-reactivity in matrices, and mass transfer limitations.

g) Liquid Chromatography Mass Spectroscopy

Liquid chromatography-mass spectrometry (LC-MS) is a conventional technique that is the combination of liquid chromatography (LC) and mass spectrometry (MS). LC is not efficient to separate the components alone, but in association with MS can identify unknown, known compounds or complex mixtures completely. LC-MS is a qualitative and quantitative analysis technique. It is extensively used in the agrochemical industry, pharmaceuticals, food industry, and environmental and forensic applications. It is also used in drug research and quality control analysis [82].

h) Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay (ELISA) is a conventional bioanalytical tool used as a biochemical assay. The principle of ELISA is based on the antibody-antigen reactions where the immunoreactions take place between antibodies (produced by

cells) and antigens. It is a highly sensitive and selective tool that permits the quantitative and qualitative analysis of antigens, proteins, hormones etc. During measurement, the molecules are labelled with enzymes using horseradish peroxidase (HRP) and β -galactosidase and are called enzyme immunoassay [83].

1.8 Limitations of Conventional Techniques

Conventional techniques have usually been applied for the detection of various analytes for clinical applications. Although these are sensitive and specific towards their analytes but also have various limitations due to their characteristics. These are very tedious, expensive, time-consuming, complicated, and require a trained technician to operate these setups. Several techniques, including ELISA, HPLC, LC-MS, and others, are heavily used in order to detect various analytes. However, ELISA has certain limitations, such as being laborious, requiring sophisticated laboratory equipment and high sample volume, which sometimes produce typical errors in the assay. Similarly, HPLC and LC-MS are very costly, require large amounts of samples for analysis, and are also difficult to handle. Analysis of samples using these methods takes a longer time and sometimes weeks. Therefore, quick, responsive techniques are required from a detection point of view.

1.9 Materials explored for the detection of *FB1*

1.9.1 Graphene Oxide

Two-dimensional graphene oxide, typically a flat or corrugated sheet with nanometer thickness, has gained much interest in the past two decades due to its scalable and low-cost synthesis. The confinement of thickness has resulted in numerous applications such as energy storage, sensors, catalysis, and water purification [84]. GO is basically a single-layer carbon sheet decorated heavily with oxygen functional groups and is produced by treating graphite with a chemical reagent through oxidation [85, 86]. Basically, carbon possesses a $1s^2$, $2s^2$, $2p^2$ electronic configuration, and it exists in three different hybridizations, namely, sp^3 (tetrahedral), sp^2 (trigonal planar), and sp^1 (linear). The particular hybridization and its bonding with neighboring atoms decide which allotrope carbon will assume. For example, carbon with sp^3

hybridization forms diamond, whereas with sp^2 hybridization it forms graphite [87, 88]. The individual allotropes exhibit unique features and are widely used in different applications ranging from drugs to synthetic materials [89, 90, 91]. The most common and naturally occurring forms of carbon are graphite and diamond. Graphite consists of stacked sheets of carbon with a hexagonal structure. **Figure 1.19** shows the schematic diagram of graphite. Graphite is a naturally occurring carbon allotrope with a layered structure. It consists of carbon atoms that are bonded with three adjacent carbon atoms via covalent bonding, resulting in hexagonal carbon rings linked together into an atomically flat layer. The distance between the planar layers is 0.33 nm, which is more than double the intra-layer distance of 0.14 nm between carbon atoms. These formed layers are interconnected with neighboring layers via the vander waals forces.

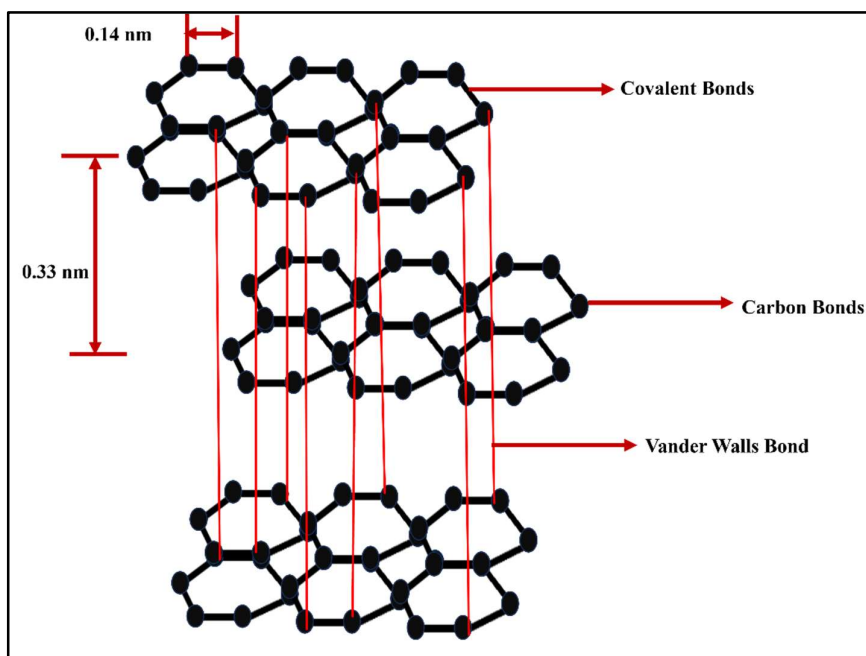


Figure 1.19: Schematic diagram of graphite

Currently, the precise atomic structure of GO is still uncertain, which is due to uncertain distribution of oxygen functional groups, non-stoichiometric atomic composition [92]. Graphene oxide was first made by British chemist Brodie nearly 150 years ago by chemical treatments of graphite and potassium chlorate ($KClO_3$), but it was not explored widely at that time. Later, with the development of graphene, the

synthesis of graphene oxide has been explored widely, as GO is considered the precursor for graphene synthesis [93]. The preparation of GO is carried out via two-step processes of oxidation and exfoliation, as shown in **Figure 1.4**. Different recipes have been developed for the synthesis of GO, such as the Brodie method [93] and the Staudenmaier method [94], Hummer's method [95], and its modification. All methods have advantages and disadvantages at the early stage [96]. Amongst all, Hummer's method was considered to be the easiest, less toxic, and cost-effective. However, GO prepared via Hummer's and its modified methods has holes due to excessive oxidation, which could be overcome by treating at a relatively low temperature [97]. Graphene oxide can be made into an insulating, semiconducting, or semi-metallic material by appropriately changing the oxidation parameters. Chemical modification of GO can be employed to disable various electroactive groups through covalent or noncovalent bonds [98, 99]. For device-based fabrication, GO-based thin conductive films deposited onto an inexpensive material led to a large surface area to volume ratio. With the aim of producing graphene from graphene oxide, various methods have been employed to reduce the number of oxygen functional groups in the graphene oxide sheet. Different methods have been developed and reported by researchers, which show that oxygen functional groups from graphene oxide are successfully reduced either by using chemical reactions, thermal reduction, hydrothermal technique, photochemical reduction, or by photoillumination reaction. The resultant product was graphene with few oxygen functional groups (reduced graphene oxide) on the surface of the graphene sheet. Chemical reduction of graphene oxide by chemical reagents depends on chemical reaction with GO. Chemical reduction is widely referred to by researchers as it is cheaper and easily available for the mass production of chemically derived graphene (reduced graphene oxide). For the reduction of GO, hydrazine hydrate is one of the most commonly used chemical reagents that was first reported by Stankovich et al. [100]. The reduction using hydrazine can be done by adding liquid reagent to a GO solution. **Figure 1.20** shows the exfoliation of graphite.

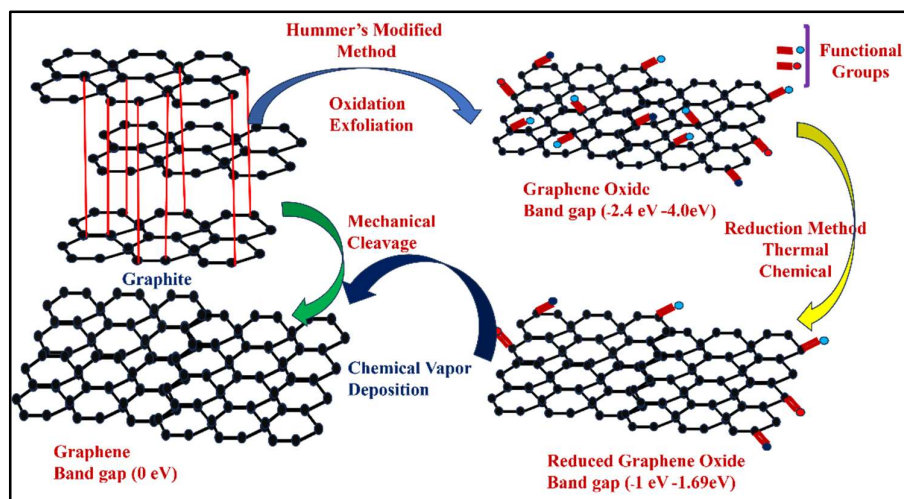


Figure 1.20: Exfoliation of graphite

The graphene exhibits electrical, mechanical, thermal, and optical properties. Graphene oxide (GO) shows advantageous characteristics as a biosensing platform [101].

1.9.2 Tri-manganese tetraoxide

Manganese is a 3d transition element with significant abundance in the Earth's crust [102]. It is a very essential nutrient found in seeds, whole grains, and smaller amounts in legumes, beans, green vegetables, etc. Manganese has many health benefits, including helping with the brain's normal functioning, the nervous system, importance in bone health, and antioxidants to minimize disease risk [102, 103, 104]. Manganese oxide is an essential material that can be processed in various structures with integrated properties. It displays a wide range of different oxide phases such as MnO , MnO_2 , Mn_2O_3 , Mn_3O_4 , and Mn_5O_8 . They exhibit optical, electrochemical, and magnetic properties, which make them useful for several applications [105, 106]. Manganese oxide is a semiconductor nanomaterial due to multiple properties like a smaller size, large surface-to-volume ratio, one-dimensional nanostructure, paramagnetic behavior, etc., with attractive technological applications [107, 108]. Several efforts have been made on the advancements of manganese oxides for technological use as a catalyst, in sensors or biosensors, biomedical devices, in water remediation, molecular sieves, drug delivery ion-sieves, in nanocomposites, in

supercapacitors, in alkaline rechargeable batteries, and other fields including imaging contrast agents, magnetic storage devices, etc. [109, 110, 111, 112, 113]. The unique properties of manganese oxides are associated with variations in bonding, electronic structure, and good thermal and chemical stability. Moreover, manganese oxide is a relatively less toxic nanomaterial than other nanomaterials based on diverse chalcogenides, environmentally compatible, specific capacitance, and cost-effectiveness, etc. [114, 115]. There are diverse structures of manganese oxide such as nanorods, nanosheets, nanowires, nanotubes, nanofibers, nanobelts, and mesoporous sieves that have been prepared using different synthesis techniques [116]. However, these metal oxides are biologically less explored and have limited use in terms of biological activities. Tri-manganese tetraoxide is a p-type semiconductor having a larger bandgap of 2.3 eV [117]. The schematic diagram of Mn_3O_4 is shown in **Figure 1.21**.

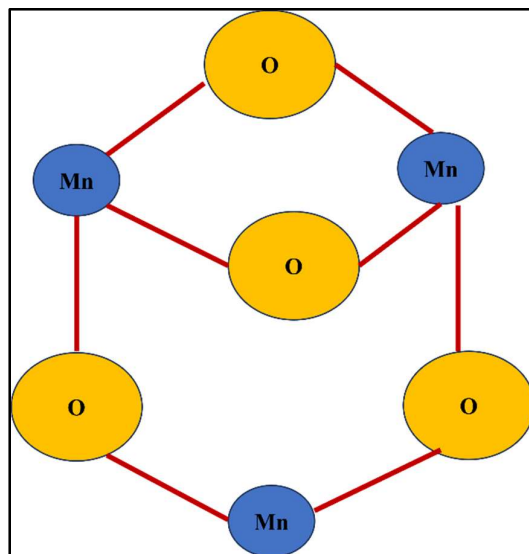


Figure 1.21: Schematic diagram of Mn_3O_4

Tri-manganese tetra-oxide (Mn_3O_4) is a very advanced phase of manganese oxide nanomaterials and more electrochemically important than other phases, MnO_2 and Mn_2O_3 . In inorganic chemistry, Mn_3O_4 has a normal spinel structure at room temperature and shows a stable tetragonal structure occupying octahedral and tetrahedral sites for Mn^{3+} and Mn^{2+} ions, respectively [118, 119]. Having a unique

structure, Mn_3O_4 offers several applications such as excellent supercapacitor, high-density magnetic storage device, used as a catalyst in oxidation or reduction, high charge carrier mobility, good biocompatibility, and chemical stability [120, 121, 122, 123]. Due to its strong oxidative and characteristic behaviour of Mn_3O_4 , interestingly used in the polymerization of aniline and degradation of organic dyes from contaminated water [124]. Mn_3O_4 nanomaterials have been investigated as effective catalysts for the oxidation of carbon monoxide (CO) and methane (CH_4) gases, and reduction of nitrobenzene [125, 126, 127]. Moreover, this phase of metal oxide is an efficient and inexpensive catalyst for minimizing the emission of NO_x and CO [127, 128, 129]. Mn_3O_4 is a non-toxic and cost-effective catalyst that is used in the decomposition of toxic and hazardous components from the wastewater [128].

1.9.3 Iron Oxide

Iron oxide nanoparticles consist of oxides of iron and belong to a class of inorganic or metal nanoparticles. Iron is positioned in the 8th group of the periodic table and belongs to the first transition metal series. It is abundantly present on Earth's crust and is known as the most frequent element as per the mass distribution. Iron shows a wide variety of polymorphism (16 types) with respect to oxides, hydroxides, and oxide-hydroxides. Magnetite (Fe_3O_4), hematite ($\alpha\text{-Fe}_2\text{O}_3$), and maghemite ($\gamma\text{-Fe}_2\text{O}_3$) are the most common polymorphs [130]. Magnetite is a mixed iron oxide and consists of iron in both Fe^{2+} and Fe^{3+} states. Maghemite bears a strong resemblance to magnetite and is formed upon the complete oxidation of magnetite. Hematite is an important type of iron oxide polymorph that consists of iron in the completely oxidized Fe^{3+} state. Details on characteristics of various iron oxides are discussed by previous researchers [131]. Hematite nanoparticles have various distinctive functional characteristics such as compatibility with biological systems, unique magnetic behavior, structural stability, relatively low toxicity, corrosion resistance, morphological polymorphism, and interesting optical properties [132]. Further, Hematite nanoparticles behave as an n-type semiconductor and display photocatalytic potential in the presence of light sources. Thus, it is widely used in various areas such as the energy sector, cleaning up the environment, water splitting, lithium batteries, biomedicine, sensing materials, mechanical field, reaction catalysis, magnetic recording, pigments, fabrics, farming

applications, electronics, etc. The structure of Fe_2O_3 is shown in **Figure 1.22**.

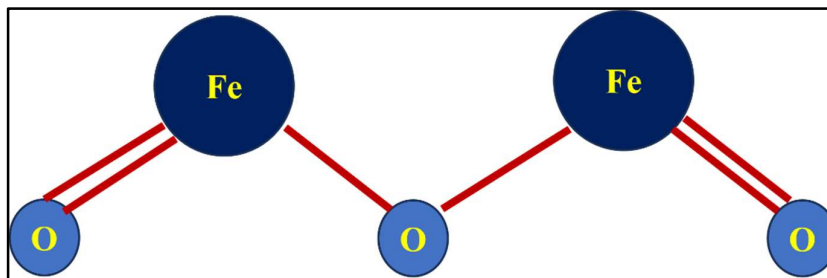


Figure 1.22: Structure of Fe_2O_3

1.10 Objectives of the present work

- Literature review of FB1 and identification of a suitable 2D nanomaterial for FB1 detection.
- Synthesis of 2D nanomaterials using different methods.
- Characterization of 2D nanomaterials and their nanocomposites.
- Thin film fabrication of 2D nanomaterials using electrophoretic deposition.
- FB1 sensing applications of 2D nanomaterials and their nanocomposites.

The outcomes of this research, aimed at achieving the aforementioned objectives, are organized into six chapters, each summarized below. This structure reflects the comprehensive optimization of synthesis parameters carried out based on the findings from characterization studies and FB1-sensing performance evaluations.

1.11 Plan of thesis

This thesis is mainly focused on the detection of FB1 based on biosensors, which is constructed in different chapters as follows:

Chapter 1

This chapter provides the overview, motivation, and objectives of the research work reported in this thesis. A brief discussion of *FB1*, their adverse effects, and various detection techniques, as well as a discussion on various nanomaterials, has been presented in this chapter.

Chapter 2

This chapter discusses the various experimental techniques and their principles that are used to carry out the characterization of nanomaterials. This chapter also covers detection techniques, such as electrochemical techniques, which are used for biosensing.

Chapter 3

In this chapter, the synthesis of trimanganese dioxide and graphene oxide is discussed. This chapter also includes the characterization of nanomaterials using electrochemical techniques.

Chapter 4

This chapter discusses the synthesis of hematite and reduced graphene oxide, and the detection of *FBI* using electrochemical techniques.

Chapter 5

This chapter discusses future research and technological development. It also discusses sensor optimization with different 2D nanomaterials for better electron mobility, potentially improving sensor sensitivity and lowering detection limits even further. The selectivity can be enhanced, and further, the density functional theory (DFT) calculations could provide a deeper understanding of charge transfer dynamics, adsorption mechanisms, and potential barrier modulation, guiding rational design of next-generation sensors. Further research can improve the long-term stability and cyclic performance under varying humidity for industrial, environmental, and biomedical applications. It can improve social impact, as exposure to these toxins can cause liver cancer, kidney damage, immune suppression, growth disorders, and neurological problems.

Chapter 2

Methodologies: Synthesis, Thin Film Fabrication and Characterization Techniques

Chapter 2 describes the various synthesis methods that are used for the synthesis of GO, rGO, Mn_3O_4 , $\alpha\text{-Fe}_2\text{O}_3$ nanomaterials, and their nanocomposites, as well as the method used for fabricating their thin films, characterization techniques used for confirming the formation of nanostructures, along with different electrochemical techniques used for biosensing applications. The formation of GO, rGO, Mn_3O_4 , and $\alpha\text{-Fe}_2\text{O}_3$ nanomaterials is examined using various characterization techniques like X-ray diffraction (XRD), Raman spectroscopy, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), high-resolution transmission electron microscopy (HR-TEM), X-ray photoelectron spectroscopy (XPS), and energy dispersive X-ray analysis (EDAX). All these characterization techniques are described in this chapter. Further, for the biosensing studies, a galvanostat/potentiostat Autolab is used, and electrochemical characterization studies of fabricated films of nanocomposites of GO, rGO, Mn_3O_4 , and $\alpha\text{-Fe}_2\text{O}_3$ are done using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV). All these techniques are also briefly reviewed in this chapter.

2.1 Synthesis of GO, rGO, Mn_3O_4 , $\alpha-Fe_2O_3$ nanomaterials, and its nanocomposites

The synthesis of GO, rGO, Mn_3O_4 , $\alpha-Fe_2O_3$ nanomaterials, and their nanocomposites can be done using a variety of techniques to obtain desired structures and properties to suit various applications. The methods of synthesis are divided into two categories, namely 1) Top-down and 2) Bottom-up. In the Top-down technique, the material in bulk form is exfoliated into layers by overcoming the weak binding forces between the layers, like Van der Waals forces, using mechanical, chemical, or ultrasonic methods. In the bottom-up method, the nanosheets are assembled from individual atoms only. The top-down approach involves milling, lithography, mechanical exfoliation, liquid-phase exfoliation, etc. However, this approach does not have control over the size and structure of NPs. Further, the bottom-up approach involves preparation of nanomaterials from the bottom, i.e., atom-by-atom, molecule-by-molecule. It is the first choice of scientists owing to its good control over nucleation and growth of nanocrystals. This approach includes chemical vapour deposition (CVD), physical vapour deposition (PVD), chemical vapour transport (CVT), atomic layer deposition (ALD), etc. **Figure 2.1** shows the schematic diagram of various synthesis procedures.

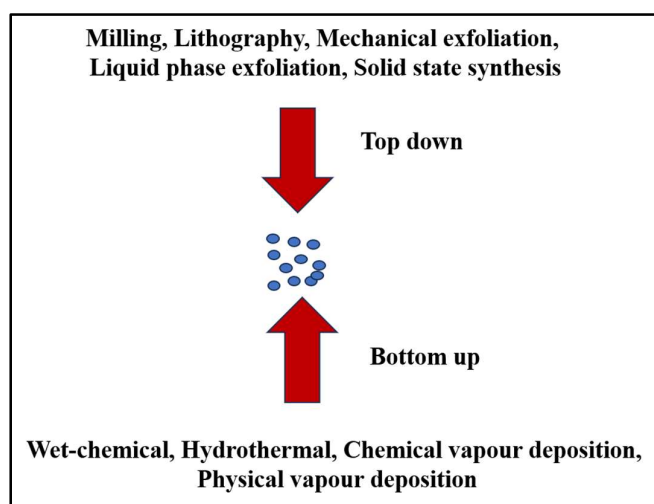


Figure 2.1: Schematic diagram of various synthesis procedures

The precursor used for the synthesis of Mn_3O_4 is manganese (II) acetate tetrahydrate, and for the synthesis of $\alpha-Fe_2O_3$ nanomaterials is Iron(III) chloride hexahydrate.

In this work, the co-precipitation method for the synthesis of Mn_3O_4 and environment friendly hydrothermal method for the synthesis of $\alpha\text{-Fe}_2\text{O}_3$ nanomaterials are used for the synthesis of nanocomposites.

Further, the synthesis of graphene oxide (GO) and reduced graphene oxide (rGO) is prepared with the standard improved Hummer's method. All the methods are discussed in brief in the following sections, and the details of synthesis have been discussed consequently in chapters 3, 4, and 5, respectively.

2.1.1 Hydrothermal method of synthesis

In the hydrothermal method, heterogeneous reaction takes place under high pressure and temperature conditions in the presence of aqueous solvents to dissolve and recrystallize various materials, which are generally insoluble under ordinary conditions. According to Rabenau and Laudise, hydrothermal synthesis is defined as the heterogeneous reactions above 100°C in aqueous media, and at a pressure of 1 bar [133, 134]. Lobachev [135] defined the hydrothermal method as a method which occurred at high pressures in superheated aqueous solutions. Roy explained hydrothermal synthesis as a method to synthesize materials at temperatures above 100°C , and pressure greater than one atmosphere [136]. Byrappa describes hydrothermal synthesis as a heterogeneous reaction in an aqueous medium conducted above room temperature and under pressures exceeding one atmosphere [137]. Yoshimura *et.al.* [138] define hydrothermal as reactions in aqueous solutions occurring under the conditions of high temperature and high pressure ($>100^\circ\text{C}$, >1 atm).

a) Apparatus in Hydrothermal Method:

The hydrothermal method requires two apparatus: first is the vessel in which precursors are placed, called an autoclave, and second is the muffle furnace. Highly corrosive salts are used for the preparation of inorganic materials. The autoclave must be corrosion resistance in that pressure and temperature. **Figure 2.2 (a), (b)** shows the photograph of the autoclave and muffle furnace, respectively, used in the present study.

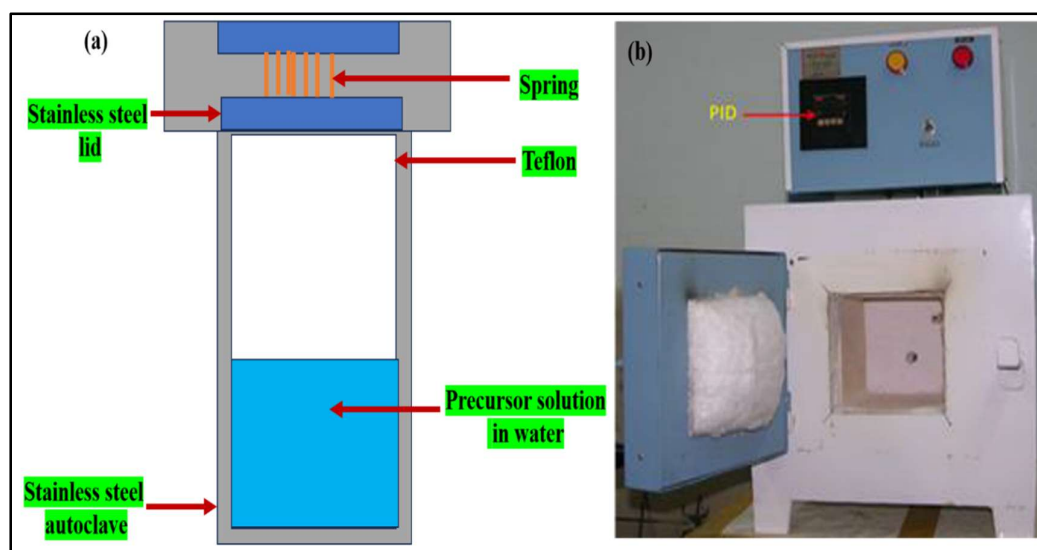


Figure 2.2: Schematic diagram of (a) autoclave and (b) muffle furnace

High-strength, corrosion-resistant materials such as stainless steel, iron, nickel, cobalt-based superalloys, and titanium alloys are commonly used for autoclave construction. To prevent corrosion inside the autoclave, the inner surfaces are coated with a non-reactive material like Teflon. Teflon has a higher thermal expansion coefficient compared to the metal autoclave body. This means Teflon expands and contracts more during heating and cooling cycles. The thermal expansion behaviour of Teflon helps it form a perfect seal inside the stainless-steel housing as the temperature rises, which prevents leaks and maintains containment. Teflon cannot be used above 300°C because it melts or dissociates at higher temperatures, which can alter the pH of the solution inside. Also, the Teflon coating tends to wear out or tear after a few uses and needs to be reapplied. The most successful corrosion-resistant materials with very high strength are used. Teflon has a higher thermal coefficient of expansion with respect to the material in which the liner is enclosed. Teflon has more thermal stability. The teflon beaker should sit exactly inside the autoclave without leaving any gap. As the temperature of teflon rises, the thermal expansion behaviour of teflon provides perfect sealing within the stainless-steel housing.

Merits of Hydrothermal Method:

The advantages of hydrothermal synthesis compared to conventional and non-conventional methods are:

- (i) The equipment required for hydrothermal synthesis is generally less expensive.
- (ii) Hydrothermal methods are more environmentally friendly compared to many other synthesis methods, reducing harmful impacts.
- (iii) These avoid issues common with high-temperature methods, such as loss of stoichiometric control caused by volatilization of components.
- (iv) Hydrothermal synthesis can produce powders with controlled size and different morphologies, which benefits industries like materials manufacturing, pigments, pharmaceuticals, and medical diagnostics.
- (v) The method can be combined with other techniques such as microwave, electrochemistry, and optical radiation to enhance reaction rates and enable the creation of new materials. This method does not require any harmful and expensive surfactant.
- (vi) Hydrothermal reactions do not require much time compared to conventional methods like solid-state reaction, which requires a few weeks; rather, the hydrothermal method requires just a few days.
- (vii) Hydrothermal synthesis also has the advantage of fast diffusion processes.

2.1.2 Co-precipitation Method

Co-precipitation is a very simple and cost-effective method for synthesizing a variety of nanomaterials. Uniform and desired nanoparticles can be obtained by using this process. An aqueous medium is required for the synthesis of nanoparticles during precipitation. This process involves the mixing of water-soluble precursors of metal ions such as salts of acetate, nitrate, or chloride, etc. These water-soluble precursors are treated with reducing agents such as sodium hydroxide and undergo a reaction, and finally get precipitated. During the reaction, the solution needs to be stirred continuously without any heat treatment. The entire reaction procedure should be

maintained in an alkaline medium by adding the alkaline salt. The size of the nanoparticles depends on various factors such as pH, ratio of salt chosen, and reaction medium, etc. [139, 140].

2.1.3 Solid-state method

The solid-state synthesis method is a very versatile method to make a wide range of nanomaterials, including metal oxides, sulfides, nitrides, and nanocomposites as well. This method requires control of a few parameters like grinding time, annealing temperature, etc. In this method, the desired quantities of the precursors are weighed and ground in a mortar and pestle for a certain time. The time may vary for a few hours, depending on the type of samples to be prepared. After grinding, the powder is heated for a few hours to obtain the desired product. **Figure. 2.3** shows the mortar and pestle required to grind the precursors.

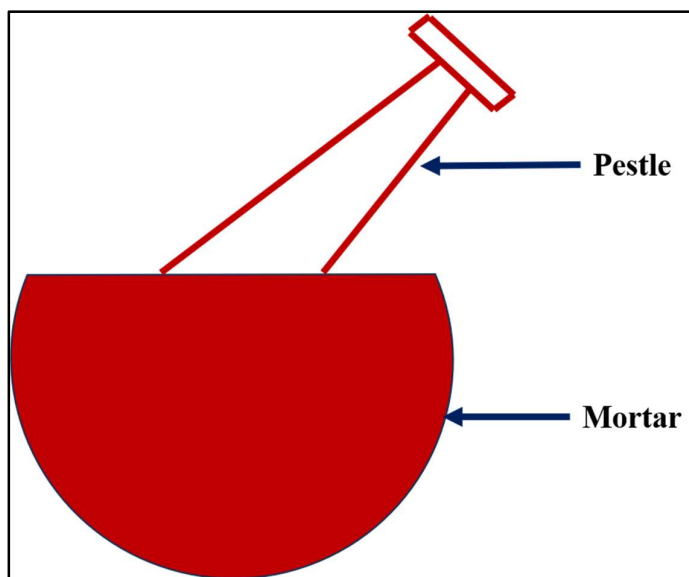


Figure. 2.3: Schematic diagram of Mortar and pestle

2.2 Method of thin film fabrication for $\text{Mn}_3\text{O}_4/\text{GO}$, $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}$ nanocomposites

2.2.1 Electrophoretic deposition (EPD) technique

EPD is a thin film formation technique where stable colloidal suspended charged particles migrate under the influence of an electric field and deposit onto the

oppositely charged conductive surface. It is a very cost-effective two-step process. The first step involves the movement of particles having electric charge in the colloidal suspended liquid solution towards the electrode by applying the electric field. While the second step allows the coherent deposition of particles on the electrode. EPD enables the fabrication of various desired structures using traditional to advanced materials. It can be used in the fabrication of nanometric thin films to 1 mm thick films. EPD is heavily used in the fabrication of electrodes for application in biosensing. **Figure 2.4** shows the schematic diagram of the EPD technique.

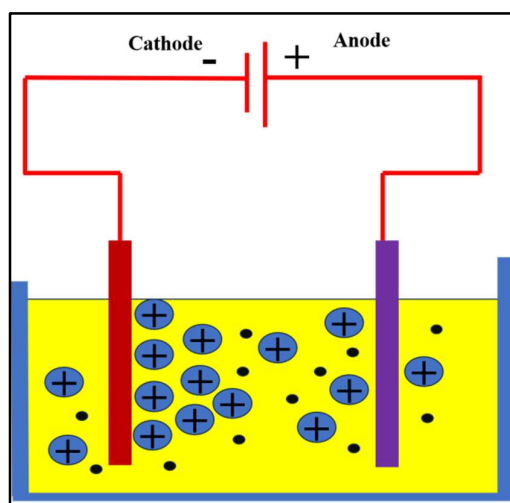


Figure 2.4 Schematic diagram of EPD

2.3 Characterization techniques used for structural, morphological, topographical, and spectroscopic investigation of *GO*, *rGO*, *Mn₃O₄*, *α -Fe₂O₃* nanomaterials and their nanocomposites

The confirmation of the successful formation of *GO*, *rGO*, *Mn₃O₄*, *α -Fe₂O₃* nanomaterials and their nanocomposites is examined using various characterization techniques. First of all, the structural study is done using X-ray diffraction (XRD), followed by morphological investigations using scanning electron microscopy (SEM), field emission scanning electron microscopy (FESEM), X-ray photoelectron spectroscopy (XPS), and high-resolution transmission electron microscopy (HR-TEM). Then, the confirmation of layered structures is probed using Raman spectroscopy. The examination of attachment of desired functional groups is studied using Fourier transform infrared spectroscopy (FTIR). Elemental analysis is done

using energy dispersive X-ray analysis (EDAX). The working and principles of all these characterization techniques are discussed in brief in the following section.

2.3.1 X-ray diffraction (XRD) for structural investigations

X-rays were first invented by the German physicist Wilhelm Conrad Rontgen in 1895. These rays are a form of invisible, highly penetrating electromagnetic radiation with wavelengths much shorter than those of visible light, around 10-10 meters. In 1912, Van Laue showed that X-rays could be diffracted by crystals, and by 1935, Le Galley had developed the first X-ray powder diffractometer.

This advancement enabled metallurgists and mineralogists to use X-ray powder diffractometers to investigate structural imperfections. The XRD technique is specifically used to analyze crystalline states of matter. XRD is a very powerful technique for investigating the crystal structures of solids, powders, and thin films. It is particularly effective for detecting crystalline materials with crystal domains larger than 3-5 nm. This technique is used to characterize various nanomaterials and determine their chemical phase compositions. A specimen is exposed to a collimated beam of X-rays, usually with wavelengths ranging from 0.7 to 2 Å. This XRD system is mainly based on X-ray generation and its monochromatic diffraction. The XRD system consists of three main components: cathode ray tube, sample holder, and detector. X-rays are produced by an X-ray tube or cathode ray tube due to applied voltage on the filament (as copper material), electrons accelerating towards the target material and bombarding the target material at the angle θ_{hkl} . In a crystalline solid, the atoms are arranged in a regular pattern, and the smallest volume element is called a unit cell, and its repetition in 3D forms a crystal. The 'd' spacing of atomic planes of the crystal is different for different materials. When a monochromatic X-ray beam is incident on the crystalline material, some part of the beam is diffracted. These diffracted X-ray beams interfere constructively as well as destructively. On destructive interference, waves are out of phase, and there will be no resultant energy leaving the crystal lattice. Those atoms which are arranged in a crystal in a regular pattern will form constructive interference, and occurs when the path difference is an integral number n of wavelengths λ , so that this is Bragg's law. Bragg reflection can

occur only when $\lambda \leq 2d$. The diffraction of X-rays by the crystalline phases in the sample is according to Bragg's law. The diffraction of a crystal is described by Bragg's **equation 2.1**, and the schematic explaining the diffraction from a crystal is shown in **Figure 2.5**.

$$2d_{hkl} \sin \theta_{hkl} = n\lambda \quad \dots\dots\dots(2.1)$$

where,

λ stands for the wavelength of the incident light, d stands for the interplanar spacing, θ stands for the diffraction angle, h , k , and l are the Miller indices for the planes of crystals, and n is the order of diffraction [141].

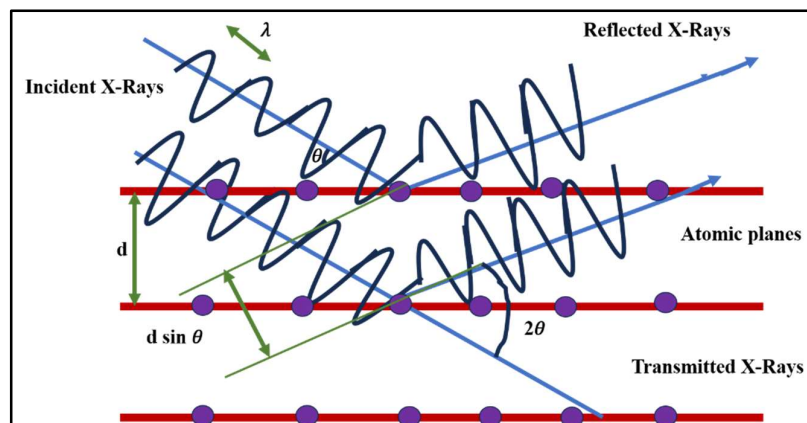


Figure 2.5: Schematic diagram of X-Ray Diffraction

Phase identification of a crystalline substance is accomplished by this analytical method, which can also yield dimensions of unit cells. The phases in the film can be identified by comparing the d -spacings with the standard JCPDS powder diffraction file, and the reflections are indexed using Miller indices. When the size of the diffracting crystal is small, complete destructive interference does not occur, resulting in the broadening of the diffraction peak. This peak broadening is proportional to the size of the tiny crystals, which can be calculated using the Scherrer **equation 2.2**. The crystallite size (D) is determined from the peak width.

$$D = K\lambda/(\beta \cos (\theta)) \quad \dots\dots\dots 2.2$$

Where D is the particle size, θ is the diffraction angle, λ is the wavelength of the X-

rays, and β is the line broadening at full width half maxima (FWHM) in radians.

In the present research work, a PANalytical (UK) powder diffractometer (**Figure 2.6**) with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) has been employed to record the XRD data in terms of intensity and 2θ angles. The diffractometer's operating current and voltage were set at 30 mA and 40 kV, respectively. To get a noise-free and clean XRD pattern of the samples, scanning was performed with a 0.01° step size and an integration time of 8 seconds per step.

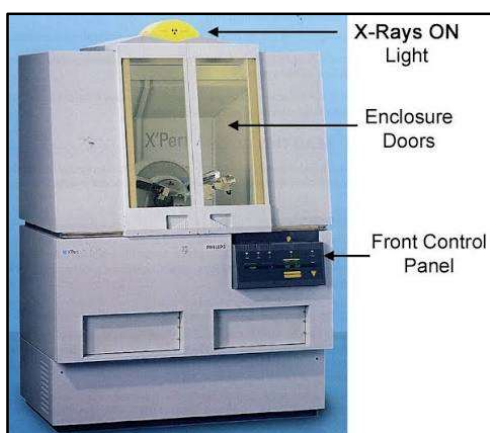


Figure 2.6: Picture of PANalytical X-Ray Diffractometer

Applications of XRD

XRD is a very powerful technique that provides extensive information about crystalline materials. Its capabilities include:

- a) **Crystal structure:** XRD is used in determining the crystal structure of a material, offering insights into the arrangement of atoms and the dimensions of the unit cell. It can also assess the degree of crystallinity, distinguishing between crystalline and amorphous phases in a sample.
- b) **Phase identification:** The technique is used in identifying various phases present in a mixture of crystalline materials. This capability is particularly valuable in fields such as materials science and geology, where phase composition is very important.
- c) **Lattice parameters:** XRD allows for the precise determination of interplanar spacing (d-spacing) between crystal planes. This information is vital for

crystallographic studies, contributing to the understanding of material properties.

- d) **Texture analysis:** XRD can reveal the orientation of crystallites within a polycrystalline material, providing information about the material's texture. This is important for applications where the directional properties of the material influence performance.
- e) **Stress and strain analysis:** The technique can investigate stress and strain in crystalline materials, which is essential for materials engineering, quality control, and ensuring reliability in structural applications.
- f) **Defects and dislocations:** XRD can offer insights into crystal defects and dislocations. Understanding these imperfections is crucial in materials science, as they can significantly affect the mechanical, thermal, and electrical properties of materials.

2.3.2 Scanning electron microscopy (SEM) for morphological investigations

The SEM works by scanning the surface of the sample with a focused beam of high-energy electrons. The focusing of the electron beam through the column is done with the electromagnetic lenses and apertures. The deflection of the electron beam is done with the scanning coils, which are controlled by a scan generator. Various types of signals are produced due to the interaction of the electron beam with the surface of the specimen, e.g., X-rays, secondary and backscattered electrons, heat, and light. The signals are collected by detectors, processed, and translated into a series of pixels, which is displayed on the display monitor. The scanning of the sample is in a raster pattern.

(a) Main components of a standard SEM

(i) Electron gun

The electron gun in a scanning electron microscope (SEM) produces a stable electron beam with adjustable energy and is positioned at the top of the SEM column. There are two main types of electron guns, viz. thermionic Emission Guns, which are used in conventional SEMs. Electrons are generated by heating the filament tip, which is made of tungsten or Lanthanum Hexaboride (LaB_6). The accelerating voltage, a voltage difference between the filament and the anode plate, speeds up the electrons down the column. The second is field emission guns, which are used in field emission scanning electron microscopes (FESEM). Electrons are generated by applying an electrostatic

field rather than heat. This method produces a beam with higher brightness and lower energy spread compared to thermionic guns. These features make FESEM especially suitable for very high-resolution imaging. In summary, thermionic guns rely on heat for electron production, while field emission guns use an electric field to produce a brighter, more focused beam ideal for detailed imaging [142].

(ii) Lenses and apertures

Electromagnetic lenses and apertures in a scanning electron microscope (SEM) are used to narrow the electron beam produced by the electron gun and focus it precisely onto the specimen. Their purpose is to reduce the beam diameter to create a small, focused electron spot on the sample. Smaller spot sizes enable higher resolution imaging of the sample surface. The lens system includes condenser lenses, objective lenses, apertures, and scanning coils. Together, these elements control and steer the electron beam to achieve fine focus and detailed imaging [143]. **Figure 2.7** shows the schematic diagram of SEM.

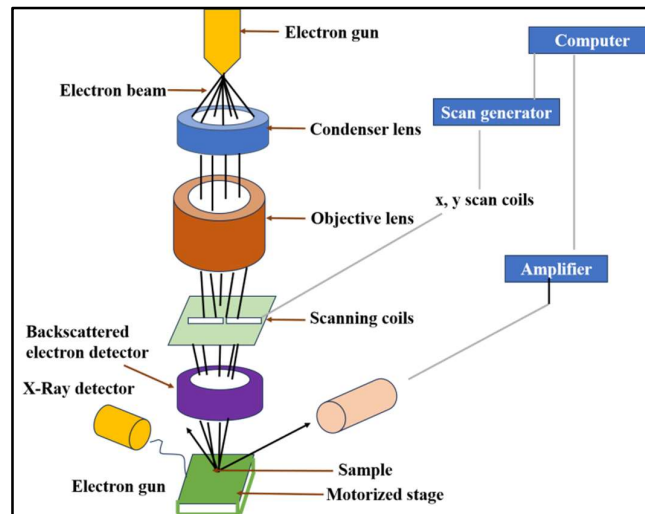


Figure 2.7: Schematic diagram of SEM

(iii) Beam-Specimen Interaction

Signals are created in an interaction volume within the specimen when an electron beam scans the sample. **Figure 2.8** shows the schematic diagram of the interaction of the electron beam with the sample. The ejection of an electron due to the deexcitation process is called an Auger electron. For biological sample measurements, low accelerating voltages are used to avoid signals beneath the surface. Secondary

electrons (SE) are produced on collision of a beam of electrons with the atom of the specimen, and it loses energy to that atom, which results in a transfer of energy to the specimen atom, and the specimen atom becomes ionized. Secondary electrons are related to energies less than 50 eV. These are created throughout the interaction volume, but most of them are absorbed by specimen atoms due to their low energy, and SEs are created near the surface of the specimen. Backscattered electrons (BSEs) occur when a primary beam of electrons comes into proximity with the nucleus of a specimen atom [144]. BSEs go through various scattering events in the interaction volume and escape back with energy greater than 50 eV. BSEs are collected and imaged by BSE detectors and are very useful for compositional contrast. The yield of BSE's increases as the atomic number increases, and the higher atomic numbered elements will appear brighter in the BSE image. When an electron from the primary electron beam hits an electron in the inner shells of an atom, the atom becomes ionized and it reaches in an unstable state, and the atom regains stability when an electron from an outer shell occupies the inner shell vacancy, and due to this effect, an X-ray photon is emitted [145]. The continuum X-rays result from an interaction of the electron beam with the Coulomb field inside the nucleus of the specimen atom.

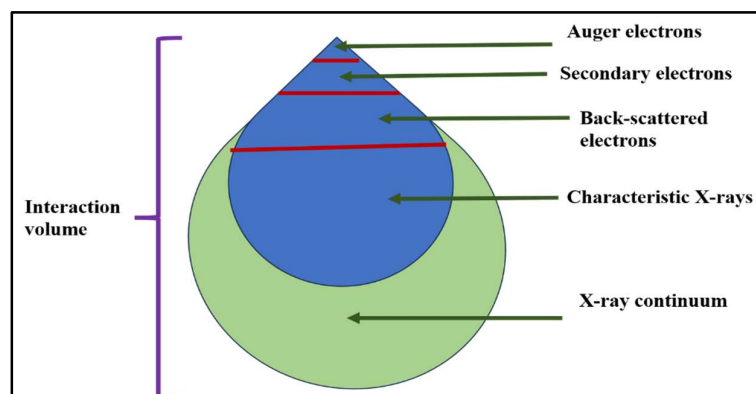


Figure 2.8 Schematic diagram of interaction of electron beam with sample

In this research, Carl Zeiss EVO 40 is used and is shown in **Figure 2.9**. The scanning electron microscope (SEM) operates under a high vacuum environment, typically around 10^{-6} to 10^{-7} torr. This high vacuum is essential to allow the electron beam to travel through the microscope's column without being disturbed or scattered by dirt, debris, or air molecules. Maintaining this vacuum ensures a clear and precise electron beam for accurate imaging.



Figure 2.9: Scanning Electron Microscope Carl Zeiss EVO 40

2.3.3 Field Emission Scanning Electron Microscope (FESEM) for morphological investigations

FESEM, like the SEM, is also a microscopy technique that uses a field emission source, which scans the specimen in a zig-zag pattern. When field emission occurs, electrons will be emitted from the cathode and attracted towards the anode. The electron emitters of the field emission gun have an emission capacity up to 1000 times greater than that of a tungsten filament used in ordinary SEM [146]. Like in SEM, here also secondary electrons are collected by a scintillator detector, which produces photons. Ultra-high vacuum (UHV) is used to minimize the chance of breakdown across the gap between anode and cathode. **Figure 2.10** shows the FESEM.



Figure 2.10: Picture of Tescan Lyra3 FESEM

2.3.4 Energy dispersive X-ray spectroscopy (EDAX) for elemental analysis

EDAX is a method used to find the elemental composition of a sample based on the interaction of X-rays with the atoms in the sample. Atoms consist of a nucleus (with positively charged protons and neutral neutrons) surrounded by electrons arranged in shells or orbitals: the innermost **K-shell**, followed by **L-shell** (with sub-shells L_I , L_{II} , L_{III}), then **M-shell** (with sub-shells M_I , M_{II} , M_{III} , M_{IV} , M_V), and so on. The K-shell can hold 2 electrons, the L-shell 8, and the M-shell 18. When the atom is irradiated by X-ray photons or electrons, an electron can be ejected from one of the inner shells, such as the K-shell, creating a vacancy or "hole." This makes the atom unstable, and it tries to restore stability by transferring an electron from a higher-energy outer shell (e.g., the L-shell) to fill the hole in the inner shell. Since the electron from the L-shell has higher energy than the vacant K-shell position, the excess energy is released in the form of characteristic X-rays. These emitted X-rays are used to identify and quantify the elements present in the sample based on their unique energy signatures. This process allows precise identification of elemental composition by detecting the characteristic X-rays emitted as electrons transition between atomic shells. (**Figure 2.11**) [147]. This is seen as a line in the spectrum. The emitted X-rays are characteristic of the element.

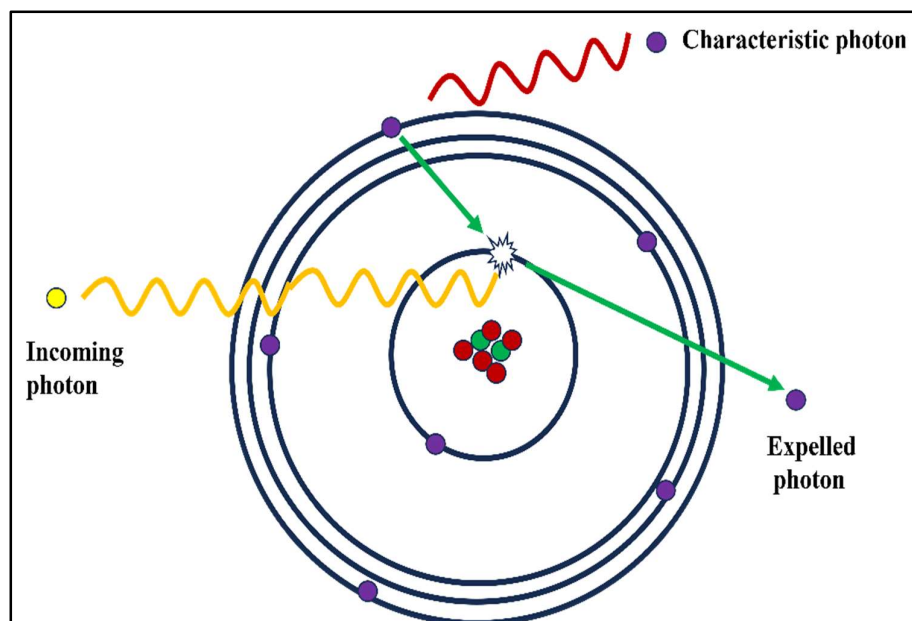


Figure 2.11: Irradiation of an atom

There are two main notations used to describe X-ray emission lines in elemental analysis: Siegbahn notation and IUPAC notation. The Siegbahn notation indicates an emission line by the element symbol followed by the shell with the initial electron vacancy and a Greek letter (α , β , γ , etc.) representing the relative intensity of the line. Fe- K_{α} means the strongest iron X-ray emission line caused by a vacancy in the K-shell. Its limitation is that it does not specify from which outer shell the electron transitions to fill the vacancy. The IUPAC notation gives more detailed information, indicating the element, the shell where the hole initially occurred, followed by the shell from which the electron transitions to fill the hole. Cr- K_{LIII} means chromium emission caused by an electron transitioning from the LIII shell to fill a vacancy in the K-shell. K-lines are more intense than L-lines. L-lines are more intense than M-lines. Not all transitions are allowed by quantum mechanics—for example, an electron transition from the L_I shell directly to the K-shell is forbidden. This notation system helps accurately describe and interpret X-ray spectra in elemental analysis. **Figure 2.12** gives an overview of the most important lines with their transitions.

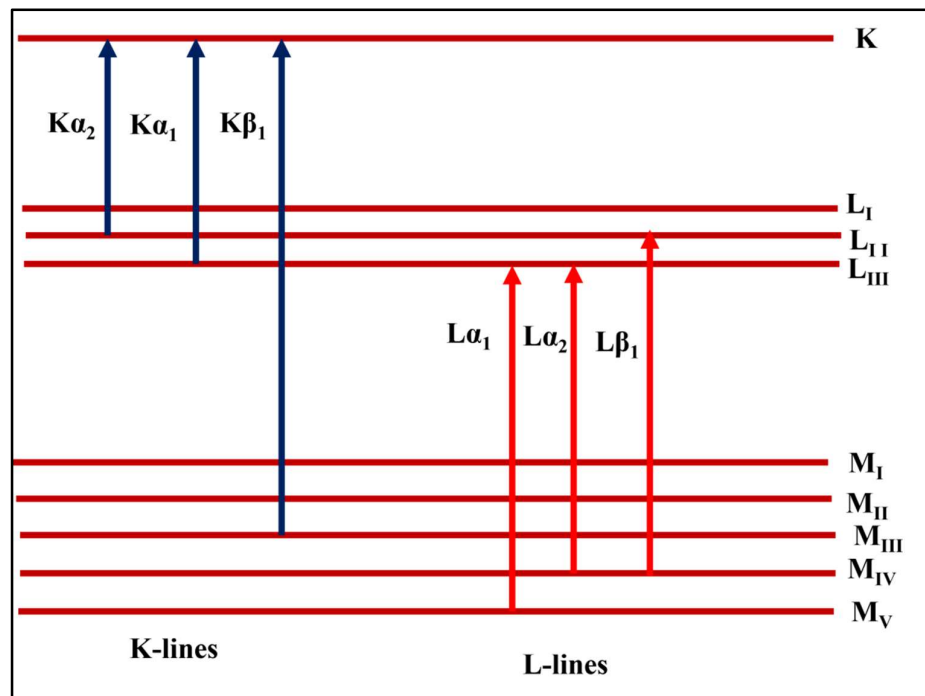


Figure 2.12: Overview of the most important lines with their transitions

Applications of EDAX

a) Elemental composition analysis:

It is widely used to determine the elemental composition of materials, including metals, ceramics, polymers, and biological samples.

b) Microanalysis:

EDAX allows for the analysis of the elemental composition of specific microscopic regions of a sample, providing spatially resolved compositional information.

c) Material characterization:

It is used to study the distribution of elements in complex materials, detect impurities, and analyze the effects of processing conditions on material composition.

d) Failure analysis:

It is employed to investigate the causes of material failures by identifying contaminant elements or compositional changes at failure sites.

2.3.5 Transmission electron microscopy (TEM) for morphological and crystallographic investigations

TEM is a highly powerful technique used to obtain detailed information about the microchemistry, crystal structure, and defect structures of crystalline materials at the microscopic scale. It allows researchers to observe fine structural details and analyse the composition of materials with very high resolution. TEM is a highly advanced imaging technique provide the morphological, topographic, compositional, and crystalline information of a variety of nanomaterials, including nanoparticles, nanorods, nanocubes, and also has the capability to determine the size of quantum dots (the smallest nanomaterials). Unlike SEM, TEM is based on the transmission process. TEM has limitless applications and is also useful in the biological field to determine the cellular function and structure of cells and tissue. When electrical potential is applied, the filament is heated, and consequently a stream of electrons is produced by an electron gun, it passes through the condenser lens, then the condenser aperture, it filters out the unwanted scattered electrons that strike the sample. Following the angle of incidence, the beam is partially transmitted or diffracted. The objective lens plays an important role in focusing the transmitted electrons from the specimen into an image. The objective aperture enhances the contrast of the image and eliminates the high-angle diffracted electrons. The transmitted beam passes through the projector lens, and the final image is obtained. The magnified image is obtained on a fluorescent screen by a CCD camera. TEM produces high-resolution black and white images in vacuum chambers during interaction with specimens and energetic electrons. The vacuum chamber pumps out the air and creates a space where electrons can move. High-resolution TEM (HRTEM) is capable of providing atomic-resolution lattice images. TEM is a highly powerful technique used to obtain detailed information about the microchemistry, crystal structure, and defect structures of crystalline materials at the microscopic scale. It allows researchers to observe fine structural details and analyze the composition of materials with very high resolution [148]. A conventional electron microscope operates in a vacuum of 10^{-5} torr or less.

Transmission Electron Microscopy (TEM) typically uses thermionic electron guns that accelerate electrons with a voltage between 40 and 200 kV. The condenser lenses located below the electron gun reduce (demagnify) the electron beam size and control its diameter (usually between 1 and 50 micrometers). This controls the intensity and

coherence of the electron beam passing through the specimen. This objective lens forms the first intermediate image and diffraction pattern of the sample. The objective aperture limits the angular range of scattered electrons, improving the contrast. The objective lens produces the initial image at about 50-100 times magnification. A series of intermediate and projector lenses further magnify the image by up to 20 times more. Total magnifications up to one million times are possible, allowing extremely detailed imaging at the atomic and nanoscale. This combination of lenses and apertures enables TEM to produce high-resolution images and diffraction patterns for microstructural analysis. **Figure 2.13** shows the schematic diagram of TEM.

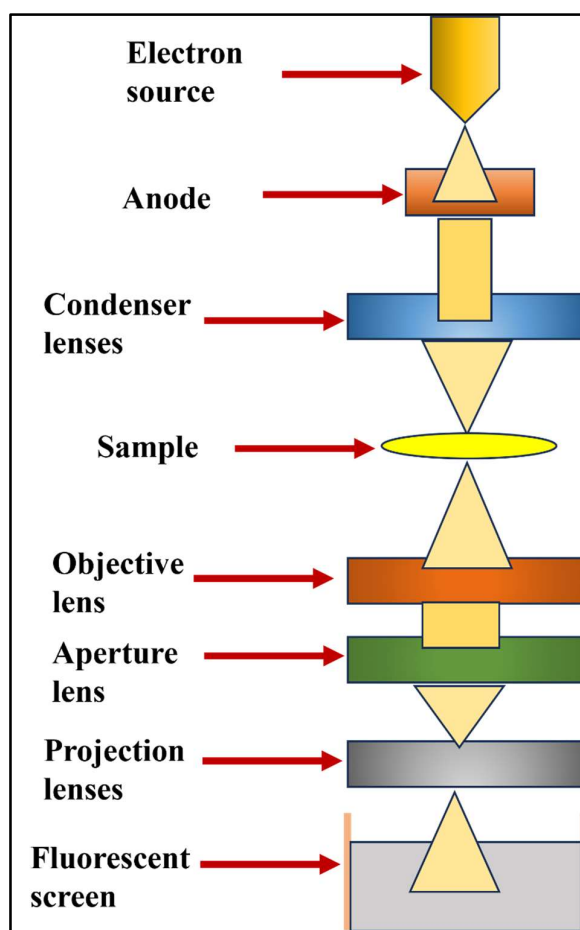


Figure 2.13: Schematic diagram of TEM

In the present research, a JEOL 2100 HRTEM (**Figure 2.14**) is used. The transmission

electron microscope (TEM) operates at a voltage of 200 kV and offers a user-friendly, simple operation with excellent expandability for various applications. It is equipped with a high-quality lanthanum hexaboride (LaB_6) electron gun, which balances excellent performance and reasonable cost. The TEM boasts an ultrahigh resolution of up to 0.19 nanometers, allowing atomic-level observations and analysis of materials. This makes it ideal for detailed structural studies at the atomic scale.



Figure 2.14: Picture of JEOL 2100 HRTEM

2.3.6 Raman spectroscopy

Raman spectroscopy is a technique that records the unique vibrational fingerprint of a material by analysing inelastically scattered light. It is based on the Raman Effect, discovered in 1928 by the Indian physicist Chandrasekhara Venkata Raman. It is a non-destructive method that studies how light interacts with matter by measuring inelastic scattering. It can probe the structures of gases, liquids, and solids, whether they are amorphous or crystalline. The sample is irradiated with a monochromatic photon beam, commonly a laser nowadays, to excite vibrations in the material. The scattered light contains information about the vibrational modes of the molecules, providing a detailed molecular fingerprint. This makes Raman spectroscopy a

powerful tool for material identification and structural analysis. Raman scattering occurs with changes in vibrational, rotational, and electronic energy in the molecules. This spectroscopy deals with a fraction of light scattering (nearly 1 in 10^7 photons) at a particular wavelength of incident light. In Raman spectroscopy, elastic scattering is known as Rayleigh scattering. If the specimen is transparent to the incident radiation, most of the radiation is transmitted, a small fraction is elastically (known as Rayleigh) scattered, and a very small fraction is inelastically (Raman) scattered. In the inelastic scattering event, the incident photons decrease or increase energy by the Stokes or anti-Stokes process due to excitation of phonons in the medium. The Stokes and anti-Stokes processes are key concepts in understanding Raman spectroscopy:

a) **Stokes Process**

If the molecule ends up in a higher vibrational energy state after interaction with the photon, the emitted photon loses some energy. This means the emitted photon has a lower frequency (or longer wavelength) compared to the incident light. This decrease in frequency is called the Stokes shift.

b) **Anti-Stokes Process**

If the molecule starts in an excited vibrational state and ends in a lower vibrational energy state, the emitted photon gains energy. Thus, the emitted photon has a higher frequency (or shorter wavelength) than the incident light. This increase in frequency is known as the anti-Stokes shift.

Both these shifts provide information about the vibrational modes of the material, critical for identifying molecular structures in Raman spectroscopy. The schematic representation of these processes is shown in **Figure 2.15**.

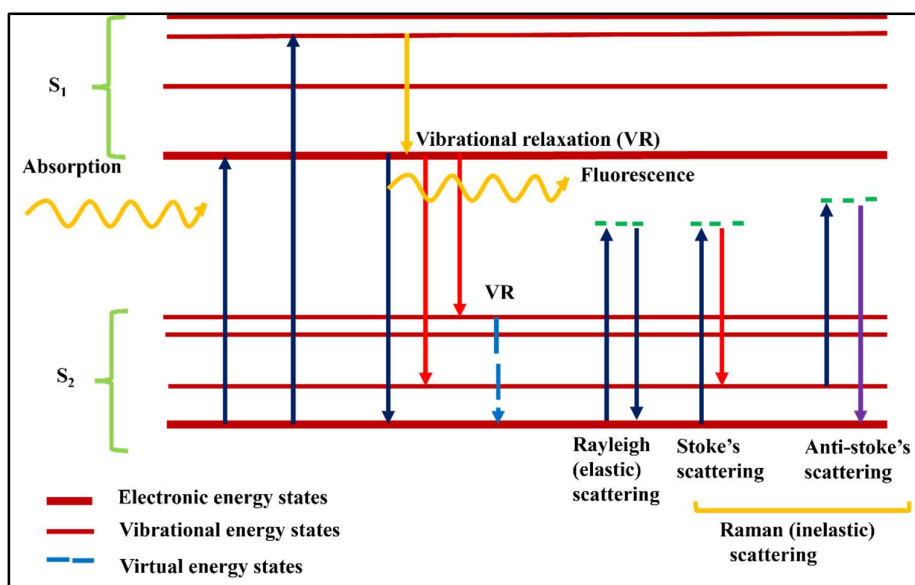


Figure 2.15: Schematic diagram of Raman lines



Figure 2.16: Picture of Witec Raman spectrometer

The energy difference between the wavelengths of incident and scattered photons is represented by the following equation and is defined in terms of Raman shift ($\bar{\nu}$) given in **equation 2.3**:

$$\bar{\nu} = \frac{1}{\lambda_{incident}} - \frac{1}{\lambda_{scattered}} \quad \dots\dots\dots 2.3$$

where $\lambda_{\text{incident}}$ is the wavelength of the incident light, $\lambda_{\text{scattered}}$ is the wavelength of scattered photons, respectively, and $\bar{\nu}$ is the Raman shift in wavenumber (cm^{-1}). Raman scattering intensity is primarily determined by the change in polarizability of the material during vibration. The intensity of inelastically scattered light is collected and plotted against wavenumber (reciprocal centimeters), which relates to the momentum of the scattered photons. Each peak in the Raman spectrum corresponds to one or more vibrational modes of the sample. The number of peaks is limited by the number of Raman-active modes allowed by the material's symmetry. Raman activity depends on the point group symmetry in molecules and the space group symmetry in crystalline solids. The Raman spectrum can reveal the strength of interatomic and intermolecular bonds, mechanical strain in solids, composition of multicomponent materials, and degree of crystallinity in solids. Raman spectroscopy is especially valuable for characterizing carbon-based materials [149, 150]. In the present research, a Witec Raman spectrometer (**Figure 2.16**) is used.

2.3.7 X-ray Photoelectron Spectroscopy (XPS)

XPS is an exceptional surface-sensitive electron emission spectroscopic method. It identifies the elemental composition, electronic structure, and chemical state of the material's surface based on the photoelectric effect [151]. The photoelectric effect was first observed experimentally in 1887 by Heinrich Hertz. However, it was not before 1905 that Elbert Einstein explained the photoelectric effect. For that, he was granted the Nobel Prize in 1921. In 1907, P.D. Innes recorded the first XPS spectrum, whereas R.G. Steinhardt Jr. was the first to acknowledge that the XPS spectra of materials' surfaces are enormously influenced by their physical and chemical nature [152]. In XPS, when a sample is illuminated by low-energy (~ 1.5 keV) X-rays, it causes the emission of electrons photoelectrons. A high-resolution electron spectrometer records the energy spectrum of the released photoelectrons. The high vacuum conditions are necessary to facilitate the transfer of photoelectrons to the analyzer and avoid sample contamination. The X-ray photon energy is sufficient to penetrate the sample's surface. Where a core electron absorbs and emits this X-ray

photon. Consequently, when an X-ray photon's energy surpasses the binding energy (E_b) of the material's core electron, the excess energy is transformed into the kinetic energy (E_k) of the photoelectron. The E_b of the core electron can be computed using the incident X-ray photon energy, the material's work function (Φ), and the E_k of the produced photoelectron, defined in **equation 2.4** as follows:

$$E_b = h\nu - E_k - \Phi \quad \dots\dots\dots 2.4$$

As a result, the incident X-ray beam that is directed toward a sample's particular area produces a broad spectrum of energies with various orientations. **Figure 2.17** illustrates the components of the XPS instrument. The XPS system contains an X-ray source to produce photoelectrons (X-rays of Al) and an electron spectrometer to measure electron momentum. The electron optics could consist of a series of magnetic and/or electrostatic lens units, which collect a portion of the emitted electrons indicated by the transferred rays *via* the apertures, which are then focused onto the analyzer entry slit. To ensure that only electrons with a particular energy (referred to as the Pass Energy PE) can pass through the detector slits and onto the detectors, electrostatic fields are set up within the hemispherical analyzer (HSA).

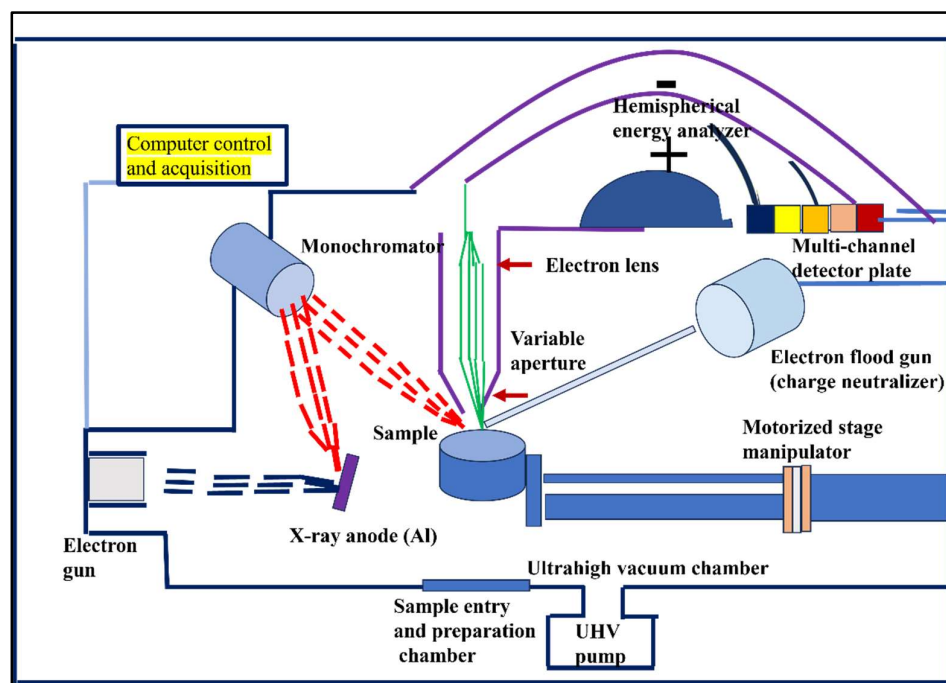


Figure 2.17: Components of the XPS instrument

In the present research, XPS (Thermo Fisher Scientific), model K-alpha (**Figure 2.18**), having a monochromatic radiation source Al K α (1486.7 eV), was used for the quantitative and qualitative analysis of the immunoelectrode.



Figure 2.18: Picture of Thermo Fisher Scientific X-ray photoelectron spectrometer

2.3.8 Fourier Transform Infrared Spectroscopy (FTIR) for identification of functional groups

In infrared (IR) spectroscopy, a specimen is irradiated by an IR laser beam. The IR beam is partially transmitted and partially absorbed by the specimen [153]. The produced spectrum depicts the molecular transmission and absorption, forming a molecular fingerprint of the specimen [154]. As two fingerprints never match, there are never two distinct molecular configurations (with unique compositions and accordingly different arrangements of atoms) that induce identical IR spectra.

Thus, an IR spectrum designates a material's identity, with absorption peaks corresponding to the vibration frequencies between atom bonds, enabling the recognition (qualitative analysis) of any material. Besides, the magnitude of a peak in the spectrum directly correlates with the amount of material present. FTIR spectrometry is the favoured technique of IR spectroscopy. It was invented to overcome the encountered limitations in dispersive instruments. The major issue was

the delayed scanning procedure. Therefore, a mechanism for recording all of the IR frequencies concurrently, rather than separately, was required. For that, a relatively simple optical apparatus known as an interferometer was built. The interferometer creates a distinctive signal that encodes all of the IR frequencies. Because the signal was produced in seconds, the duration per specimen was minimized. Most interferometers use a beam splitter, which creates two beams from the entering IR light. One beam that reflects off a fixed flat mirror. The other beam reflects off a moving flat mirror (moving merely a few millimetres back and forth). Because the path of one beam is fixed while the other is continually varying, the interferometer output signal is the consequence of interference of these two beams (**Figure 2.19**) and is referred to as the interferogram.

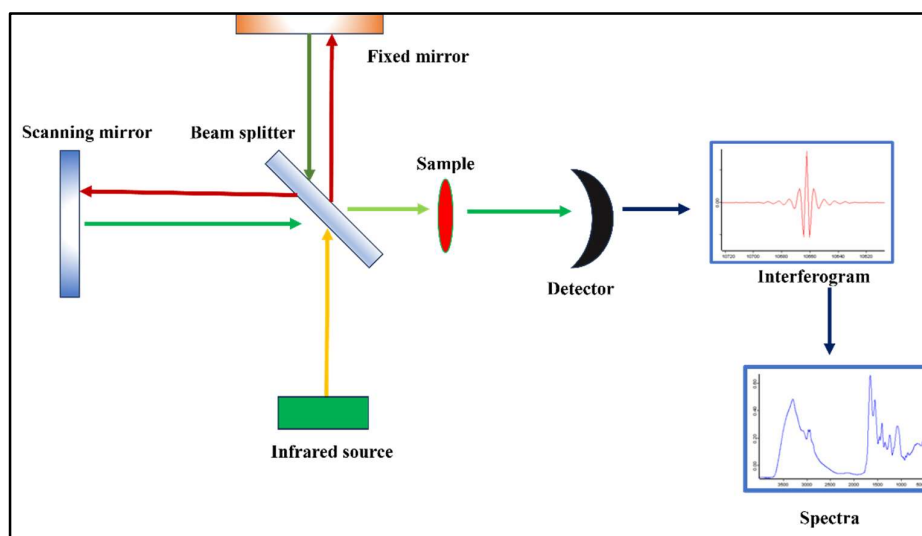


Figure 2.19: Schematic diagram of FTIR Instrument

Similar to electronic transitions, certain energies designate vibrational transitions where molecules absorb IR light exclusively at specific frequencies. Chemical bonds vibrate at particular frequencies, and when radiated by IR light, they absorb it at frequencies that correspond to their vibration modes. Measurement of radiation absorption frequency yields a spectrum that identifies compounds and functional groups. Some contaminants/impurities generate their own bands in the IR region. Spectral measurements of these bands are utilized to identify impurity concentrations and further bonding with the host material. Since the genuine requirement is a frequency spectrum (depicting intensity at every single frequency), the recorded interferogram signal cannot be directly elucidated. The various frequencies are

‘decoded’ using a recognized mathematical process, namely Fourier transformation (FT). A computer performs the transformation, and the resulting spectral information is acquired for analysis. In this research, an FTIR spectrometer (Frontier, Perkin-Elmer, USA) in potassium bromide (KBr) mode and attenuated total reflectance (ATR) mode is used as shown in **Figure 2.20** for surface adsorbed functional groups identification.

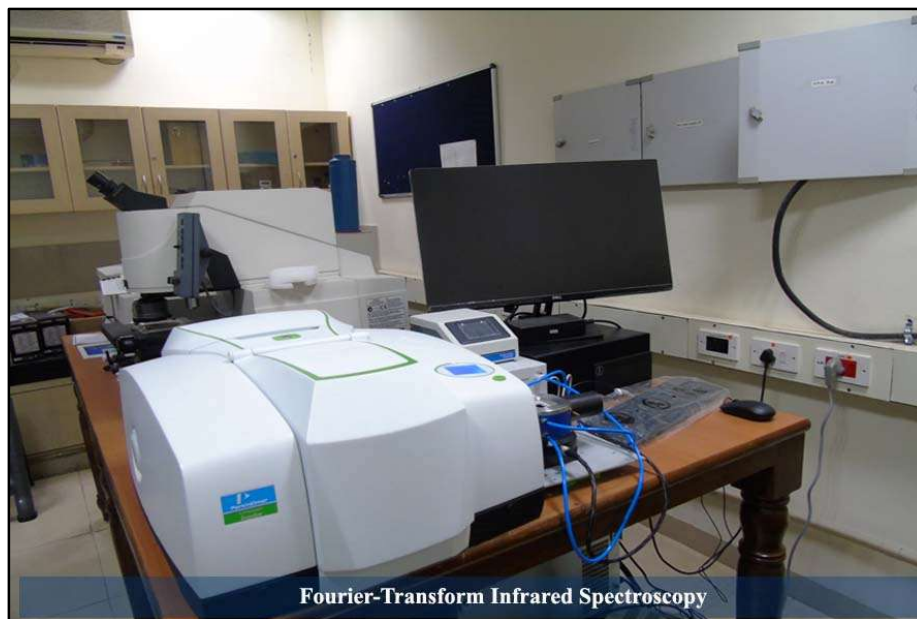


Figure 2.20: Picture of Perkin-Elmer FTIR Instrument

2.3.9 Ultraperformance liquid chromatography tandem mass spectrometer (UPLC-MS/MS)

The UPLC-MS/MS is the analytical technique used to separate, identify, and quantify complex mixtures and trace compounds. It combines the high-resolution separation power of UPLC with the precise molecular weight and structural detection capabilities of tandem mass spectrometry. In UPLC, first separation takes place; the liquid sample is pumped through a specialized chromatographic column at high pressure. This physically separates the individual chemical compounds based on how they interact with the column material before they exit. In the 1st MS scan, as the separated molecules enter the first mass spectrometer, they are ionized, and their precursor masses are measured. In fragmentation MS/MS, selected precursor ions are broken down into smaller, distinct "daughter" or product ions. In 2nd MS scan, these

fragments are measured in the second mass spectrometer, providing the chemical fingerprint for identification [155]. **Figure 2.21** shows the schematic diagram of UPLC-MS/MS. In the present research, Waters SYNAPT G2 with 2D nano ACQUITY System is used. The instrument has mobility of ion separation with very high efficiency, with hybrid quadrupole orthogonal acceleration. **Figure 2.22** shows the Waters SYNAPT G2 with 2D nano ACQUITY System.

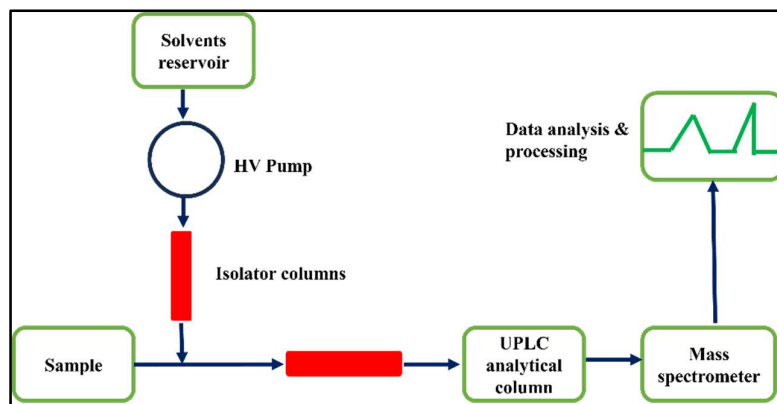


Figure 2.21: Schematic diagram of UPLC-MS/MS



Figure 2.22: Picture of Waters SYNAPT G2 with 2D nano ACQUITY System

2.4 Electrochemical (EC) Measurement techniques

EC is a technique based on the change in current, voltage, or impedance. The electrochemical techniques are very powerful and versatile analytical techniques that have various advantages like accuracy, high sensitivity, and precision. This technique is able to analyze a smaller number of biological samples such as blood, serum, urine, vitamins, and cancerous cells. Besides these, it is also explored in different phenomena like electrophoresis, electrochemical sensors or biosensors, battery and fuel cells, etc. These techniques improve the applicability of detection and determination limits of the samples. The electrochemical technique is used to study the kinetics of the electron transfer process in the system. It is a very sensitive technique and is employed for the detection of analytes in the electrolytic solution in an electrochemical cell. The response of the analyte is measured in terms of change in electrical current. This electrochemical technique recognizes the different concentrations of analyte at the electrode surface and leads to a change in current or voltage in the electrolyte solution. This technique is divided into various groups: potentiometry, voltammetry, coulometry, and impedance spectroscopy, etc. Among these, voltammetry and impedance-based techniques are often used in the detection of analytes with the modification of the electrode surface in the electrolyte solution.

The present work of the thesis is related to the study of analytes using three-electrode systems in the electrochemical cell. These electrodes are as follows: working electrode, reference electrode, and counter electrode. **Figure 2.23** shows the schematic diagram of the electrochemical cell. In this research, an Autolab galvanostat potentiostat (**Figure 2.24**) is used.

- a) Working electrode: Working electrodes provide a surface where chemical reactions take place in terms of current or voltage change. It is composed of an inert material (ITO glass or glassy electrode) where the redox reactions take place. The working electrodes act as an anode or cathode depending upon the redox reaction.
- b) Reference electrode: The reference electrode acts as a reference point against which the potential of another electrode in the electrochemical cell is measured. There are various reference electrodes used in electrochemical cells, such as

saturated calomel electrode (SCE), standard hydrogen electrode (SHE), and Ag/AgCl electrode.

- c) Counter electrode: The counter electrode helps in the flow of current at the working electrode when potential is applied to the electrode and oxidation or reduction of the analyte takes place. Generally, electron flow between the working electrode (WE) and counter electrode (CE) is recorded. CE also completes the electrical circuit. CE shows as much inertness as possible when a reduction is recorded at WE; in turn, an oxidation occurs at CE. A platinum (Pt) wire or disk carbon-based electrode is used as a counter electrode.

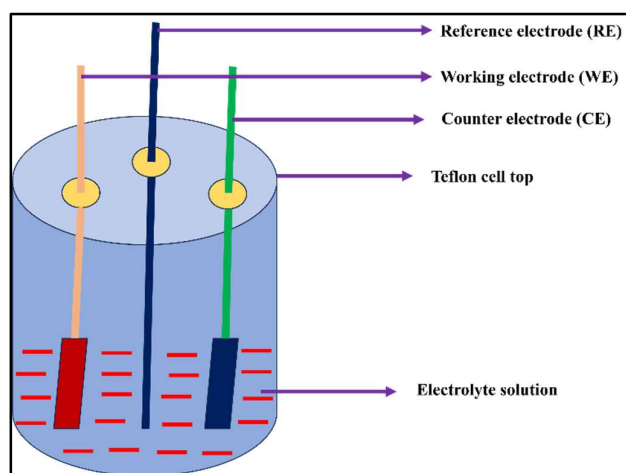


Figure 2.23: Schematic diagram of the electrochemical cell.



Figure 2.24 Picture of Autolab galvanostat potentiostat

2.4.1 Cyclic Voltammetry (CV)

CV is a very popular EC technique applied to evaluate molecular species' reduction and oxidation processes [156]. In CV, the current is measured by sweeping the potential back and forth (from positive to negative or vice versa) between a chosen potential window [157]. The CV data is used to analyze the EC behavior at the electrode-electrolyte interface. The CV plot 'voltammogram' includes the redox peaks, which are the material's reduction and oxidation peaks, inferring crucial information concerning the material and its features. The system reversibility, irreversibility, and quasi-reversibility nature can be determined using the cathodic and anodic peak currents ratio. Further, CV assists in finding the number of transferred electrons, the rate constant of the reaction, and the rate-limiting factor of the reaction rate. CV consists of two types of voltage sweep profiles: forward and reverse or backward. In the forward sweep, Fe^{2+} ions in the ferry/ferrocyanide buffer are oxidized into Fe^{3+} ions, while in the backward sweep, Fe^{3+} ions are reduced into Fe^{2+} ions. This process results in the anodic current (I_{pa}) in the forward scan and cathodic current (I_{pc}) in the backward scan. Thus, these oxidation and reduction processes show an equilibrium profile of cyclic voltammetry. CV is a reversible process and provides several characteristics such as: a) voltage separation between the current peak in forward and reverse mode, b) the current peak is directly proportional to scan rate, c) the peak voltage position does not change as a function of voltage scan rate, and d) the ratio of two peak currents, I_{pa} and I_{pc} , is nearly equal to one. **Figure 2.25** shows the schematic diagram of cyclic voltammetry: (a) applied ramp potential and (b) a typical voltammogram. In the CV, a variable voltage is applied to the working electrode (WE) relative to the reference electrode (like Ag/AgCl), and electron flow between the counter electrode and working electrode results in the electrode reaction. The range of the applied voltage can be from 10 mV/s to 100 mV/s. With the increase in potential of the solution, the current is proportionally increasing. The CV measures various electrokinetic parameters at the electrode/electrolyte interface. The electrokinetic parameters are diffusion constant (D_f), effective electroactive area (A_e), and surface concentration of redox species (I^*).

The surface concentration of the bioelectrode was determined by the Brown-Anson

model equation (2.6)

$$I_p = \frac{n^2 F^2 I^* A V}{4R} \quad \dots\dots\dots (2.6)$$

Where F is the Faraday constant ($96,485 \text{ C mol}^{-1}$), R stands for the gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), T stands for temperature (300 K), A is the area of the electrode, I^* is the surface concentration of the electroactive species (mol cm^{-2}), and V is the scan rate (V s^{-1}).

The diffusivity of the bioelectrode is calculated by **equation (2.7)** (the Randles-Sevcik equation), which will be used to find the diffusivity (D) of the bioelectrode.

$$I_p = (2.69 \times 10^5) C n^{3/2} D^{1/2} \nu^{1/2} A \quad \dots\dots\dots (2.7)$$

where I_p is the peak current of the electrode, C is the electrolyte concentration, n is the number of electrons transferred, A is the effective surface area of the working electrode, and ν is the scan rate (mV s^{-1}).

The effective surface area is crucial for understanding the density of available binding sites for antigen interaction, and is defined in **equation 2.8**.

$$Ae = \frac{S}{(2.69 \times 10^5) n^3 C D^{1/2}} \quad \dots\dots\dots (2.8)$$

Where S denotes the slope of the line as determined by plotting I_{pa} versus scan rate linearly.

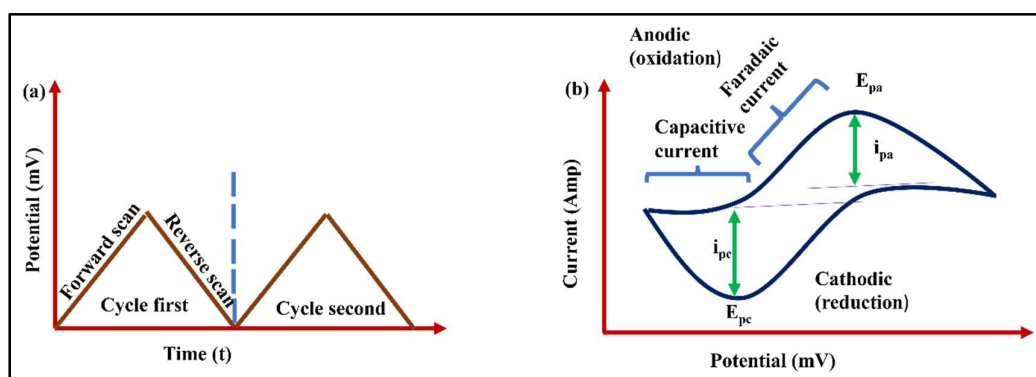


Figure 2.25: Cyclic voltammetry (a) applied ramp potential and (b) a typical voltammogram

2.4.2 Differential Pulse Voltammetry (DPV)

DPV is the most sensitive and selective method allowing analysis at the parts per billion (ppb) level. While CV is effective for qualitative determination, DPV is practical for quantitative determination. DPV is a pulse technique that is designed to eliminate the background charging current. In DPV, the excitation waveform is generally a staircase. A constant pulse amplitude superimposed on the linear potential ramp is applied to the working electrode at a time just before the end of the drop. The current is measured twice for each pulse; one just starts the pulse and another ending of the pulse. This technique measures the current in the electrochemical cell at the electrode/electrolyte interface. In the typical DPV measurement, the curve is plotted against ΔI (current) vs V (potential) [158]. **Figure 2.26** shows the (a) formation of staircase potential for DPV and (b) a typical DPV plot.

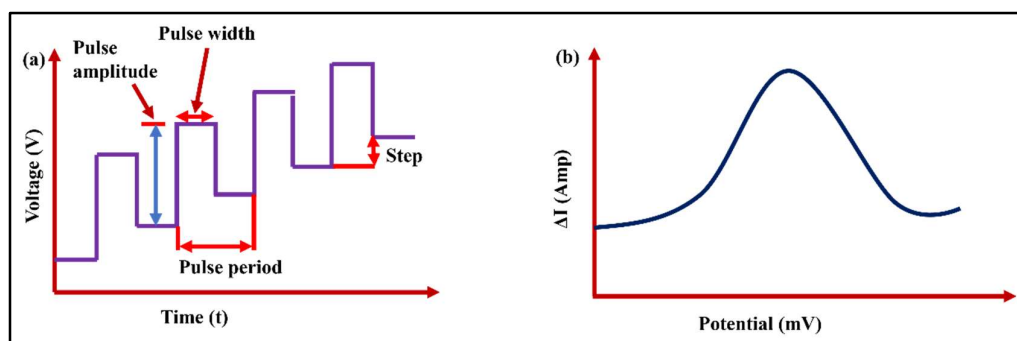


Figure 2.26: (a) Formation of staircase potential for DPV and (b) a typical DPV plot.

2.4.3 Electrochemical Impedance Spectroscopy (EIS)

EIS is a technique for investigating an electrode's interfacial characteristics in an electrolyte solution. Using this technique, the relation between the charge transfer resistance (RCT) and electrode kinetics can be studied [159]. In EIS measurement, an alternating current (AC) potential is supplied to the EC cell and the current through the cell is monitored. Accordingly, the EC reaction can be depicted using an analogous electrical circuit (a Randles circuit) (**Figure 2.27a**), which shows the electrolyte resistance (R_S), RCT, charge double layer capacitance (Cdl), and Warburg impedance (ZW) [160]. Where Cdl corresponds to the formation of an electrical

double layer of charged species and aligned dipoles at the electrode-solution interface. The charge double layer of the solution side is expected to be formed of several layers. The flow of current at the electrode and electrolyte interface is governed by the following **relation (2.9)**:



Where O is the oxidant, n is the number of transferred electrons, and R is the reductant. The plot resulting from EIS measurement (the Nyquist plot) is formed between real (Z') and imaginary (Z'') values of impedance (**Figure 2.27b**). Whereas the diameter of the Nyquist plot's semicircle indicates the amount of charge transfer resistance (R_{CT}). The sum of Z' and Z'' represents the frequency-dependent impedance $Z(\omega)$ of the Randles circuit, which originates from the resistance and capacitance of the EC cell (**equation 2.10**).

$$Z(\omega) = Z'(\omega) + i Z''(\omega) \quad \dots\dots\dots 2.10$$

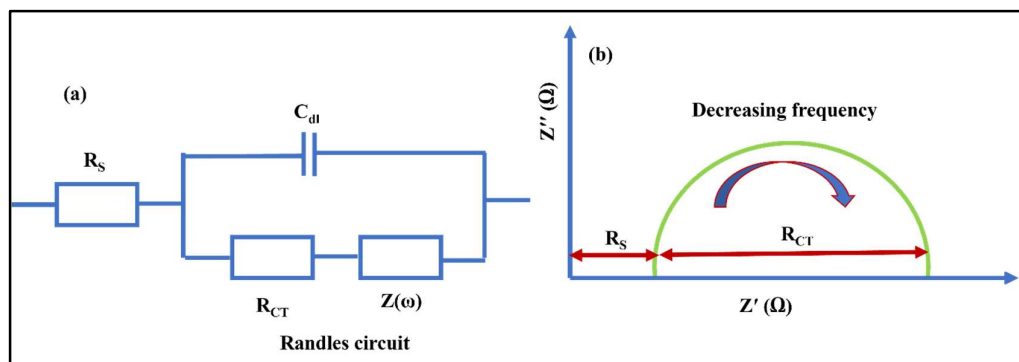


Figure 2.27: (a) Randles circuit and (b) Semicircle in Nyquist plot

Chapter 3

Graphene oxide/Mn₃O₄ nanocomposites for advanced electrochemical biosensor for *fumonisin B1* detection

The mycotoxins are a threat to food samples due to their high toxicity and carcinogenic behavior. Herein, the nanocomposite of trimanganese tetraoxide (Mn₃O₄) nanoparticles and graphene oxide (GO) was used to increase the electrochemical performance for detection of *fumonisin B1* (FBI). A thin film of GOMn₃O₄ nanocomposite was prepared on an indium tin oxide (ITO) substrate, and antibodies (ab-FBI) were immobilized onto the electrode using the electrophoretic deposition technique. The differential pulse voltammetry technique was used to measure the sensing performance. The non-binding sites of ab-FBI on the immunoelectrode were blocked with bovine serum albumin (BSA). The sensitivity of the biosensor was obtained as 10.08 $\mu\text{A mL ng}^{-1} \text{cm}^{-2}$ in the detection range of 1 pg mL^{-1} to 800 ng mL^{-1} , with a limit of detection (LOD) of 0.195 pg mL^{-1} . In addition, the recovery of the biosensor was found to be 98.91%.

This work is published as: Sandeep Sarpal, Avinash Kumar Singh, Hema Bhardwaj, Nitin Kumar Puri and Pratima R Solanki. "Graphene oxide-Mn₃O₄ nanocomposites for advanced electrochemical biosensor for fumonisinB1 detection." Nanotechnology (IOP) 34 (2023): 465708.

3.1 Introduction

The worldwide presence of mycotoxins in food represents a significant issue in food safety, as these substances are recognized as critical toxic agents that pose risks to both human and animal wellbeing. These naturally occurring poisonous organic substances emerge from *fungi* secondary metabolism—particularly from genera including *Aspergillus*, *Penicillium*, *Fusarium*, and *Claviceps*—where they accumulate in food and feed commodities [161,162]. Every year, approximately 25 % of global agricultural yields are impacted by mycotoxin contamination, resulting in substantial financial repercussions and contributing to various health conditions—both short-term and long-term—across populations worldwide. Within the spectrum of existing mycotoxins, *Fumonisin B1* (*FBI*) stands out as a particularly significant mycotoxin, accounting for around 70% of all *fumonisin*s. This compound is naturally synthesized by the *fungi* species *Fusarium verticillioides* and *Fusarium proliferatum*, and it commonly appears in numerous food and feed applications, including but not limited to peanuts, corn-derived products, and grain-based items [163,164]. *FBI* has been officially classified as a carcinogenic substance by the International Agency for Research on Cancer (IARC), which has categorized it within Group 2B—the classification for possible human carcinogens. The harmful effects of *FBI* exposure lead to multiple adverse health outcomes, including neurological damage, pulmonary and renal dysfunction, among others [165]. Thus, the research on the detection of *FBI* has become very important. There are several analytical techniques available for detecting *FBI* mycotoxin. Mainly, these techniques belong to conventional tools such as gas Chromatography-Mass Spectrometry (GCMS) [165], enzyme-linked immunosorbent assay (ELISA) [166], high-performance liquid chromatography (HPLC) [167], liquid chromatography-mass spectrometry (LCMS) [168], and polymerase chain reaction (PCR) [169], etc. These techniques are sensitive, specific, and selective toward their analytes. However, these techniques have several disadvantages, such as being sophisticated, requiring more samples for detection, very tedious processing, and, more importantly, requiring a well-trained person to operate the system. To overcome the aforementioned difficulties, an electrochemical biosensor is a very promising tool for detecting analytes at very low concentrations

and has several advantages over conventional techniques. Nanomaterials have been of great interest in the development of sensors or biosensors. Among a variety of nanomaterials, graphene-based nanomaterials have emerged as the most advanced materials for sensing applications due to their enhanced signal response. Graphene-based nanomaterials provide a large surface area and excellent biocompatibility with a wide range of biomolecules such as proteins, cells, DNA, enzymes, and antibodies [169]. Graphene also has high electrical conductivity, high electron transfer rates, and the ability to immobilize various molecules [170]. Graphene-based biosensors also offer extremely high sensitivity, stability, and fast response times, leading to advances in healthcare and diagnostics.

Manganese-based materials have unique redox properties that give them significant importance in applications related to energy, environment, and biomedical fields [171]. Trimanganese tetraoxide (Mn_3O_4) nanomaterial is also known as an oxidizing and reducing agent, and it is a spinel-structured stable oxide with unique catalytic, electrochemical, and magnetic properties. It is a p-type semiconductor having a larger bandgap of 2.3 eV, which provides supercapacitor properties to it [172]. It has several physical properties like thermodynamic stability, high electrochemical conductivity, chemical stability, paramagnetic behavior, etc [173,174]. Mn_3O_4 is a unique phase of manganese oxide nanomaterial which is explored in various applications such as excellent supercapacitors, high-density magnetic storage devices, high charge carrier mobility, good biocompatibility, etc. [174,175]. Moreover, Mn_3O_4 nanomaterial exhibits distinguished properties like environmental compatibility and cost-effectiveness. However, the Mn_3O_4 phase of manganese oxide is biologically less explored. Graphene Oxide (GO) sheets have more functional groups on their basal plane and edges, resulting in the enhancement of the loading amount of Mn_3O_4 , surface area, and porosity [176]. GOMn_3O_4 nanocomposite exhibits higher energy density and specific capacitance as compared to GO, while having larger specific capacitance and power density as compared to Mn_3O_4 . Their results of cell viability analysis showed significant cell death under laser irradiation. The elemental composition of GOMn_3O_4 nanocomposites showed intense signals, which showed that 'Mn' was the major element showing optical absorption [177]. Furthermore, Chen et al. developed nanocomposites of reduced graphene oxide wrapped Mn_3O_4 as anode

materials for high-performance lithium-ion batteries, which showed the specific capacity of 795.5 mAh g⁻¹ after 200 cycles. The nanocomposites showed excellent cyclic performance and good electrochemical activity [178].

Several research groups have reported various methods for detecting mycotoxins. For instance, Ahmed et al recently developed an electrochemical biosensor for detecting the naturally occurring histamine based on the molecularly imprinted polymer using the gold inverse opal electrode and the detection limit of 1.07 µM [179]. Dhiman et al developed an Ag-CeO₂ nanocomposite-based electrochemical biosensor for *FBI* detection [180]. Furthermore, Singh et al developed a molecularly imprinted polymer-based electrochemical sensing tool for *FBI* detection [163]. Alsulami et al developed a homogeneous luminescence immunoassay for *FBI* detection [181]. Zhang et al developed graphene and gold nanocomposites based on label-free DNA for the detection of *FBI* [182]. In recent years, Mao et al developed GO-CdS heterojunctions based on an ultrasensitive molecularly imprinted photoelectrochemical sensing platform for detecting *FBI* [183]. Wu et al developed the fluorescence resonance energy transfer (FRET) system for the detection of *FBI* [184]. Jodra et al developed the electrochemical magneto-immunosensor involving magnetic beads for the detection of *fumonisin* [185]. For the detection of *FBI*, Lu et al created a competitive fluorescence enzyme-linked immunosorbent test based on the quenching of CdTe quantum dots [186]. Yang et al created an electrochemical immunosensor using single-walled carbon nanotubes and chitosan to detect *FBI* in corn samples [187]. Chen et al created a colloidal gold-based lateral flow immunoassay using modified gold nanobeads to detect *FBI* [188]. Furthermore, Qu et al created a magnetic beads-assisted fluorescence aptasensing method to detect *FBI* [189]. Table 1 shows comparison data between current work and previously reported results for *FBI* detection.

Herein, a simple and sensitive electrochemical immunosensor was fabricated for the detection of *FBI* mycotoxins using a GOMn₃O₄ nanocomposite. This nanocomposite was synthesized at room temperature, which is robust and cost-effective. The phase and crystallinity of the synthesized GOMn₃O₄ nanocomposite were observed by XRD and Raman spectroscopy. The immunosensor was fabricated onto the ITO glass substrate using a GOMn₃O₄ film by an electrophoretic deposition method, which was

used for the immobilization of antibodies (ab-*FBI*). The non-specific binding sites of antibodies were blocked by BSA protein. The fabricated immunosensor was BSA/ab-*FBI*/GOMn₃O₄/ITO, which showed a wide detection range for the detection of *FBI*. Here, results suggest that the newly designed immunosensor is robust, cost-effective, sensitive, and selective towards *FBI*. A better and remarkable increase in the biosensing response for *FBI* detection happens due to the use of GOMn₃O₄ nanocomposite in comparison to other surface matrices summarized in **Table 3.1**.

Table 3.1 illustrates the comparison of the present work with other reported methods for the detection of *FBI*

Method	Procedure	Detection Range (ng mL ⁻¹)	Sensitivity (μA mL ng ⁻¹ cm ⁻²)	LOD	Ref.
HIS/MIP/Au/IO	CV	50nM-500 μM	-	1.07 nM	[179]
CeO ₂ -Ag/ab <i>FBI</i> /ITO	CV	0.01 -100	7.33	1.5 pg mL ⁻¹	[180]
<i>FBI</i> /MIP	CV	0.001 -500	9.09	0.32 pg mL ⁻¹	[163]
<i>FBI</i> /FnanoBIT assay	HPLC	0.533-6.81	-	0.079 ng mL ⁻¹	[181]
<i>FBI</i> /GO-Au	CV	0.01 – 0.01	-	0.01 ng mL ⁻¹	[182]
<i>FBI</i> /GO-CdS	CV	0.01 – 1000	-	4.7 pg mL ⁻¹	[183]
AuNPs-MB-UCNPs	FRET	0.01 -100	-	0.01 ng mL ⁻¹	[184]
<i>FBI</i> -HRP/ ab <i>FBI</i> / MB and protein G/ CSPE	DPV	0.73 -11.2	-	0.33 ng mL ⁻¹	[185]
FELISA/CAT/QDs	ELISA	0.39-12.50	-	0.22 ng mL ⁻¹	[186]
GCE/SWCNTs/CS	EIS	0.01-1000	-	2 pg mL ⁻¹	[187]
GNB/LFIA	TOF-MS	3.9-125	-	1.76 ng mL ⁻¹	[188]
MBs/DNA	Fluorescence	0.1-40	-	0.061 ng mL ⁻¹	[189]
BSA/ab- <i>FBI</i> / GOMn ₃ O ₄ / ITO	CV	0.001- 800	10.08	0.195 pg mL ⁻¹	This work

HIS: Histamine; IO: Inverse opal electrode; ab*FBI*: Antibody FBI; MIP: Molecularly imprinted polymer; FnanoBIT assay: Ab-*fumonisin* antibody nano luminescent binary technology; GO-CdS: Graphene oxide– cadmium sulfide; ECL-ELISA: Enhanced chemiluminescent/Enzyme-linked immunosorbent assay; AuNPs-MB-UCNPs: Gold nanoparticles-methylene blue-upconversion nano particles; PPy/ErGO-SPE: Polypyrrole/electrochemically reduced graphene oxide-screen printed electrode; HRP: Horseradish peroxidase; Ab-FBI/MB: Antibody-FBI/methylene blue; PG/CSPE: Protein guanine/carbon screen-printed electrode; BSA: Bovine serum albumin; FELISA: Fluorescence enzyme-linked immunosorbent assay; CAT: Catalase; QDs: Mercaptopropionic acid-modified CdTe quantum dots; GCE: Glass carbon electrode; SWCNTs: Single-walled carbon nanotubes; CS: Chitosan; GNB: Gold nanobeads; LFIA: Lateral flow immunoassay; TOF-MS: Time-off-flight mass spectrometry; MBs: Magnetic beads

3.2 Experimental Section

3.2.1 Material used

Graphite powder (20-micron), hydrochloric acid (HCl), orthophosphoric acid (H_3PO_4), potassium permanganate (KMnO_4), sulfuric acid (H_2SO_4), *fumonisin B1*-specific antibody (ab-*FBI*), *ochratoxin A* (*OTA*), *deoxynivalenol* (*DON*), cellulose, fructose, starch, *fumonisin B1* (*FBI*), manganese (II) acetate tetrahydrate ($(\text{CH}_3\text{COO})_2\text{Mn} \cdot 4\text{H}_2\text{O}$), sodium hydroxide (NaOH) was purchased from M/S Sigma Aldrich. The glass substrate coated with indium tin oxide (ITO) was used with a sheet resistance of $10 \Omega \text{ sq}^{-1}$ having a thickness of 1.1 mm. Sodium phosphate monobasic anhydrous (NaH_2PO_4) and sodium phosphate dibasic dehydrate (Na_2HPO_4), N-ethyl-N-(3-dimethylamino propyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), sodium chloride, potassium chloride, and bovine serum albumin (BSA) were purchased from SRL, India. Potassium ferricyanide [$\text{K}_3(\text{Fe}(\text{CN})_6)$] and potassium ferrocyanide [$\text{K}_4(\text{Fe}(\text{CN})_6)$] were obtained from Fischer Scientific. Deionized water with a resistivity of $18.2 \text{ M}\Omega \cdot \text{cm}$ was purified by a Millipore water purification assembly and used for the preparation of buffer and all other required solutions. Phosphate buffered solution (PBS, 0.2 M) was prepared containing 0.9% NaCl and 5 mM of redox probe $[\text{Fe}(\text{CN})_6]^{3-/4-}$ with pH 7.0

3.2.2 Synthesis of Graphene Oxide (GO)

GO was synthesized by a modified Hummer's method using pure graphite powder (20 μm) [190]. In the typical procedure, 240 mL of H_2SO_4 and 27 mL of H_3PO_4 were mixed in a beaker under continuous magnetic stirring for 30 minutes. Then, 12 gram of KMnO_4 was added very slowly in small steps. The mixture was stirred using a magnetic stirrer for 15 hours until the solution became dark green. After this, 2 mL of H_2O_2 was slowly added dropwise and stirred for 30 minutes. The ice was placed outside to prevent heating due to the occurrence of an exothermic reaction. Then, it was allowed to cool down. After this, the mixture was washed several times with 70 % distilled water and 30 % HCl, and centrifuged using a Remi Make Eppendorf centrifuge at 400 rpm for 10 minutes. Then the supernatant was decanted away and

was rewashed again and again until the pH reached 7. The washed GO solution was dried using a vacuum oven at 60 °C for 24 hours, and powder of GO was collected.

3.2.3 Synthesis of Mn₃O₄ nanoparticles

The manganese oxide nanoparticles (Mn₃O₄ nps) were synthesized using the co-precipitation method [191]. In the typical synthesis, 0.2 M of ((CH₃COO)₂Mn.4H₂O) and 0.5 M of NaOH were used as precursors. NaOH was added dropwise to manganese acetate using constant magnetic stirring at 800 rpm at room temperature until the pH of the solution reached ~ 12. Then the precipitate was filtered out and washed many times using distilled water until the pH of the solution reached 7. The obtained precipitate was then dried at room temperature using the vacuum oven.

3.2.4 Synthesis of GO/Mn₃O₄ nanocomposite

The GOMn₃O₄ nanocomposite was synthesized using the one-step *in-situ* preparation process [192]. GOMn₃O₄ nanocomposite was prepared in the presence of GO. In the typical synthesis, 200 mg of GO was mixed in 100 mL of distilled water at room temperature and sonicated. Then, dispersed GO was added slowly to the solution containing 0.2 M manganese acetate and 0.5 M reducing agent (NaOH) until the completion of the reaction. The obtained pH of the solution reached ~ 12. Then the precipitate was filtered out and washed several times until the pH reached 7. The obtained precipitate was then dried at room temperature in an oven and collected in a vial as GOMn₃O₄ nanocomposite.

3.3 Characterization Techniques

DC power supply of 60 V was used to fabricate a thin film of nanocomposite on the ITO glass substrate. The crystallinity of the GOMn₃O₄ nanocomposite was investigated using an X-ray diffraction (XRD) spectrometer (PANalytical Model X'pert Pro with Cu K_α radiation at $\lambda = 1.5406$ Å). The working voltage was 45 kV, and the current was 40 mA. The XRD patterns were recorded in the range of 10°–80° with a step size of 0.033° and a scan rate of 2° per minute. The Raman spectra were captured using the Alfa 300 Raman spectrometer with a 532 nm laser. A JEOL 2100 F high-resolution transmission electron microscopy (HR-TEM) with a

200 KV accelerating potential was used to capture images. The Carl-Zeiss EVO40 was used to study the energy-dispersive X-ray (EDX) spectra of the nanocomposite. Nova nano FE-SEM-450 was used to capture the Field-emission scanning electron microscopy (FE-SEM) images of the graphene and the nanocomposite. Fourier transform infrared (FTIR, UATR TWO) spectroscopy from Perkin-Elmer was used for the investigation of the vibrational spectra of graphene and the nanocomposite. The electrochemical analyzer (Autolab-AUT-Metrohm) was used to investigate the electrochemical characteristics (AUT86014) in a three-electrode system composed of ITO as a working electrode, Platinum as a counter, and Ag/AgCl as a reference electrode in PBS containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

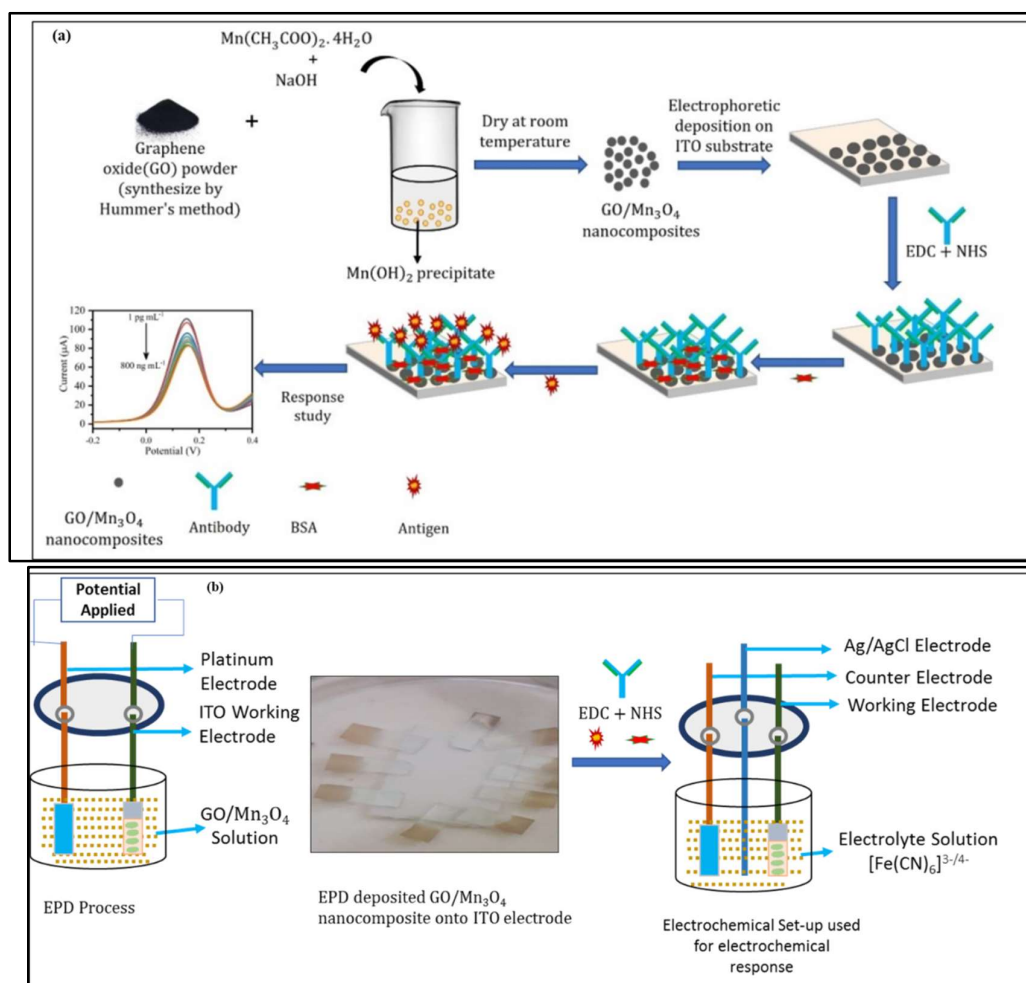
3.4 Thin film fabrication of $\text{Mn}_3\text{O}_4/\text{GO}/\text{ITO}$ immunoelectrode using electrophoretic deposition Technique

Glass surfaces coated with an ITO electrode were first hydrolyzed using the mixture of $\text{H}_2\text{O}:\text{NH}_3:\text{H}_2\text{O}_2$ in the ratio of 5:1:1 to enhance the hydrophilic nature of ITO-coated glass and kept in the oven for about 1 hour at 80°C . Then the ITO-coated glass substrates were washed with a mixture of ultra-pure water and ethanol and dried at 60°C for about 1 hour. These hydrolyzed ITO-coated glass substrates were further used for the fabrication of an immunosensor for *FBI* detection. For this, the synthesized GOMn_3O_4 nanocomposite was deposited onto the ITO-coated electrode using the electrophoretic deposition (EPD) technique. The EPD method constitutes an economical, eco-friendly, low-energy-consuming alternative to the high-energy-consuming evaporative techniques [193]. To obtain a uniform film of GOMn_3O_4 onto the ITO surface, 5 mL of GOMn_3O_4 solution was mixed with 3 mL of acetonitrile solution, then deposited onto the ITO surface by applying an optimized D.C. 60 volt for 5 minutes to obtain smooth and uniform thin films.

3.5 The fabrication process of Immunoelectrode

A solution of 5% ab-*FBI* (in sodium phosphate buffer), EDC (0.4 M), and NHS (0.1 M) was mixed in a 2:1:1 ratio and placed at 4°C for 1-hour. For the immobilization process, 20 mL of a solution of activated EDC-NHS was evenly dropped onto the surface of the $\text{GOMn}_3\text{O}_4/\text{ITO}$ electrode and placed at room temperature in a humidity chamber for 6

hours. The positively charged GOMn_3O_4 nanocomposite and the carbonyl group of *ab-FBI* create an electrostatic interaction that leads to the formation of metal ester bonds. PBS was used to clean the *ab-FBI*/ GOMn_3O_4 /ITO bioelectrode surface of unbound *ab-FBI* (pH 7.0). *Ab-FBI*/ GOMn_3O_4 /ITO bioelectrode non-specific sites were blocked with BSA (10 mL), and incubation was initiated at room temperature for 4 hours in a humidifying chamber. PBS with a pH of 7.0 was used to rinse the BSA/*ab-FBI*/ GOMn_3O_4 /ITO immunoelectrode, which was then kept at 4°C for later examination. The procedure for preparing the BSA/*ab-FBI*/ GOMn_3O_4 /ITO immunoelectrode is described in **Scheme 3.1**.



Scheme 3.1: (a) Schematic of synthesis of GOMn_3O_4 nanocomposite and fabrication of immunoelectrode on the ITO electrode surface for *FBI* detection; (b) illustrates the EPD set-up used for the electrophoretic deposition of GOMn_3O_4 nanocomposite onto the ITO electrode surface under the applied voltage for a period of time, followed by surface activation, immobilization and detection of *FBI* using the electrochemical set-up of three electrode system comprised of working, counter and reference electrode

3.6 Results and discussions

3.6.1 Structural analysis of GOMn₃O₄ nanocomposite

Figure 3.1 (a) shows the XRD of synthesized GO sheets. The characteristic intense peak appeared at 10.67° corresponding to the (001) plane, and data were recorded between the 5° and 80° range at a 2θ angle. The interlayer spacing of GO was calculated using Bragg's law (**equation 3.1**):

$$n\lambda = 2d \sin\theta \quad \dots\dots\dots [3.1]$$

Where n is the order of diffraction, λ is the wavelength of the X-ray, d is the interlayer spacing, and θ is the diffraction peak. Using the above equation, the interplanar distance between the graphene sheets was found to be 0.828 nm [194]. **Figure 3.1(b)** shows the XRD data of Mn₃O₄ nanoparticles, and **Figure 3.1(c)** shows the XRD data of GOMn₃O₄ nanocomposites. It was observed that the XRD pattern of Mn₃O₄ consists of several diffraction peaks at 13.82°, 29.13°, 31.32°, 32.60°, 36.33°, 44.69°, 51.01°, 58.76°, 60.13°, and 64.88°, which were corresponding to the lattice planes (101), (112), (103), (211), (004), (220), (105), (321), (224) and (400), respectively. Tetragonal-phase Mn₃O₄ nanoparticles matched well with the reported JCPDS number 80-0382 [195-198]. The average crystallite size (D) was calculated to be 20.92 nm using the Scherrer equation, described below (**equation 3.2**):

$$D = \frac{K\lambda}{\beta \cos(\theta)} \quad \dots\dots\dots [3.2]$$

Where K = crystallite shape factor = 0.9, wavelength (λ) of X-rays = 1.5406 Å, β = full width at half maximum (FWHM) of the diffraction peaks, and θ = Bragg's diffraction angle. The XRD peaks of Mn₃O₄ matched well with the XRD peaks of the GOMn₃O₄ nanocomposite. All the same XRD peaks appeared in **Figure 3.1(c)**, attributed to the presence of Mn₃O₄ nanoparticles in the GO sheets. However, the intense peak of GO disappeared in the XRD pattern of the nanocomposite. It indicated that the Mn₃O₄ was mainly formed on the surface of graphene [192, 199-203].

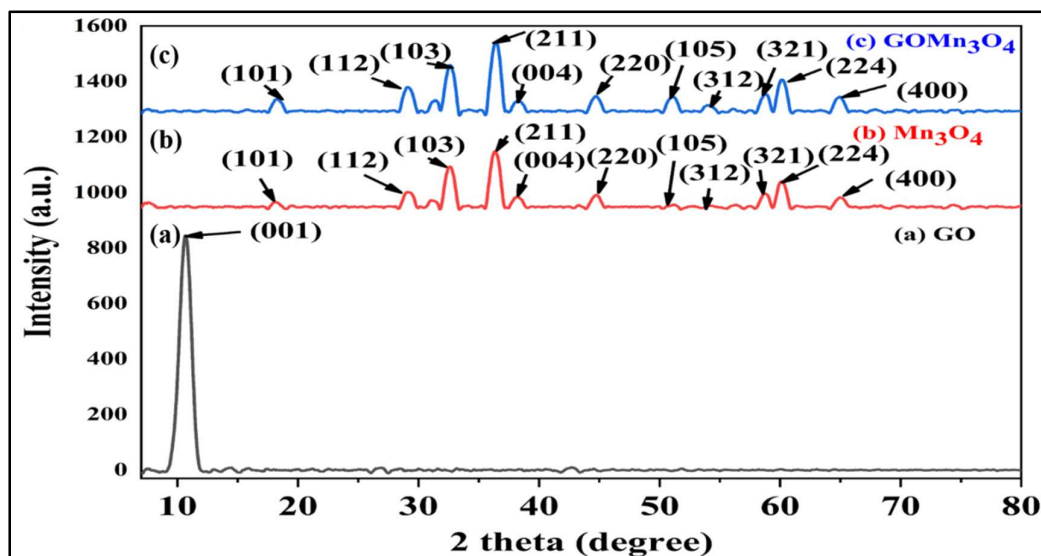


Figure 3.1: XRD spectrum of (a) GO, (b) Mn_3O_4 , and (c) GOMn_3O_4 nanocomposite

The GOMn_3O_4 nanocomposite pure phase development was confirmed by the Raman spectrum captured in **Figure 3.2**. **Figure 3.2(a)** shows that the synthesized GO contains two characteristic bands, i.e., D and G bands, corresponding to 1346 and 1598 cm^{-1} , respectively [204, 205]. The intensity ratio of the D-band to the G-band is 0.84, which is less than 1, showing a lower defect concentration. Raman spectrum of synthesized Mn_3O_4 nanoparticles was recorded and depicted in **Figure 3.2(b)**; a very sharp peak was observed at 634 cm^{-1} , which corresponds to the breathing vibration of divalent manganese ions in tetrahedral coordination [206]. **Figure 3.2(c)** illustrates the Raman spectrum of the synthesized GO- Mn_3O_4 nanocomposite. The D-band and G-band shifted from 1346 to 1351, 1598 to 1591 cm^{-1} , respectively, due to the specific interaction of GO with Mn_3O_4 . The sharp peak at 647 cm^{-1} corresponds to the A_{1g} breathing vibration of the Mn-O tetrahedral spinel structure. At 305 and 360 cm^{-1} , there were also two additional oscillation peaks seen. The presence of the Mn-O bending mode was indicated by the first two low-intensity peaks, 305 and 360 cm^{-1} [207, 208].

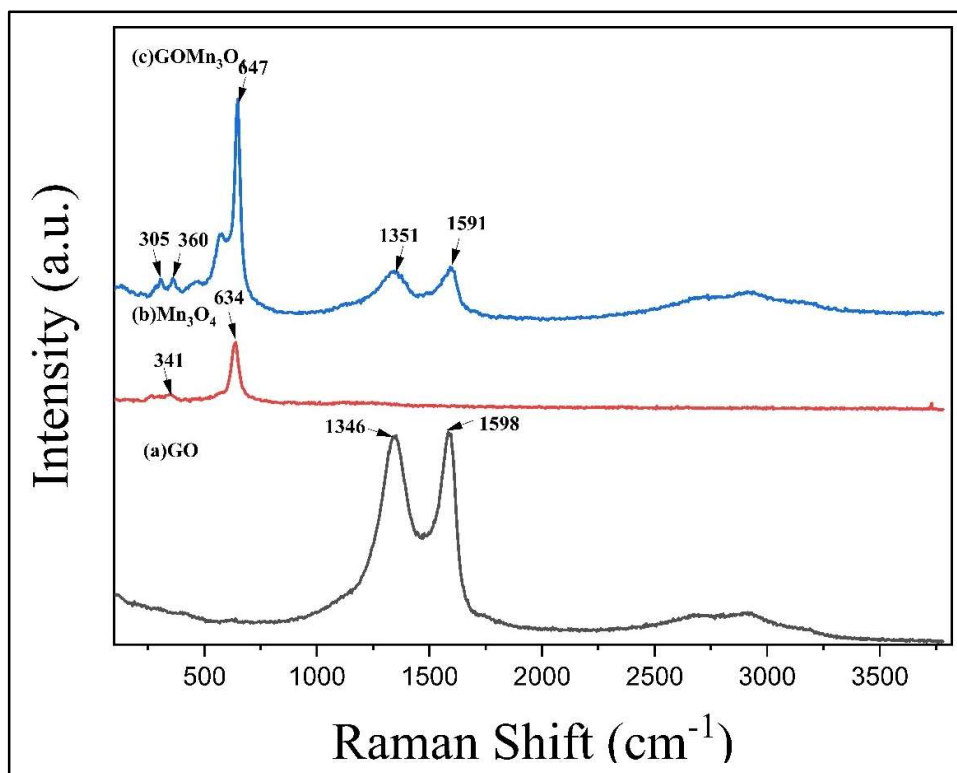


Figure 3.2: Raman spectrum of (a) GO, (b) Mn_3O_4 , and (c) GOMn_3O_4 nanocomposite

Figure 3.3 shows the FE-SEM of EPD GO onto the ITO-coated glass electrode surface. The appearance of GO sheets on the surface of the ITO electrode confirms successful deposition of the material by the EPD technique. It can be seen that individual graphene sheets stack together and appear smooth over the ITO electrode surface. The EPD technique is simple and fast for depositing the material by applying a certain voltage and time in comparison to the drop-cast method, that have a lot of limitations, including poor uniformity of the film, and limited coverage area. EPD is a simple, economical, and eco-friendly technique that is used for uniform deposition of materials onto the conducting surface. Therefore, for deposition of GO materials, the EPD technique was used and deposited onto the ITO electrode surface under the optimized parameters of 60 volts for 5 minutes. Moreover, it can be seen that graphene sheets form a flat-like sheet structure and appear smooth. A clear appearance of GO sheets over the ITO glass substrate confirms the synthesis and deposition of GO.

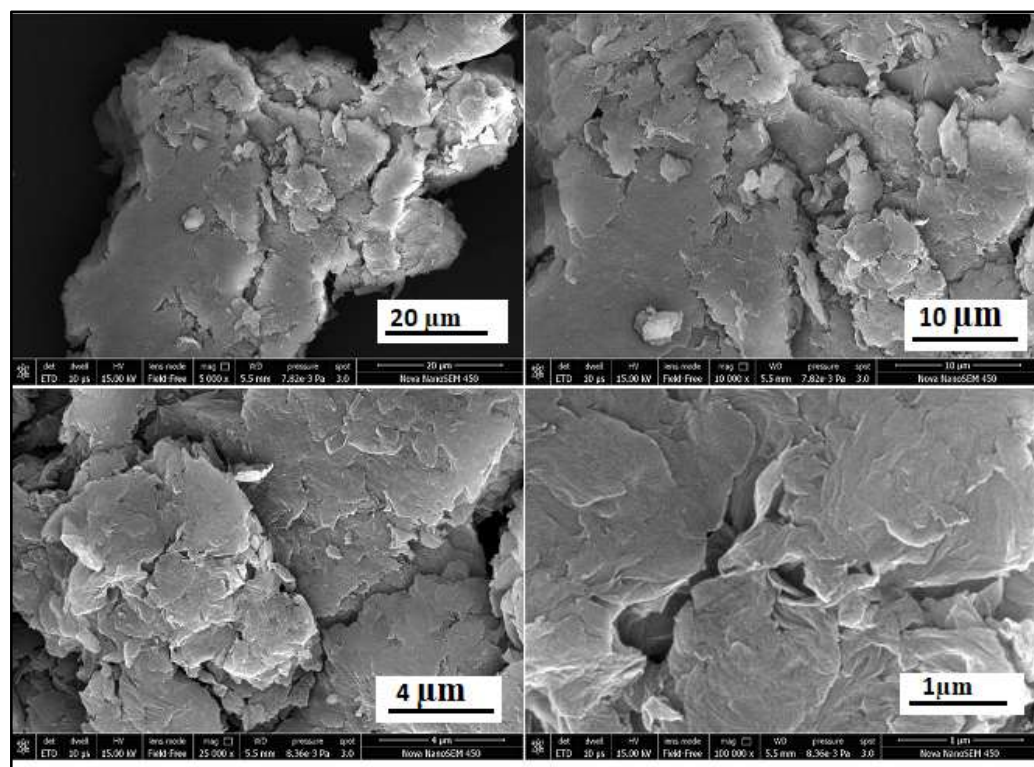


Figure 3.3: Picture of FESEM Images of synthesized GO

3.6.2 FTIR Study of GO, Mn_3O_4 , GOMn_3O_4

The FTIR study of GO, Mn_3O_4 , and GOMn_3O_4 was observed using KBr mode in the 400 to 4000 cm^{-1} wavelength range, as shown in **Figure 3.4**. FTIR spectrum of the GO sample is shown in Figure 4(a) accorded well with the previous works [190]. There were various oxygen configurations in the structure. They include hydroxyl (namely phenol, C-OH) (3050-3800 cm^{-1}), ketonic species (C = O) (1600-1650 cm^{-1}), and C-OH vibration at 1083 cm^{-1} . In Figure 4(b) of Mn_3O_4 , three main absorption peaks occurred. The peak at 3430 cm^{-1} is related to O-H stretching and bending modes of absorbed water [209]. The peak at 615 cm^{-1} was related to the vibration of manganese species (Mn^{3+}) in a tetrahedral site. The peak at 487 cm^{-1} was attributed to the vibration of manganese species (Mn^{3+}) in an octahedral site [210]. FTIR spectrum was recorded for the GOMn_3O_4 composite also, and the result is shown in Figure 4(c). The peak at 3447 cm^{-1} corresponds to O-H stretching and bending modes of absorbed water, and peaks appeared at 1590 and 1411 cm^{-1} attributed to the C=O and C=C bonding, respectively. The two sharp, intense peaks occurred at 618 and 496 cm^{-1}

corresponds to the vibration of manganese species (Mn^{3+}) in a tetrahedral site and in an octahedral site, respectively.

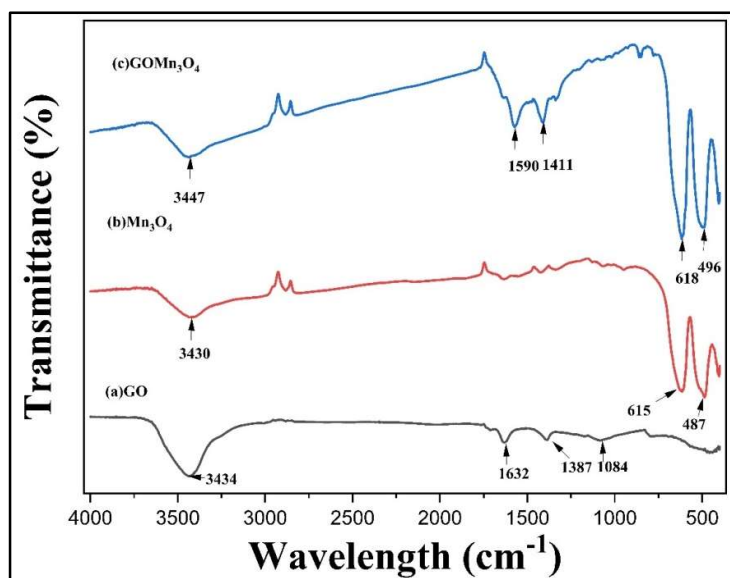


Figure 3.4: FTIR spectrum of (a) GO, (b) Mn_3O_4 , and (c) GOMn_3O_4 nanocomposite

3.6.3 Elemental and morphological study of GOMn_3O_4 nanocomposite

Figure 3.5 illustrates the shape and crystallinity of the GOMn_3O_4 nanocomposite as determined by TEM (a-c). The distribution of Mn_3O_4 nanoparticles on the graphene sheet surface is depicted in **Figure 3.5 (a, b)**. The arrow points to a layer of graphene oxide with Mn_3O_4 nanoparticles decorated onto the GO sheets, and the average size of the nanoparticle was found to be 52.38 ± 1.40 nm. **Figure 3.5 (c)** shows an HRTEM image of the GOMn_3O_4 nanocomposite showing the thickness of the gap. The HRTEM images were assigned to the (101) lattice plane of the Mn_3O_4 tetragonal phase, and the d spacing was calculated to be 0.45 nm. **Figure 3.5(d)** illustrates energy dispersive X-ray (EDX) imaging used to find the elemental composition of the GOMn_3O_4 nanocomposite. The carbon (C), manganese (Mn), and oxygen (O) elements could be found in the GOMn_3O_4 nanocomposite hybrid structure, according to EDX spectra. The elements Mn and O were observed in the EDX spectra due to the presence of Mn_3O_4 , while C was seen due to the presence of graphene nanosheets.

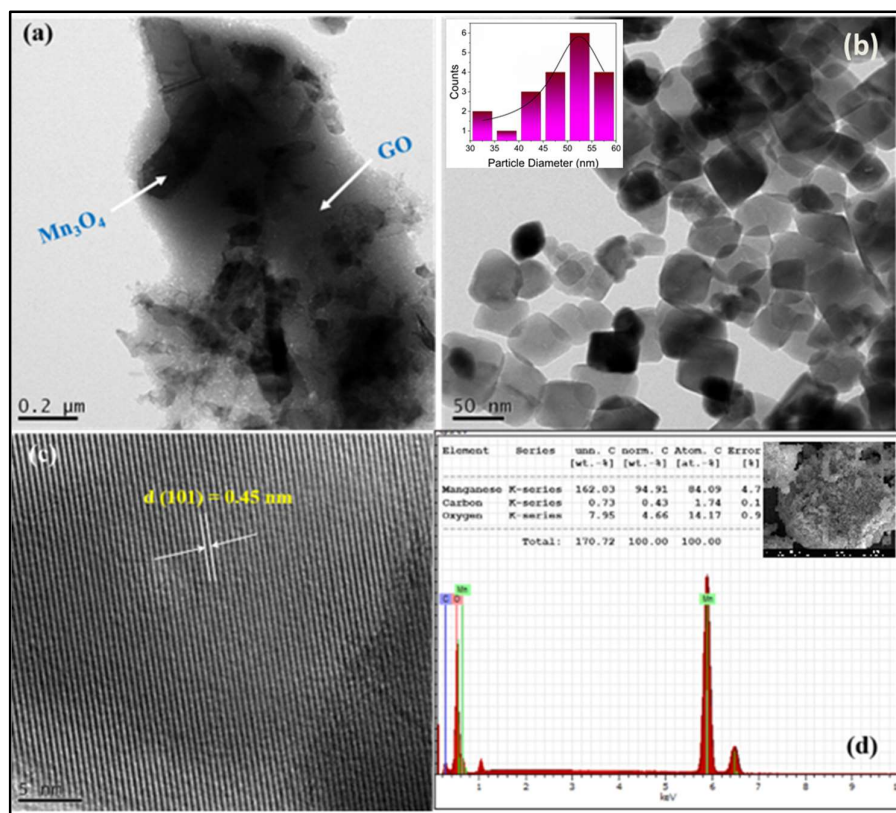


Figure 3.5: (a, b) describe the HR-TEM images of GOMn₃O₄ nanocomposite [inset image: particle size column graph], (c) HR-TEM lattice fringe image of GOMn₃O₄, and (d) EDX image of GOMn₃O₄ nanocomposite.

3.6.4 FTIR study of ab-FBI onto GOMn₃O₄/ITO

The immobilization of ab-FBI and BSA on the surface of the GOMn₃O₄/ITO immunoelectrode was investigated using attenuated total reflection (ATR) FTIR spectroscopy in the 400 to 4000 cm⁻¹ range; the results are shown in **Figure 3.6**. The FTIR spectra of the GOMn₃O₄/ITO immunoelectrode are shown in **Figure 3.6 (i)**, which includes distinctive peaks at 530, 612, 1085, and 1412 cm⁻¹. The two peaks that appeared at 1085 and 1412 cm⁻¹ correspond to the C-O stretching vibration and the -OH group in the carboxylic acid, respectively, present in the graphene oxide, while the other two peaks at 530 and 612 cm⁻¹ correspond to the presence of Mn-O bonds in the Mn₃O₄ nanoparticles. In **Figure 3.6(ii)**, the peaks that appeared at 1068, 1570, and 1642 cm⁻¹ were attributed to C-N stretching, NH₂ group deformation (amide-II bond), and primary amide N-H of the fabricated ab-FBI/GO-Mn₃O₄/ITO immunoelectrodes [211]. These results were obtained due to the presence of ab-FBI on the surface of the GOMn₃O₄/ITO

immuno-electrode. The FTIR spectrum of the BSA/Ab-*FBI*/GOMn₃O₄/ITO immuno-electrode was also performed to confirm that the non-specific binding site of the antibody on the electrode surface was blocked by BSA [212]. As shown in **Figure 3.6(iii)**, the FTIR spectrum showed a characteristic decrease in the peak intensity of ab-*FBI*, and the peak position of the Mn-O bond was shifted from 530 to 536 cm⁻¹ at the Mn₃O₄ nanoparticle [213]. The above studies showed that ab-*FBI* and BSA could be successfully immobilized on the surface of the GOMn₃O₄/ITO electrode.

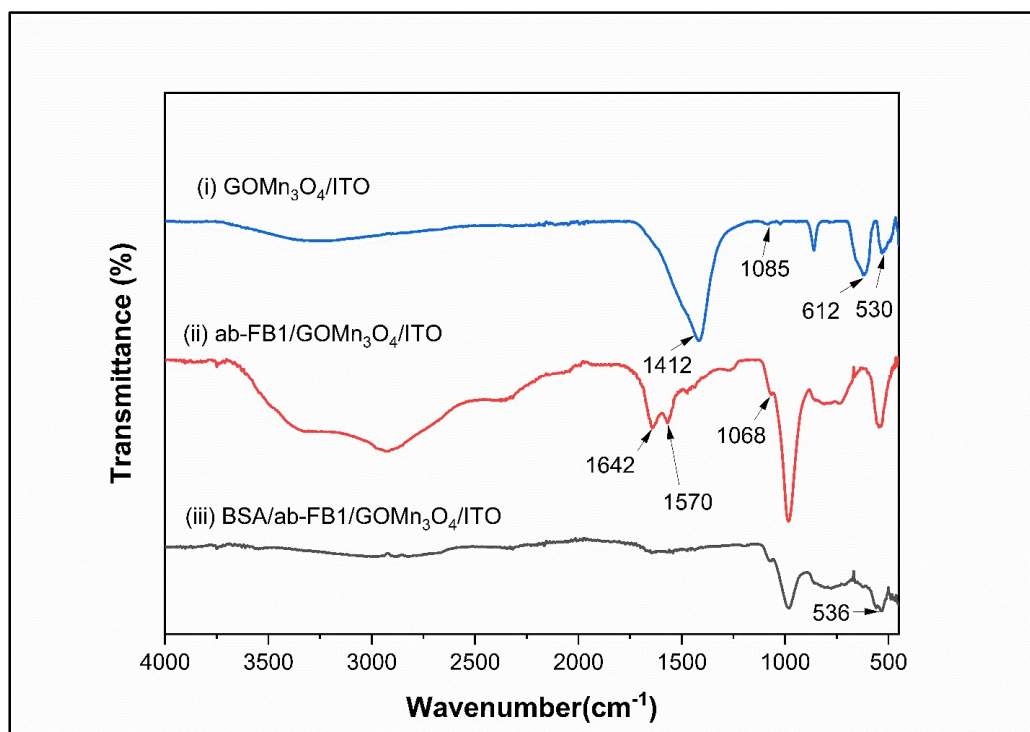


Figure 3.6: FTIR spectrum of (i) GOMn₃O₄/ITO, (ii) ab*FBI*/GOMn₃O₄/ITO, and (iii) BSA/ab-*FBI*/GOMn₃O₄/ITO immuno-electrode.

3.7 Electrochemical study

3.7.1 Optimization conditions

To carry out the electrochemical biosensing studies of the fabricated immunosensor BSA/ab-*FBI*/GOMn₃O₄/ITO for *FBI* detection, optimization studies are important to optimize certain parameters for the fabrication of the sensor. Therefore, a few parameters such as pH study and incubation time study were carried out using the BSA/ab-*FBI*/GOMn₃O₄/ITO immuno-electrode. **Figure 3.7(a)** presents the electrochemical responses of the immunosensor BSA/ab-*FBI*/GOMn₃O₄/ITO at

different pH values ranging from 6.0 to 8.0 of PBS containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$ due to the effect of pH on the protein molecules. Changing from a lower to a higher pH value, i.e., from acidic to basic medium of the electrolytic solution PBS containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$, can lead to altering the conformation of ab-*FBI* and BSA present on the surface of the immunoelectrode. Therefore, it is important to evaluate the specific pH for the fabrication of the immunosensor. By adjusting the pH from 6.0 to 8.0, DPV electrochemical responses were recorded under the potential range from -0.2 to +0.4 V, and pH study results are depicted in **Figure 3.7(a)**. The maximum current was obtained in the specified pH range at pH 7.0. Since the composition of antibodies might be altered by acidic or basic media, pH 7.0 of PBS containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$ was used for the fabrication of an immunosensor for FB1 detection.

The incubation time was optimized at 5-minute intervals of time to analyze the interaction between the immunoelectrode and analyte. The incubation study plot is shown in **Figure 3.7(b)**. The interaction of the immunoelectrode (BSA/ab*FBI*/GOMn₃O₄/ITO) and *FBI* (10 pg mL⁻¹ fixed concentration) mycotoxin in PBS containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$ buffer solution was studied using the DPV method. The decrease in peak current for the immunoelectrode was observed and saturated after 35 minutes. As a result, the incubation period for the *FBI* analyte to interact with the ab-*FBI* immobilized on the surface of the GOMn₃O₄/ITO electrode was optimized to 35 minutes.

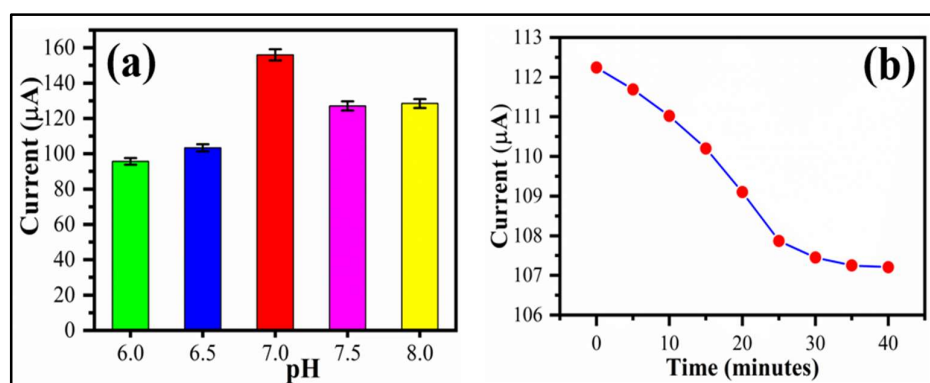


Figure 3.7: (a) The effect of different pH from 6.0 to 8.0 in PBS containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$, and (b) incubation plot for BSA/ab-*FBI*/GOMn₃O₄/ITO immunoelectrode, in PBS containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

3.7.2 An electro-kinetic rate study

The electro-kinetic interface parameters were measured for electrodes GOMn₃O₄/ITO, ab*FBI*/GOMn₃O₄/ITO, and BSA/ab*FBI*/GOMn₃O₄/ITO using the

cyclic voltammetry technique with changing the scan rate from 20 to 100 mV s^{-1} , which is shown in **Figures 3.8 (a-c)**. Peak anode (I_a) and cathode (I_c) redox currents were calculated for the electrodes $\text{GOMn}_3\text{O}_4/\text{ITO}$, $\text{abFBI}/\text{GOMn}_3\text{O}_4/\text{ITO}$, and $\text{BSA}/\text{abFBI}/\text{GOMn}_3\text{O}_4/\text{ITO}$ immunoelectrode at 50 mV/s .

The inset **Figures 3.8 (a-c)** show a plot of the anode (I_a) and cathode (I_c) currents that are linearly proportional to the square root ($v^{1/2}$) of the scan rate. The peak current ratio (I_a/I_c) was determined to be 1.11, 1.12, and 1.13, which corresponds to the immunoelectrodes $\text{GOMn}_3\text{O}_4/\text{ITO}$, $\text{ab-FBI}/\text{GOMn}_3\text{O}_4/\text{ITO}$, and $\text{BSA}/\text{ab-FBI}/\text{GOMn}_3\text{O}_4/\text{ITO}$, respectively. Thus, the obtained results of the current ratio indicate the quasi-reversible electron transfer process. Furthermore, the peak potential was also determined using the relation $\Delta E_p = E_{pa} - E_{pc}$ for the immunoelectrode and found to be 0.35 V. The determined potential is higher than that of the $\text{ab-FBI}/\text{GOMn}_3\text{O}_4/\text{ITO}$ electrode, which ascribes the efficient electron transfer between immunoelectrode and the redox solution.

The diffusivity (D) of the immunoelectrode was found using the Randles-Sevcik equation described below in **equation no. 3.3**:

$$I_p = (2.69 \times 10^5) C n^{3/2} D^{1/2} v^{1/2} A \quad [3.3]$$

Where I_p is the peak current of the electrode, C is the concentration of the electrolyte, n is the number of electrons, A represents the surface area of the working electrode, and v is the scan rate (mV/s). The diffusivity for $\text{BSA}/\text{abFBI}/\text{GOMn}_3\text{O}_4/\text{ITO}$ immunoelectrode is found to be $2.08 \times 10^{-11} \text{cm}^2 \text{s}^{-1}$ which indicates that at the electro-analytical interface, the rate of electron transfer is higher.

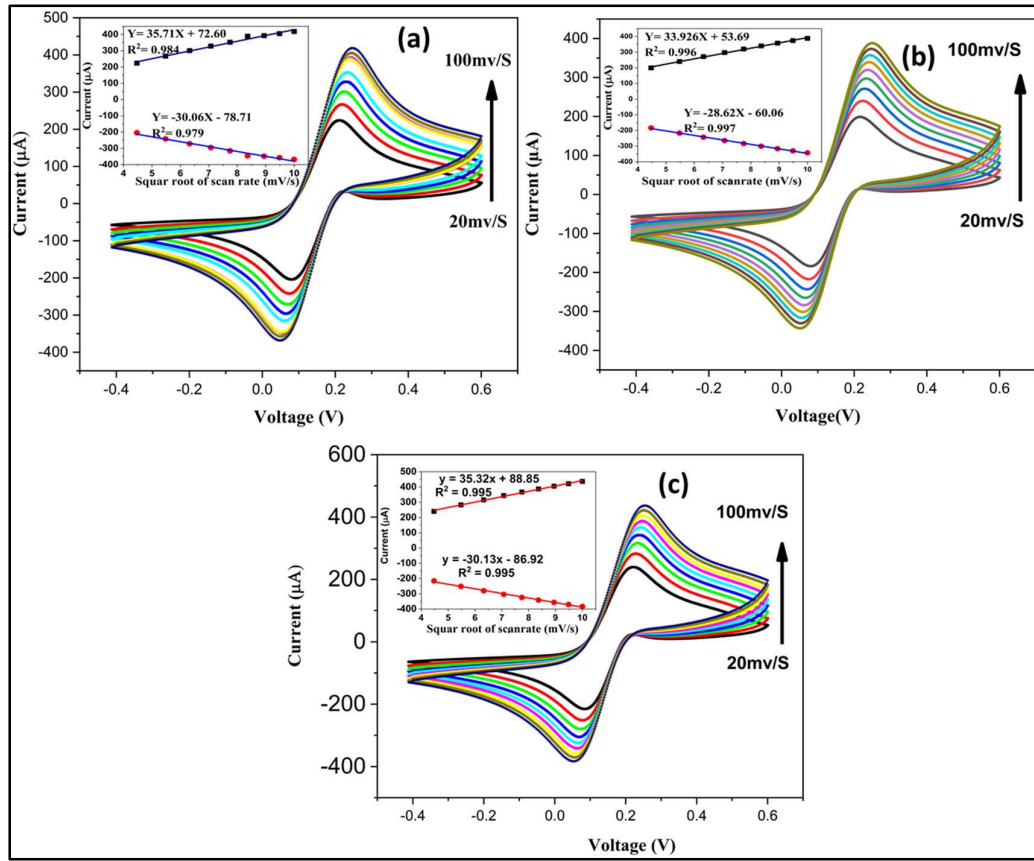


Figure 3.8: Scan rate study from 20 to 100 mV/s and linear plot between the square root of scan rate vs peak current of (a) GOMn₃O₄/ITO, (b) ab-FBI/GOMn₃O₄/ITO, and (c) BSA/ab-FBI/GOMn₃O₄/ITO immunoelectrode, respectively.

The availability of binding sites present at the immunoelectrode was calculated with the help of the above-mentioned diffusion-controlled parameter, and the area (A_e) was determined using the Randles-Sevcik equation, described below in **equation no.3.4**:

$$A_e = \frac{S}{(2.69 \times 10^5)n^3 CD^{1/2}} \quad [3.4]$$

Where S is the slope of the straight line obtained from the linear plot of I_{pa} vs scan rate. The obtained results show a 4.11 mm² area, which indicates the presence of more binding sites per unit volume at the immunoelectrode.

Further, the concentration at the surface of ionic species (I^*) was also determined using Brown-Anson **equation no. 3.5**, which is as follows:

$$I_p = \frac{n^2 F^2 I^* A V}{4RT} \quad [3.5]$$

Where F is the Faraday constant ($96,485 \text{ C mol}^{-1}$), R stands for the gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), T stands for temperature (300 K), and the meaning of additional symbols is the same as that given in the previous equation. The determined result was found to be $2.94 \times 10^{-9} \text{ mol cm}^{-2}$ for the BSA/ab-FBI/GOMn₃O₄/ITO immunoelectrode, which is higher for the reactive species at the interface. Electrochemical characterization studies of GOMn₃O₄/ITO, ab-FBI/GOMn₃O₄/ITO, and BSA/ab-FBI/GOMn₃O₄/ITO surfaces were carried out using the DPV technique. The DPV spectra of GOMn₃O₄/ITO, ab-FBI/GOMn₃O₄/ITO, and BSA/ab-FBI/GOMn₃O₄/ITO immunoelectrodes are shown in **Figure 3.9** under the potential range from -0.2 to 0.4 V in PBS containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at pH 7.0. The magnitude of peak current values of (i) GOMn₃O₄/ITO is higher than (ii) ab-FBI/GOMn₃O₄/ITO. This happens due to the large surface-to-volume ratio, excellent adhesion, good electron conductivity, and high electro-catalytic activity of the material. After the immobilization of ab-FBI over the GOMn₃O₄/ITO electrode surface, the peak current value decreased due to the presence of a heavier, bulky molecule. Then, the modified electrode surface was washed with BSA protein to remove the non-binding sites; therefore, the peak current increased.

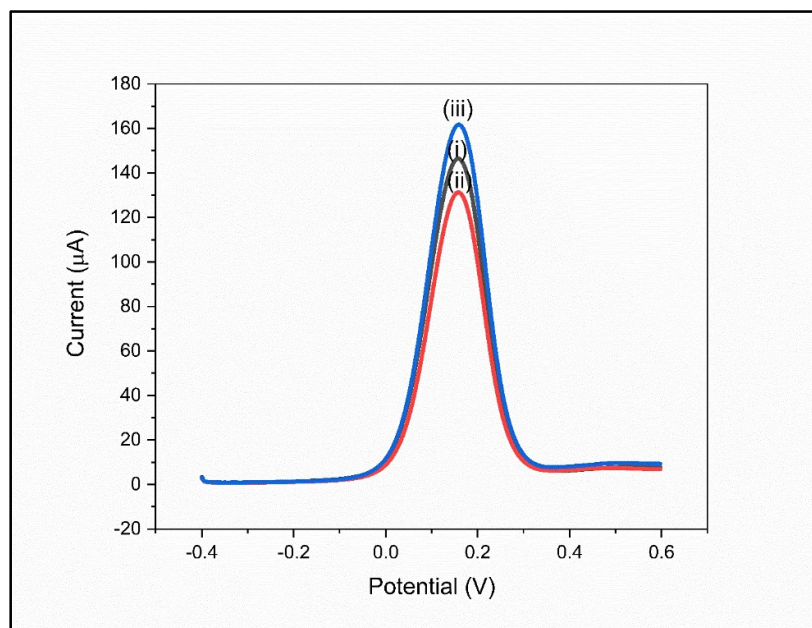


Figure 3.9: DPV response study of the (i) GOMn₃O₄/ITO, (ii) ab-FBI/GOMn₃O₄/ITO, (iii) BSA/ab-FBI/GOMn₃O₄/ITO electrode surface

3.7.3 Response study

Electrochemical biosensing response studies were carried out using the DPV technique in a three-electrode system containing BSA/ab-*FBI*/GOMn₃O₄/ITO as a working electrode, reference electrode (Ag/AgCl), and counter electrode (Pt) in a PBS containing [Fe(CN)₆]^{3-/4} in the potential range from -0.2 to 0.4 V. The DPV technique is a voltammetry method that is used to make the electrochemical measurement response in which a pulsed potential (ΔE) is applied within a linear potential scan using fixed increments. The electrochemical response is similar to the first derivative of a linear voltammogram, where a peak originates from the Faradaic reaction and can be identified near $E_{1/2}$. DPV measurements can be used to study the redox properties of extremely low concentrations of analyte because the effect of the charging capacitive current can be minimized, so higher sensitivity is achieved than the linear scanning method, and only faradaic current is extracted, so the electrode reactions can be analyzed more precisely. To carry out the sensing response study, different concentrations of analyte *FBI* were analyzed by the DPV technique to acquire higher sensitivity, better limit of detection, and better resolution for lower concentrations of analyte. Therefore, electrochemical biosensing studies were carried out using the *FBI* concentration range from 1 pg mL⁻¹ to 800 ng mL⁻¹ and result depicted in **Figure 3.10**. **Figure 3.10(a)** shows that the current response proportionally decreased as the *FBI* concentration ranged from 1 pg mL⁻¹ to 800 ng mL⁻¹. As the concentration of *FBI* was increased on the immunoelectrode surface of BSA/ab-*FBI*/GOMn₃O₄/ITO, interaction took place between the immobilized ab-*FBI* and *FBI*, which led to the formation of an electrically insulating immunocomplex between antigen and antibody that hinders the electron transfer between electrode and electrolyte; correspondingly, the current response signal decreased. Figure 10 (b) shows the calibration curve between *FBI* concentration and current response. The linear calibration plot presents the linear correlation observed between current responses with a range of analyte concentrations from 1 pg mL⁻¹ to 800 ng mL⁻¹. The fabricated immunoelectrode electrochemical response in terms of sensitivity was calculated using the following: Sensitivity = slope/area of the electrode, assuming a 0.25 cm² electrode area. The immunoelectrode sensitivity was determined to be 10.08 $\mu\text{A mL ng}^{-1} \text{cm}^{-2}$, with a regression coefficient (R^2) of 0.97. The measurements were carried out in triplicate

under the same experimental conditions, and the signal-to-noise ratio (S/N) was three. LOD was calculated using the standard formula of $3\sigma/m$ (σ is the standard deviation and m is the slope), and the LOD value was found to be 0.195 pg mL^{-1} . The biosensing response obtained from the current fabricated immunoelectrode BSA/ab-*FBI*/GOMn₃O₄/ITO for *FBI* detection is comparable to the previously reported articles for *FBI* detection, and the findings are summarized in **Table 1**. GOMn₃O₄ nanocomposite increased surface-to-volume ratio [203], improved loading of ab-*FBI* over the GOMn₃O₄/ITO surface, provided good electrical conductivity [176], electron transfer efficacy [202, 214], and abundant functional groups available for conjugation with antibodies, thereby enhancing the sensitivity, linear detection range, limit of detection, specificity, selectivity, and stability of the immunosensor. The electrochemical biosensing signal response parameters are found to be better in comparison to the previously published work due to the integration of GOMn₃O₄ nanocomposite for the fabrication of an immunosensor for *FBI* detection.

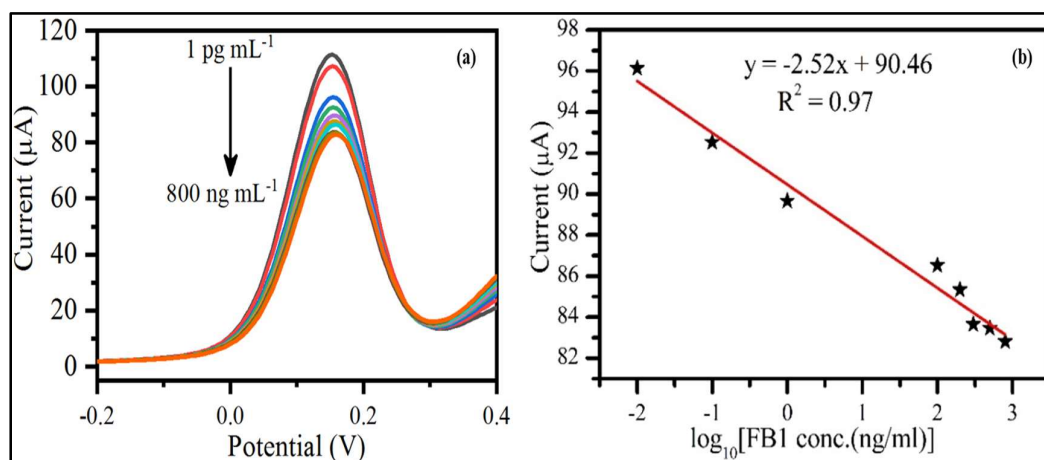


Figure 3.10: (a) DPV biosensing response, and (b) a linear plot of the BSA/ab-*FBI*/GOMn₃O₄/ITO immunoelectrode, respectively, against *FBI* in the concentration range from 1 pg mL^{-1} to 800 ng mL^{-1} .

3.7.4 Repeatability, reproducibility, control, and interferent study

Reproducibility studies of immunosensor BSA/ab-*FBI*/GOMn₃O₄/ITO were performed on five different immunoelectrodes using the DPV technique. **Figure 3.11(a)** displays the current response obtained from five immunoelectrodes. The column graph demonstrated remarkable consistency for a specific immunoelectrode

under the same settings, with a relative standard deviation (RSD) of 2.49%. Due to the stability and reproducibility of the immunoelectrodes, almost the same current response with minimal variation was obtained with the five different immunoelectrodes. **Figure 3.11(b)** shows the repeatability study of the fabricated immunoelectrode BSA/ab-*FBI*/GOMn₃O₄/ITO, which was measured at a *FBI* concentration of 5 ng mL⁻¹ using the DPV technique. A virtually steady current was obtained with a relative standard deviation (RSD) of 1.06 % by five consecutive readings. This study verified that the designed BSA/ab-*FBI*/GOMn₃O₄/ITO immunosensor has remarkable repeatability. It is important to investigate the interference study of the fabricated immunosensor to measure the specificity and selectivity of the sensor towards *FBI* only. Therefore, an interference study was carried out with various interfering analytes to see the influence of other analytes on the electrode surface of BSA/ab-*FBI*/GOMn₃O₄/ITO. For this study, various interferents, including other mycotoxins such as *AFBI*, *OchA*, and *DON*, and other carbohydrates like cellulose, fructose, and starch, which are found in the food sample, were used, and DPV response obtained as shown in **Figure 3.11(c)**. The selectivity study was measured with the addition of 20 µL of interferents in the electrolytic solution in the presence of *FBI* mycotoxins, and their response was recorded using the DPV technique. The obtained results showed that the fabricated immunosensor is highly selective towards the presence of *FBI*. These results indicate that ab-*FBI* is highly specific for *FBI*. Also, specificity studies were carried out to investigate the binding of *FBI* only with ab- *FBI* [**Figure 3.11(d)**]. To perform this study, a control study using the GOMn₃O₄/ITO electrode was tested with various series of concentrations of *FBI* from 1 pg mL⁻¹ to 800 ng mL⁻¹. It is observed that no change in the current signal was found, indicating that the *FBI* analyte is highly specific for the immobilized ab-*FBI* onto the GOMn₃O₄/ITO electrode.

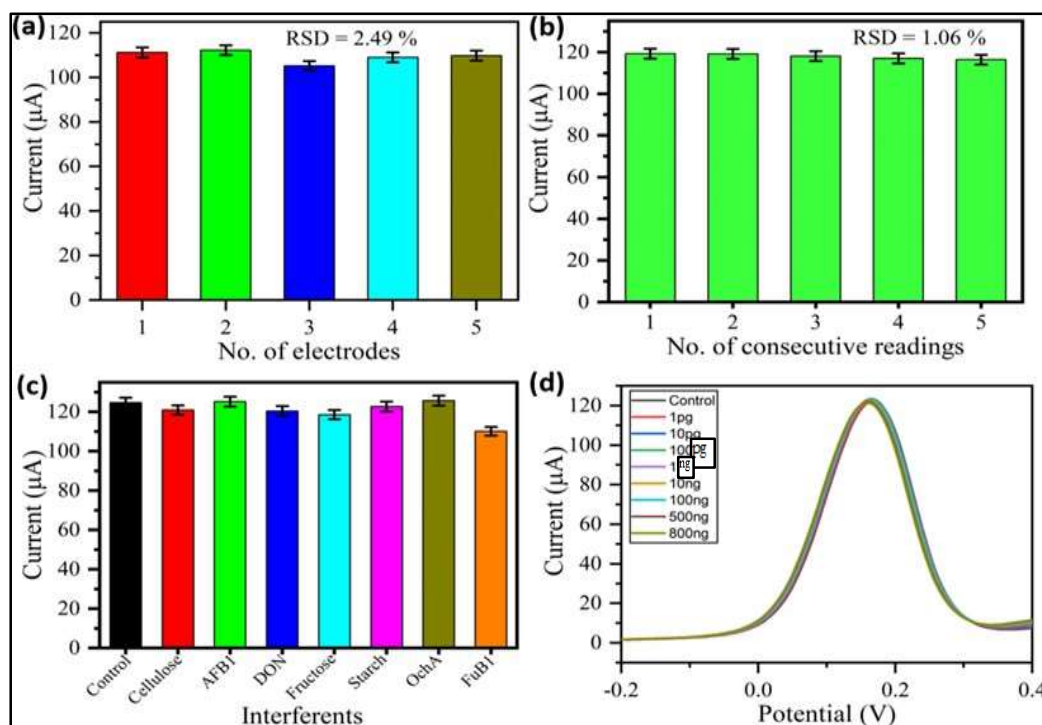


Figure 3.11: (a) Reproducibility plot for five different immunoelectrodes, (b) shows the repeatability study measured for five different consecutive readings with a 1.06% RSD value, (c) describes the interferent study plot with various interfering analytes, and (d) depicts the GOMn₃O₄/ITO control measurement plot with different concentration ranges from 1 pg mL⁻¹ to 800 ng mL⁻¹.

3.7.5 Spiked study

To determine the real sample study, BSA/ab-*FBI*/GOMn₃O₄/ITO immunoelectrode responses were observed in spiked maize samples with different *FBI* concentrations. The fresh sweet maize was purchased from the local market, crushed thoroughly, and diluted in D.I. water. Further, the diluted maize extract was taken and mixed with different concentrations of *FBI* (including 1 pg mL⁻¹, 10 pg mL⁻¹, 100 pg mL⁻¹, 1 ng mL⁻¹, 100 ng mL⁻¹, 300 ng mL⁻¹ and 500 ng mL⁻¹), and measurement was performed using the DPV technique under similar conditions. **Figure 3.12** shows the linear calibration graph plotted for both spiked and sensing responses obtained for various *FBI* concentrations from 1 pg mL⁻¹ to 500 ng mL⁻¹ in sweet maize samples. The linear regression coefficient (R^2) values were calculated for the spiked and sensing results and found to be 0.82 for spiked and 0.85 for sensing studies. The lower regression coefficient for spiked sample studies is due to the small change in the current values

with changing the concentration of *FB1* in spiked maize samples, while in the case of *FB1* sensing studies, current is decreasing linearly with the increase of *FB1* concentration, resulting in an improvement in the regression coefficient. However, it is observed that in both spiked samples and with *FB1*, current values decrease as the concentration of *FB1* antigen indicates that the fabricated immunoelectrode BSA/ab-*FB1*/GOMn₃O₄/ITO shows specific binding for *FB1* analyte in sensing and spiked samples. The percentage recovery rate was calculated and found to be in the range of 98.91% to 123.3 %. These results show that an excellent recovery, and this fabricated immunoelectrode BSA/ab-*FB1*/GOMn₃O₄/ITO can be used for *FB1* detection.

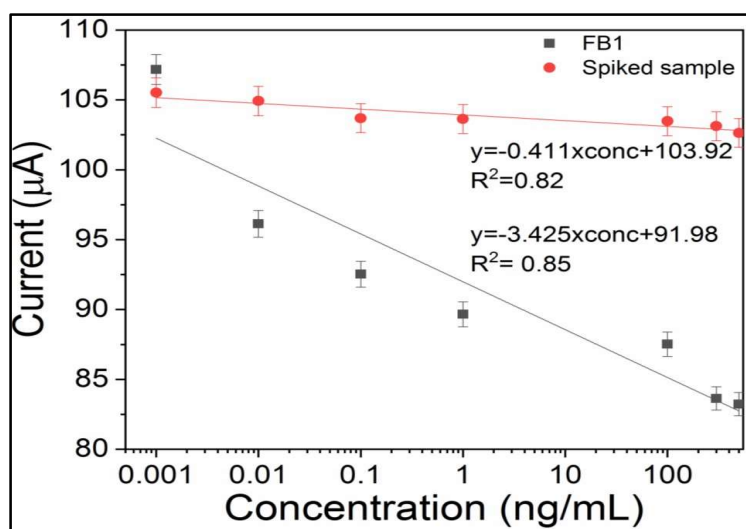


Figure 3.12: Linear plot of *FB1* and spiked sample studies for concentrations of 1 pg mL⁻¹, 10 pg mL⁻¹, 100 pg mL⁻¹, and 1 ng mL⁻¹, 100 ng mL⁻¹, 300 ng mL⁻¹, and 500 ng mL⁻¹ performed in the sweet maize samples using the fabricated BSA/ab*FB1*/GOMn₃O₄/ITO immunoelectrode

3.8 Conclusions

BSA/ab-*FB1*/GOMn₃O₄/ITO immunoelectrode was fabricated for the sensitive and precise detection of *FB1* in sweet corn samples in the concentration range from 1 pg mL⁻¹ to 800 ng mL⁻¹. GOMn₃O₄ nanocomposite was successfully synthesized by the wet chemical co-precipitation method. XRD and HR-TEM characterization techniques were used to validate its tetragonal phase structure. A GOMn₃O₄ nanocomposite thin film was developed on an ITO-coated glass surface using an electrophoretic deposition method. Ab-*FB1* and BSA were immobilized on GOMn₃O₄/ITO electrodes, and the immobilization was confirmed using DPV and

FTIR spectroscopy. The fabricated immunoelectrode exhibits a high sensitivity of $10.08 \mu\text{A mL ng}^{-1}\text{cm}^{-2}$ for *FBI* detection, and a LOD was found to be 0.195 pg mL^{-1} with a regression coefficient (R^2) of 0.97. The *FBI* spiked maize samples recovery rates were found in the range from 98.91% to 123.3%. This shows that the BSA/ab-*FBI*/GOMn₃O₄/ITO immunoelectrode system is fully applicable to detect *FBI* in actual samples. Various naturally occurring interferences in food (along with *FBI*), such as *AFBI*, *OchA*, *DON*, cellulose, fructose, and starch, were used in interference studies to confirm the specific detection of *FBI*. With 5 electrodes and continuous measurements, the BSA/ab-*FBI*/GOMn₃O₄/ITO immunoelectrode was likewise shown to be very reliable and reproducible. In a controlled study, the GOMn₃O₄/ITO electrode showed no tendency to detect *FBI*, and the BSA/ab-*FBI*/GOMn₃O₄/ITO immunoelectrode confirmed the specific interaction with *FBI*.

3.9 Acknowledgments

The authors thank the Advanced Instrumentation Research Facility (AIRF), JNU, for assisting with the XRD, SEM-EDAX, Raman, and TEM characterization. The authors are thankful to MNIT, Jaipur, for FESEM image analysis. The authors are very grateful to the Special Centre for Nanosciences, JNU, for FTIR and electrochemical data analysis.

Chapter 4

Hematite-reduced graphene oxide Nanocomposite-Based Electrochemical Biosensing Platform for Rapid Detection of *Fumonisin B1* in Corn Samples

Naturally occurring mycotoxins present significant health risks to humans and animals due to their toxic and carcinogenic properties, often contaminating food supplies. Therefore, the development of a highly sensitive and selective detection method for mycotoxins is essential. In this study, we report an electrochemical biosensing platform for the detection of the most abundant mycotoxin, *fumonisin B1* (*FBI*), using a nanocomposite of hematite (α -Fe₂O₃) and reduced graphene oxide (rGO) nanosheets. The successful synthesis of the α -Fe₂O₃/rGO nanocomposite was validated using multiple characterization techniques, including X-ray diffraction (XRD), field-emission scanning electron microscopy (FESEM), Raman spectroscopy, and high-resolution transmission electron microscopy (HRTEM). A smooth, uniform, and thin layer of α -Fe₂O₃/rGO was deposited onto an indium tin oxide (ITO) glass substrate using the electrophoretic deposition (EPD) process. Anti-*FBI* antibodies (ab-*FBI*) were covalently immobilized onto the α -Fe₂O₃/rGO/ITO surface using EDC:NHS chemistry. To block nonspecific binding sites, the immunoelectrode was treated with bovine serum albumin (BSA). The biosensor exhibited a decreasing electrochemical response with increasing concentrations of *FBI* in the range of 1×10^{-3} - 700 ng mL⁻¹, with a limit of detection (LOD) of 0.0052 ng mL⁻¹. The fabricated BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO biosensor demonstrated a sensitivity of 6.36 μ A mL ng⁻¹ cm⁻² and a high correlation coefficient ($R^2 = 0.98831$). When tested with spiked sweet corn samples, the biosensor achieved a recovery rate between 97.2% and 99.4%, indicating its potential for reliable real-sample analysis.

This work is published as: Sandeep Sarpal, Hema Bhardwaj, Nitin Kumar Puri, Vinod Singh, and Pratima R Solanki. "Hematite-reduced graphene oxide nanocomposite-based electrochemical biosensing platform for rapid detection of fumonisin B1 in corn samples" Chemistry Select (Wiley publishing) 11(23)(2026): p.e06376.

4.1 Introduction

Mycotoxins are naturally occurring toxic compounds that contaminate a wide range of food products, especially under warm and humid conditions [215]. Moreover, these compounds are chemically stable and can withstand conventional food processing methods [216, 217]. Corn and corn-based products are especially prone to mycotoxin contamination, with *fumonisin B1* (*FBI*) frequently occurring at high levels and comprising about 70% of total *fumonisin*s [218, 219]. *FBI* is produced by the fungus *Fusarium verticillioides* and has been classified by the International Agency for Research on Cancer (IARC) as a Group 2B carcinogen [220]. The U.S. Food and Drug Administration (FDA) has set the maximum permissible limit of *FBI* in food products at 2–4 parts per million (ppm), equivalent to 2000–4000 ng/mL [221]. Therefore, the ability to detect *FBI* at concentrations significantly below these regulatory thresholds is essential for early warning.

There are numerous traditional techniques for identifying *FBI*, including thin-layer chromatography, mass spectroscopy, molecular fluorescence, and high-performance liquid chromatography. However, these methods have low sensitivity and poor selectivity and necessitate an experienced analyst [222]. These limitations restrict the use of conventional techniques for the routine analysis of food products. The electrochemical biosensor is one of the advanced detection techniques for sensitive and selective detection [223]. Among various low-cost and biocompatible metal oxides, hematite ($\alpha\text{-Fe}_2\text{O}_3$) nanoparticles are chemically stable and are considered promising materials for biosensing applications because of having n-type semiconducting properties, a narrow bandgap (2.1 eV), good biocompatibility, and excellent catalytic activity. However, pristine hematite exhibits relatively low electrical conductivity and rapid electron-hole recombination, which can limit its electrochemical performance. Therefore, the incorporation of $\alpha\text{-Fe}_2\text{O}_3$ on graphene oxide (GO) significantly enhances the charge transfer efficiency between $\alpha\text{-Fe}_2\text{O}_3$ and GO, resulting in improved electrochemical conductivity and enhanced sensitivity. Herein, a novel approach is demonstrated for the synthesis of a nanocomposite without the use of highly toxic elements [221, 224–226]. Furthermore, the incorporation of $\alpha\text{-Fe}_2\text{O}_3$ nanoparticles onto a 2-D graphene sheet provides a

substantial number of active sites that enhance the immobilization of antibodies over the electrode surface. The use of such nanocomposites offers cost-effectiveness and improved catalytic activity, leading to enhanced adsorption capabilities as well as improved electrical and structural properties [227]. Various research groups have reported the detection of *FBI* using electrochemical sensors and biosensors. For example, in 2022, Naghshbandi et al. developed a thiol-modified single-stranded DNA for the detection of *FBI*, which was immobilized on screen-printed carbon electrodes [228]. A good stability, excellent recovery, high selectivity, and acceptable repeatability and reproducibility were obtained using the designed aptasensor. Further, Yingkai et al. reported a biosensor for the estimation of *FBI* using fluorescence resonance energy transfer between GO and upconversion energy particles, which developed a very strong fluorescence signal [229]. The signal was achieved at 980 nm and developed a very high-throughput detection of *FBI*. Chengquan et al. reported a multiplexed FRET aptasensor using magnetically controlled GO/Fe₃O₄ for the estimation of *FBI* in peanut samples [230]. Traun et al. fabricated an electrochemical biosensor with a microfluidic biosensing platform of silver-ceria nanocomposite using maskless lithography for *FBI* detection [231]. Xianxian et al. utilized a single-walled carbon nanotube/chitosan nanocomposite for the development of an electrochemical sensor [232]. Kadir et al. reported screen-printed gold electrodes with a carbon counter and a silver-silver chloride electrode immunoelectrode for the estimation of *FBI* using an electrochemical technique [221]. Masikini et al. utilized polyaniline-carbon nanotubes doped with palladium telluride quantum dots for the development of an immunoelectrode capable of detecting *FBI* [233]. Sarpal et al. synthesized the nanocomposite of graphene oxide and manganese tetroxide nanoparticles, used for the detection of *FBI* [234]. In this regard, there is a wide scope to explore other nanocomposites, including α -Fe₂O₃/rGO for *FBI* detection [235]. Lu et al. used a chemically reduced graphene oxide thin film for the detection of *FBI* and found good sensitivity [236]. Zheng et al. developed a label-free electrochemical sensor using the nanocomposite of gold and graphene for the detection of *FBI* [237]. Xue et al. developed a photoelectrochemical sensor using the nanocomposites of α -Fe₃O₄@Cu@Cu₂O for the detection of *FBI* [238]. However, despite the remarkable progress have been made for the development of *FBI*

biosensors, α -Fe₂O₃/rGO nanocomposites have not yet been extensively explored. Owing to the excellent biocompatibility, large surface area, enhanced electron transfer capability, and superior electrochemical properties provides a promising sensing platform for sensitive and selective *FBI* detection. Therefore, the present work focused on the development of sensitive and selective detection of *FBI* using an electrochemical technique.

For this, α -Fe₂O₃ was synthesized using the co-precipitation method at room temperature, and a nanocomposite formed with graphene oxide (GO) nanosheets (α -Fe₂O₃/rGO) was used for the detection of *FBI*. This study aims to explore the synthesis of an α -Fe₂O₃/rGO nanocomposite, the direct electrophoretic deposition process on an ITO glass surface to obtain uniform deposition, and use it for the development of electrochemical biosensors with stability, specificity, and high sensitivity. EPD is a very common method of preparing thin films, as the surface coating can be easily controlled with the deposition weight, deposition voltage, and deposition time. EPD allows for precise control over the chemical interactions between α -Fe₂O₃ and rGO, resulting in uniform, dense, and well-distributed materials across the substrate. Unlike bulk powder composites, EPD-fabricated thin films maintain high purity of crystalline structure with a porous morphology that facilitates faster electron transport phenomena. The sensor prepared by this method gives very good linearity, stability, and good selectivity [239]. As per our best knowledge, this is the first time we are reporting the work for the EPD of a synthesized α -Fe₂O₃/rGO material-based platform for the development of electrochemical biosensors for *FBI* detection in food samples. The combination of α -Fe₂O₃ and rGO offers excellent redox reversibility, biocompatibility, and stability. Compared to the traditional drop-casting approach, the EPD method provides superior control over the thickness and uniformity of the α -Fe₂O₃/rGO film, resulting in improved reproducibility and sensing performance of the biosensor. The high surface area and excellent electrical conductivity of rGO facilitate rapid electron transport, whereas the α -Fe₂O₃ nanoparticles provide excellent biocompatibility and catalytic sites for effective immobilization of anti-*FBI* and *FBI* interaction. Also, the developed electrochemical biosensor was validated with real sweet corn samples, and the percentage recovery

was found to be acceptable. Also, an ultra-performance liquid chromatography/tandem mass spectroscopy (UPLC-MS/MS) experiment was performed to assess the accuracy of the developed sensor for the detection of *FBI*.

4.2 Experimental Section

4.2.1 Chemicals

Graphene oxide (GO) powder (4%–10% edge-oxidized, 15–20 sheets), iron(III) chloride hexahydrate, sodium acetate, *FBI*-specific antibodies (anti-*FBI*), starch, fructose, *ochratoxin-A* (*OTA*), sodium hydroxide (NaOH), and ITO-coated glass substrates of surface resistivity 8–12 Ω /square were procured from M/S Sigma-Aldrich. All necessary solution preparations were done using de-ionized water that had a resistivity of 18.2 M Ω through the Millipore water purification system. The following products were acquired from SRL, India: potassium chloride, sodium chloride, BSA, 1-ethyl-3-(dimethyl-aminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and sodium phosphate monobasic dehydrate (NaH_2PO_4). Potassium ferrocyanide ($\text{K}_4[\text{Fe}(\text{CN})_6]$) and potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$) were procured from Fisher Scientific. A phosphate-buffered solution (PBS, 0.2 M, pH 7.4) containing NaCl (0.9%), with $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (5 mM) as the redox probe was prepared.

4.2.2 Synthesis of $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}$ Nanocomposite

The nanocomposites of $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}$ have been prepared using a simple co-precipitation method in which $\alpha\text{-Fe}_2\text{O}_3$ nanocrystals were deposited onto rGO nanosheets using probe sonicators. Firstly, 0.6 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 1.8 g of $\text{C}_2\text{H}_3\text{NaO}_2$ were added to the solution containing 25 mL of ethanol and 6 mL of DI water. Now, the solution was agitated for 2 h at ambient temperature (25°C) using the magnetic stirrer. This solution was then transferred into a Teflon-lined high-pressure hydrothermal reaction vessel at 180°C for 12 h. After the reaction was over, it was allowed to cool to room temperature, and the red powder was collected and washed thoroughly with ethanol five to six times to remove unreacted impurities. Then, the red powder was dried in a heating oven at 70°C for 8 h, and $\alpha\text{-Fe}_2\text{O}_3$ nanoparticles were obtained [240–243]. Furthermore, 300 mg of EOGO was reduced to reduced

graphene oxide by adding 10 μL of hydrazine hydrate in 300 mL of DI water under continuous stirring for 12 h. Further, 150 mg of as-synthesized red powder $\alpha\text{-Fe}_2\text{O}_3$ was mixed with 15 mg of rGO in 50 mL of ethanol and subjected to probe sonication for 60 min. After sonication, the mixture was washed three to four times with ethanol, centrifuged, and dried at room temperature in an oven to obtain the nanocomposite of $\alpha\text{-Fe}_2\text{O}_3$ and reduced graphene oxide.

4.2.3 Characterization Techniques

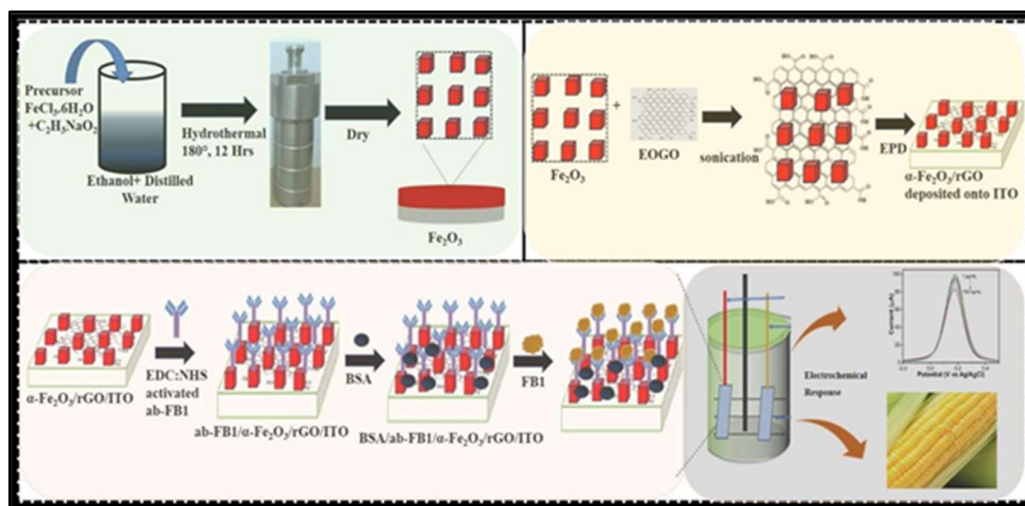
For deposition of synthesized material onto the ITO glass surface, EPD of GeNei equipment was used. Cu K_α radiation at $\lambda = 1.54 \text{ \AA}$, X-ray diffraction (XRD) using a Rigaku Miniflex 600 were used to examine the crystallinity of the nanocomposite. The measurements were carried out at an operational voltage of 40 KV, and a current of 15 mA. To monitor the XRD patterns from 10° to 80° , a scan rate of 2° per min with a step size of 0.02° was employed. Raman spectra were recorded using an Alfa 300 Witec Raman spectrometer that used a 532 nm laser. The Fourier transform infrared spectrophotometer (FTIR) manufactured by PerkinElmer was used to examine the vibrational spectra of GO, $\alpha\text{-Fe}_2\text{O}_3$, and $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}$ nanocomposite. To determine the crystallographic structure of the nanocomposites, high-resolution transmission electron microscopy (HR-TEM) using the JEOL 2100F was used. Using a field-emission scanning electron microscope (FE-SEM, Nova nano-450), the elemental analysis and surface morphology of nanocomposites were observed. X-ray photoelectron spectroscopy (XPS) using a Thermo Scientific K-Alpha was used to analyze the elemental composition and chemical state of the material. The electrokinetics studied were performed using cyclic voltammetry and differential pulse voltammetry techniques. The single-channel electrochemical analyzer (Metrohm Autolab) was used for all of the electrochemical measurements. The three-electrode electrochemical cell system contained platinum as a counter, Ag/AgCl as a reference electrode, and ITO as a working electrode in 0.2 M PBS containing 5 mM of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as a redox probe. Moreover, UPLC-MS/MS was performed using SYNAPT G2 connected to a nanoACQUITY UPLC System, Waters, equipped with a BEH-C18 column having a $1.7 \mu\text{m}$ particle size and $130 \text{ \AA}$ pore size using the polarity mode as ES negative with the acquisition mass ranging from 100 to 200 Da.

4.2.4 Fabrication of α -Fe₂O₃/rGO Thin Film Using EPD Technique

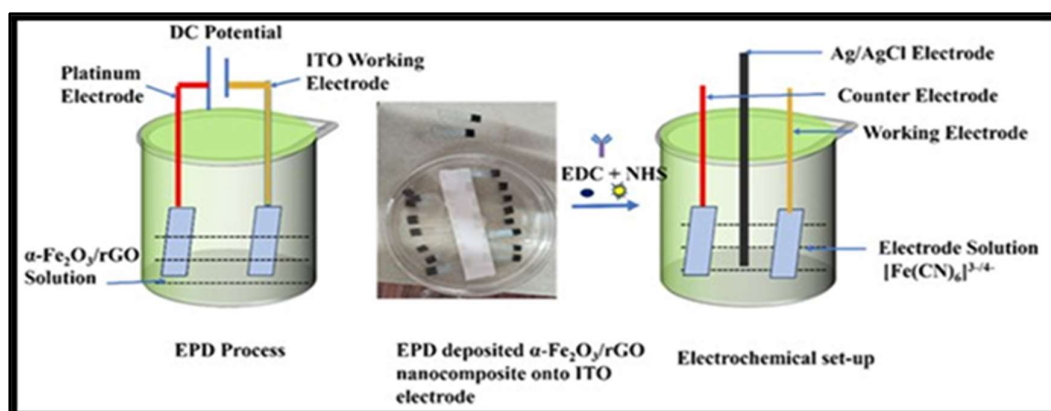
Firstly, the ITO-coated glass electrodes were hydrolyzed with a combination of de-ionized water, liquid ammonia, and hydrogen peroxide in a 5:1:1 ratio to increase the hydrophilicity of ITO-coated glass substrates. After being heated to 80°C for approximately 2 h, these hydrolyzed electrodes were rinsed with ethanol and distilled water and then dried for an additional 2 h at 60°C. These hydrolyzed ITO glass electrodes were used as such for the fabrication of immunoelectrodes for FB₁ detection. For deposition of nanocomposite onto the ITO electrode, the EPD process was used. In detail, 140 μ L of a 1 mg/mL suspension of synthesized α -Fe₂O₃/rGO nanocomposites was deposited onto ITO-coated glass substrates at room temperature. A DC power supply with a voltage of 60 V was applied for 6 min to achieve a smooth and uniform deposition of the α -Fe₂O₃/rGO nanocomposite. Compared to other evaporative methods, the EPD method of thin film deposition is more energy-efficient, low-cost, requires minimal apparatus, and is very adaptable [244, 245].

4.2.5 Thin Film Fabrication Process of Immunoelectrode

Antibodies specific to FB₁ (ab-FB₁) in a sodium phosphate buffer, NHS (0.1 M), and EDC (0.4 M) were mixed in a 2:1:1 ratio, respectively, which was then left at 4°C for approximately 1 h. Subsequently, 20 μ L of EDC-NHS activated anti-FB₁ solution was uniformly drop-casted onto the α -Fe₂O₃/rGO surface and kept at room temperature in a humid environment for about 6 h. During this, an electrostatic interaction occurred between the positively charged α -Fe₂O₃/rGO nanocomposites and the carbonyl group of ab-FB₁, resulting in stable metal ester bonds, that is, amide bond (–CO–NH) formation. Then the ab-FB₁/ α -Fe₂O₃/rGO/ITO immunoelectrode was washed with PBS (pH 7.4) to wash out free ab-FB₁ from the electrode surface. Further, the non-specific sites on the surface of the ab-FB₁/ α -Fe₂O₃/rGO/ITO immunoelectrode were blocked by BSA (10 μ L) and kept for 4 h in a humid chamber at room temperature (25°C). After the fabrication of the BSA/ab-FB₁/ α -Fe₂O₃/rGO/ITO immunoelectrode, it was rinsed with PBS with a pH value of 7.4. After rinsing, the immunoelectrode was stored at 4°C for further study. The complete steps followed for the fabrication of the immunoelectrode (BSA/ab-FB₁/ α -Fe₂O₃/rGO/ITO) are mentioned in Scheme 4.1a,b.



Scheme 4.1(a): Diagram illustrating the synthesis of $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}$ nanocomposite and the subsequent fabrication of an immunoelectrode onto an ITO-coated glass surface to detect *FBI*.



Scheme 4.1(b): EPD set-up used for the EPD of $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}$ nanocomposite onto the ITO electrode surface under the applied voltage for a period of time, followed by surface activation, immobilization, and detection of *FBI* using the electrochemical set-up of a three-electrode system comprised of working, counter, and reference electrode

4.2.6 Preparation of Real Corn Samples for Biosensor and UPLC-MS/MS Study

Fresh sweet corn was purchased at the neighborhood market, Munirka, Delhi. Purchased sweet corn was processed for real sample preparation to carry out the validation study of the fabricated electrochemical biosensor. Firstly, 1 g of sweet corn was crushed and then dispersed in 20 mL of a methanol and DI water (8:2 v/v)

mixture and shaken for 30 min on a vortex mixer, and then centrifuged at 4000 rpm for 16 min. The resultant supernatant was collected, and several dilutions were made with various concentrations of *FBI* (such as 1 and 10 ng mL⁻¹, 100 ng mL⁻¹, and 400 ng mL⁻¹). Then, the different prepared dilutions were tested against the fabricated immunoelectrode BSA/ab-*FBI*/α-Fe₂O₃/rGO/ITO and measured under similar conditions with the DPV technique. Also, the prepared dilutions of 2 mL volume were tested with the UPLC-MS/MS technique.

4.3 Results and discussion

4.3.1 XRD Studies

XRD spectrum of GO nanosheets for the structural analysis was recorded as illustrated in **Figure 4.1A(a)**. The diffraction pattern of GO at 26.32° and 54.8° corresponds to the (002) and (004) (JCPDS 41-1487), and the data were recorded over a 2θ angle from 10° to 80° range [240]. There is no peak at 2θ = 10.67° because GO was used as the edge-oxidized form (EOGO). The interlayer spacing between the adjacent graphene sheets was determined by Bragg's law (**equation 4.1**):

$$n\lambda = 2d \sin\theta \quad \dots \dots \dots [4.1]$$

where d is the distance between the atomic layers, θ is the diffraction peak, λ is the wavelength of the incident X-ray beam, and n indicates the order of diffraction (an integer). Using Equation (4.1), the d value was obtained as 0.338 nm [246]. **Figure 4.1A(b,c)** shows the XRD patterns of α-Fe₂O₃ nanoparticles and α-Fe₂O₃/rGO nanocomposite, respectively. The lattice planes (012), (104), (110), (113), (024), (116), (018), (214), (300), (1010), and (220) correlated to the several diffraction peaks found in the α-Fe₂O₃ XRD pattern, which were located at 24.06°, 33.04°, 35.52°, 40.76°, 49.34°, 53.96°, 57.36°, 62.32°, 63.86°, 71.82°, and 75.26°. The α-Fe₂O₃ nanoparticles matched well with the reported JCPDS number 33-0664, with cell parameters of a : 5.035 Å and c : 13.748 Å [247, 248]. The degree of crystallization of the α-Fe₂O₃ changed from a rhombohedral to a hexagonal shape over the surface of rGO owing to the occurrence of various functional groups in rGO [249].

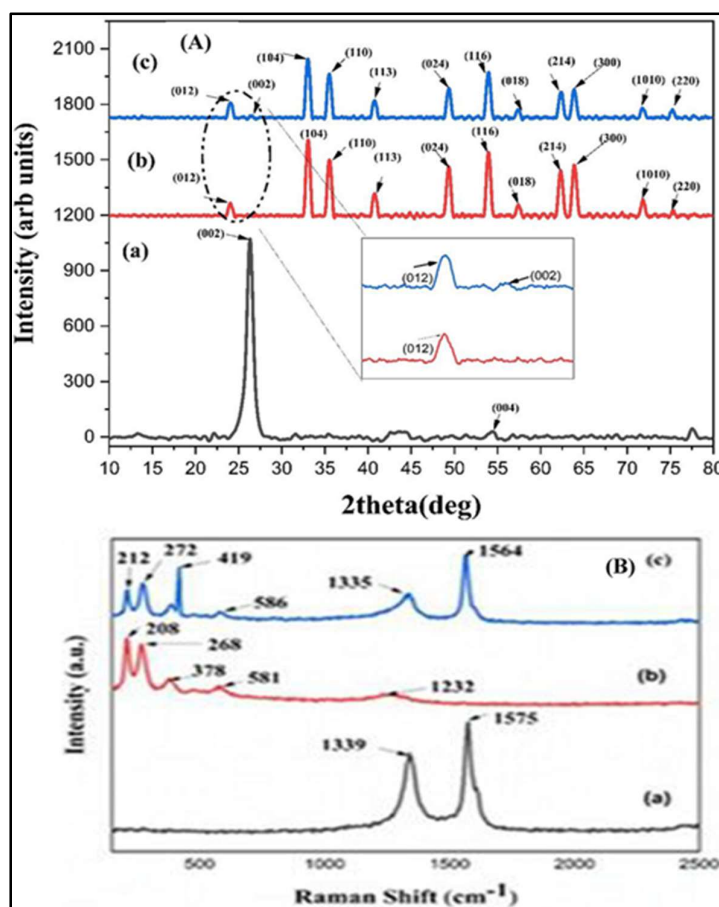


Figure 4.1: (A) XRD pattern of (a) GO, (b) α -Fe₂O₃, and (c) α -Fe₂O₃/rGO nanocomposite [Inset image: XRD of (b) α -Fe₂O₃ and (c) α -Fe₂O₃/rGO], (B) Raman spectra of (a) GO, (b) α -Fe₂O₃, and (c) α -Fe₂O₃/rGO nanocomposite

The Scherrer equation (**equation 4.2**) was used to evaluate the average crystallite size (D) of α -Fe₂O₃, which was found to be 17.97 nm:

$$D = \frac{0.9\lambda}{\beta \cos(\theta)} \quad \dots\dots\dots [4.2]$$

where θ is Bragg's diffraction angle, β is the full width at half maxima (FWHM) of the diffraction peaks, and $\lambda = 1.54 \text{ \AA}$. The α -Fe₂O₃ and α -Fe₂O₃/rGO nanocomposite XRD diffraction peaks matched quite well. The presence of α -Fe₂O₃ nanoparticles on the rGO sheets is demonstrated by the XRD peaks in **Figure 4.1A(c)**. Furthermore, it also indicates that the graphene nanosheet surface was decorated with α -Fe₂O₃ nanoparticles. Using the Scherrer equation above, the average crystallite size (D) of α -Fe₂O₃/rGO was determined to be 18.55 nm.

4.3.2 Raman Study

Raman scattering spectroscopy was used to further examine the pure phase development of α -Fe₂O₃/rGO nanocomposites; the findings are shown in **Figure 4.1B(c)**. The Raman spectra of α -Fe₂O₃ shown in **Figure 4.1B(b)** present peaks at 208, 268, 378, and 581 cm⁻¹. The peaks at 208 and 268 cm⁻¹ are assigned to the A_{1g} band due to vibrations along the *c*-axis of iron, while the E_g modes at 378 and 581 cm⁻¹ are due to symmetric breathing vibrations of the O atom [243]. In **Figure 4.1B(a)**, the D-band, which peaks at 1339 cm⁻¹, is indicative of defects and disorders in the rGO layers, and its intensity rises as the number of defects and disorders increases. The G-band is represented by the peak at 1575 cm⁻¹ (E_{2g}) [250]. The average sp² domain size and degree of disorder are assessed using the D-band to G-band intensity ratio (*I_D/I_G*). The *I_D/I_G* ratio (0.8535) of the nanocomposite α -Fe₂O₃/rGO increases as compared with the *I_D/I_G* ratio (0.8501) of GO. The reduction of the oxygen-containing functional groups and the interaction between the rGO sheets and the α -Fe₂O₃ nanoparticles are the causes of the increase in *I_D/I_G*. The successful preparation of the α -Fe₂O₃/rGO nanocomposite using the hydrothermal method is confirmed by the observation of α -Fe₂O₃ and rGO Raman peaks.

4.3.3 FTIR Study of prepared nanomaterials

FTIR was used to analyze the infrared absorption properties of α -Fe₂O₃/rGO nanocomposites. FTIR spectra of the nanocomposite were obtained in the mid-IR range of 400–4000 cm⁻¹ in high-energy mode using KBr, and the spectrum is depicted in **Figure 4.2**. The spectra of α -Fe₂O₃ nanoparticles show absorption at about 3429, 1638, 553, and 475 cm⁻¹. The absorption peaks at 3429 and 1638 cm⁻¹ are assigned to bending and stretching vibrations of OH groups of H₂O [251, 252]. The bands at 553 and 475 cm⁻¹ are assigned to the Fe-O stretching vibration modes in α -Fe₂O₃. In rGO, the peaks at 3448 and 1579 cm⁻¹ are ascribed to the O–H and C–OH stretching vibrations, respectively [253].

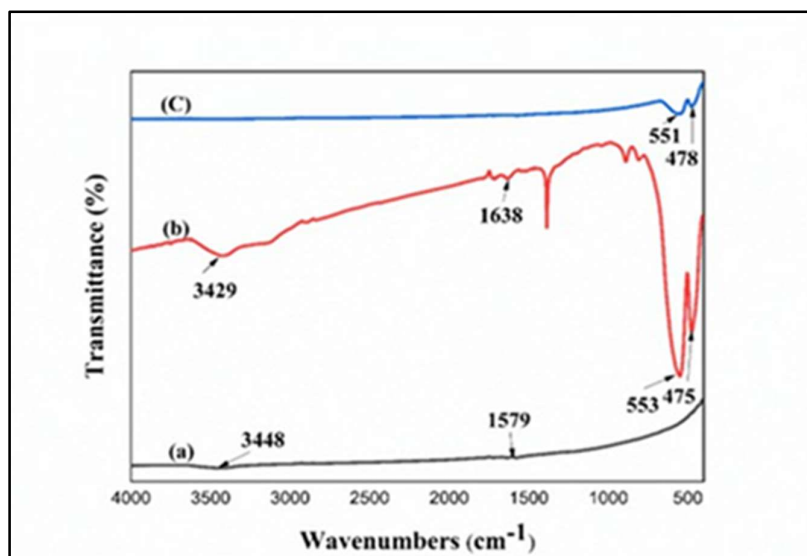


Figure 4.2: FTIR spectra of (a) GO, (b) α -Fe₂O₃, and (c) α -Fe₂O₃/rGO nanocomposite

4.3.4 FTIR study of Modified ITO Substrate

The immobilization of ab-*FBI* and BSA was studied on the surface of the α -Fe₂O₃/rGO/ITO immunoelectrode using attenuated total reflectance (ATR) FTIR technique in the mid-IR range of 4000–400 cm⁻¹. The results are shown in **Figure 4.3**. **Figure 4.3a** shows distinctive peaks at 1412, 1057, and 539 cm⁻¹. The two peaks that appeared at 1412 and 1057 cm⁻¹ correspond to O–H stretching vibration and –O– stretching vibration in the carboxylic acid, respectively, present in the graphene oxide, while the other peak at 539 cm⁻¹ corresponds to the presence of Fe–O vibration bands in the α -Fe₂O₃ nanoparticles. In **Figure 4.3b**, the peaks appeared at 3359, 1632, and 1573 cm⁻¹, which were attributed to O–H stretching vibrations, primary amide N–H of the fabricated, and NH₂ group deformation (amide-II bond), and primary amide N–H of the fabricated ab-*FBI*/ α -Fe₂O₃/rGO/ITO immunoelectrodes [259]. The FTIR spectrum of the BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO immunoelectrode was also performed to confirm that the non-specific binding site of the antibody on the electrode surface was blocked by BSA [255]. As shown in **Figure 4.3c**, the FTIR spectrum showed a characteristic decrease in the peak intensity of ab-*FBI*, and the peak position of the Fe–O bond was shifted from 539 to 534 cm⁻¹ at α -Fe₂O₃ nanoparticles [256]. The above studies showed that ab-*FBI* and BSA could be successfully immobilized on the surface of α -Fe₂O₃/rGO/ITO electrodes.

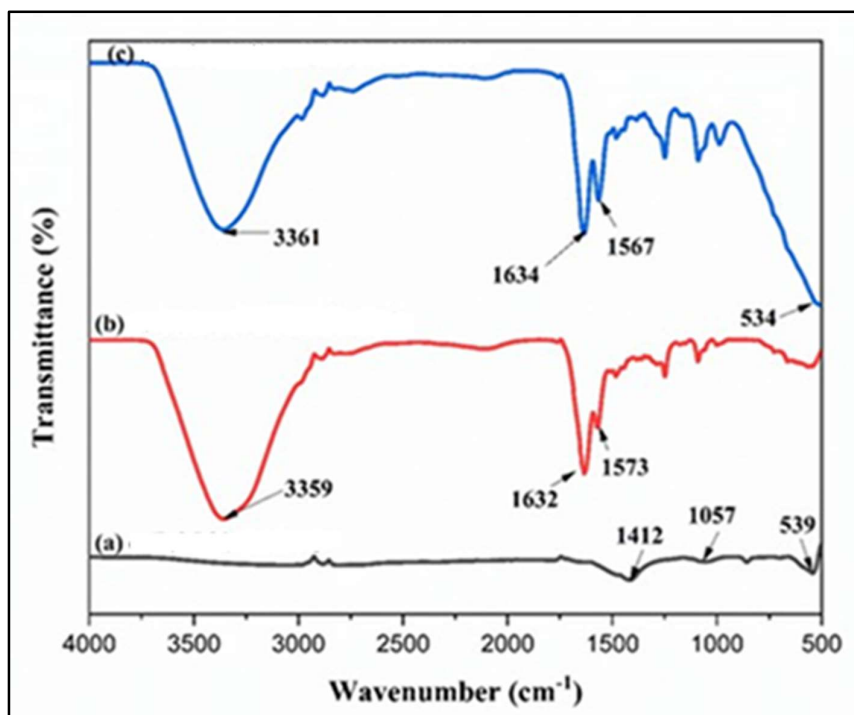


Figure 4.3: FTIR spectra of (a) α -Fe₂O₃/rGO/ITO, (b) ab-FBI/ α -Fe₂O₃/rGO/ITO, (c) BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO nanocomposite

4.3.5 Elemental and Morphological Study

The surface morphology and internal structure of the synthesized nanocomposite α -Fe₂O₃/rGO were analyzed using FE-SEM. **Figure 4.4** exhibits the FE-SEM micrographs of the nanocomposites at different measuring scales. **Figure 4.4** shows the surface morphology of the α -Fe₂O₃/rGO nanocomposite. It shows the presence of the α -Fe₂O₃ on the top surface of rGO and does not become detached due to the strong bonding between the α -Fe₂O₃ nanoparticles and the rGO sheets. **Figure 4.4** shows the EDX spectrum to analyze the elemental composition in the α -Fe₂O₃/rGO nanocomposite. The elemental composition of carbon, iron, and oxygen is mentioned in the inset of **Figure 4.4**. However, the percentage by weight and atomic weight were found to be 79.50% and 100%, respectively (Table inset of **Figure 4.4**).

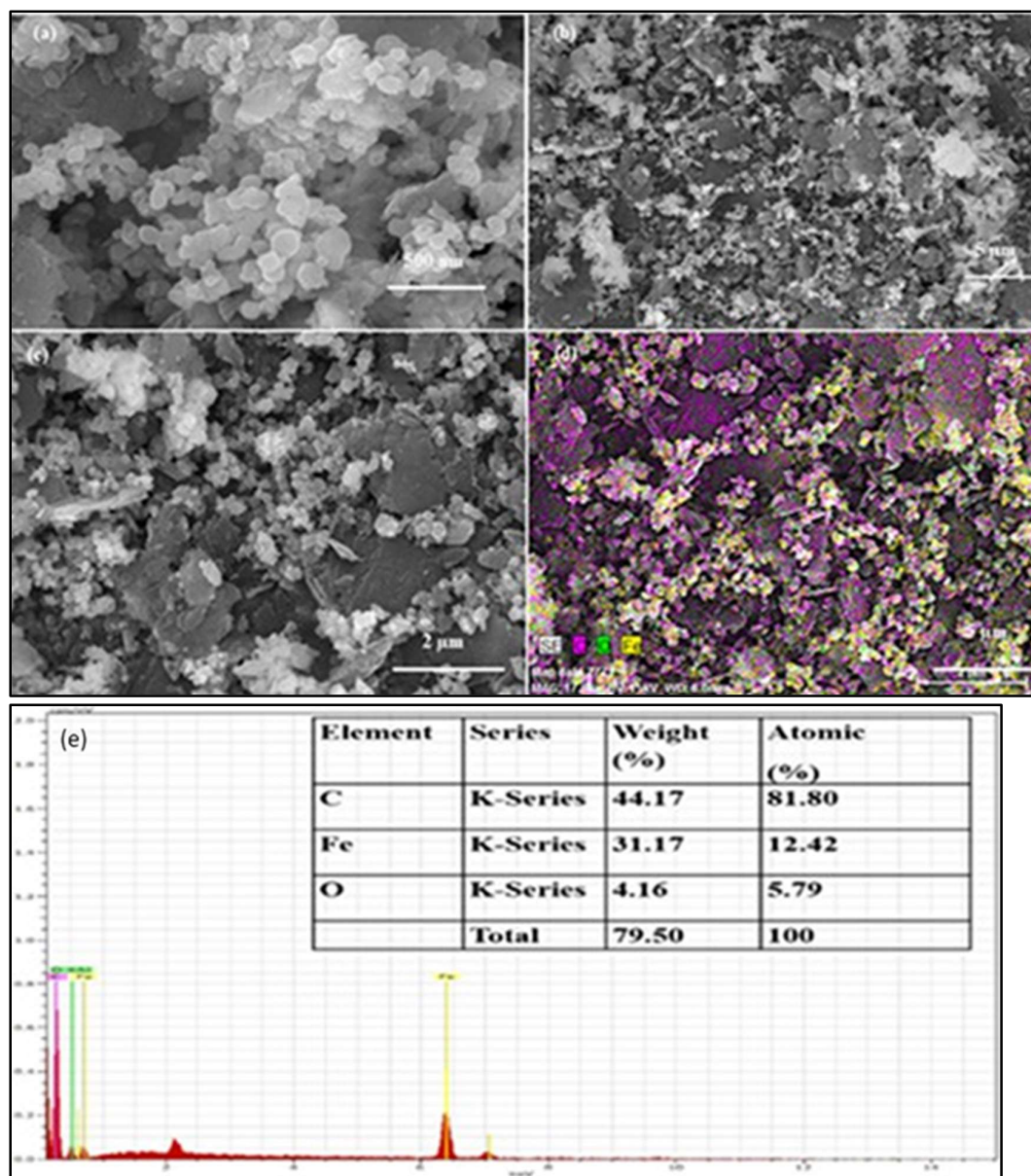


Figure 4.4: FE-SEM of α -Fe₂O₃/rGO nanocomposite at (a) 500 μ m, (b) 5 μ m, (c) 2 μ m, (d) mapping, (e) Elemental spectra of α -Fe₂O₃/rGO nanocomposite

This shows the formation of the α -Fe₂O₃/rGO nanocomposite. An intensive TEM study was also done to find out the shape and size of the as-synthesized α -Fe₂O₃/rGO nanocomposites using an in-situ synthesis method. **Figure 4.5** depicts the typical TEM micrograph of the nanocomposites α -Fe₂O₃/rGO at different measuring scales. It indicates that the synthesized nanoparticles are hexagonal in structure with an average particle size of 24.82 nm. The nanoparticles are found to be connected due to

agglomeration. It shows the dispersion of nanoparticles onto the surface of the rGO nanosheets. These TEM micrographs showed the formation of the nanocomposites α -Fe₂O₃/rGO, which was also investigated with a selected area electron diffraction (SAED) pattern of the α -Fe₂O₃/rGO nanocomposite with well-matched indices.

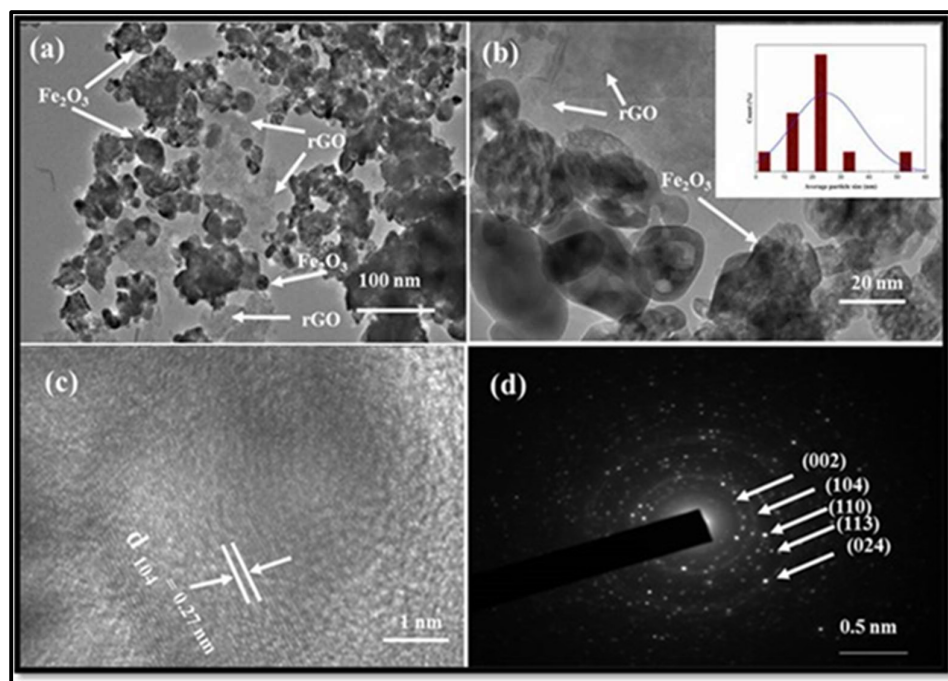


Figure 4.5: (a,b) illustrates the HR-TEM images of α -Fe₂O₃/rGO nanocomposite and particle size distribution graph [inset image]; (c) Lattice image of α -Fe₂O₃/rGO, and (d) SAED pattern of α -Fe₂O₃/rGO nanocomposite

4.3.6 XPS Study of α -Fe₂O₃/rGO nanocomposite

XPS is used to study the chemical structure and identify the functional groups in the nanocomposite of α -Fe₂O₃ and rGO, and any potential changes due to *FBI*. To explore this further and gain more insights into the properties and characteristics of these elements, a survey of α -Fe₂O₃ and rGO hybrid nanocomposites is conducted. The results of this survey, as shown in **Figure 4.6**, are depicted as high-resolution Fe2p, O1s, C1s, and S2p peaks, which are then deconvoluted into individual components for further analysis. Specifically, the Fe2p spectra (**Figure 4.6**) show two separate peaks representing Fe2p_{3/2} at 703.1 eV and Fe2p_{1/2} at 715.5 eV, respectively [257]. It shows that the composition of the iron particles remains unchanged by the graphene. The predicted graphitic carbon peaks for O=C–OH and sp² are found to be present in the high-resolution XPS spectrum of C1s at 289.3 and 284.3 eV,

respectively (**Figure 4.6c**) [258]. Regarding the O1s spectra, a peak appears at 530.8 eV that shows the Fe-O bond present in α -Fe₂O₃ (**Figure 4.6d**) [259]. **Figure 4.7** shows the XPS spectra of *FBI* onto the α -Fe₂O₃/rGO immunoelectrode. It shows that after interaction of *FBI*, the relative intensity of the C 1s peak increases, while the Fe2p signal peak decreases, as it may attenuate the underlying hematite due to the formation of an organic bio-layer [260].

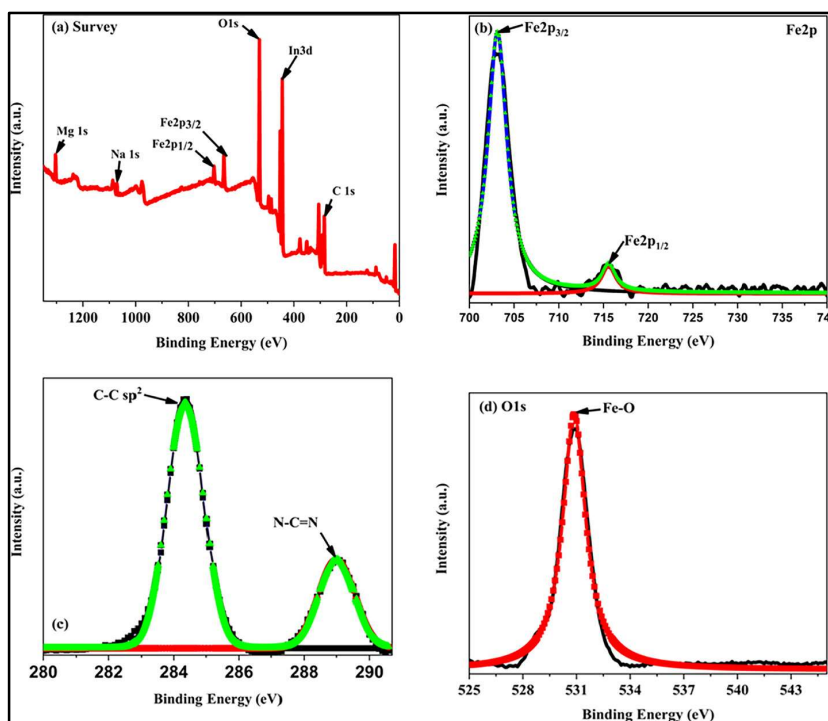


Figure 4.6: XPS spectra (a) Survey spectrum of α -Fe₂O₃/rGO nanocomposite, (b) Deconvolution spectra of Fe2p, (c) C1s spectra, and (d) O1s spectra of α -Fe₂O₃/rGO nanocomposite

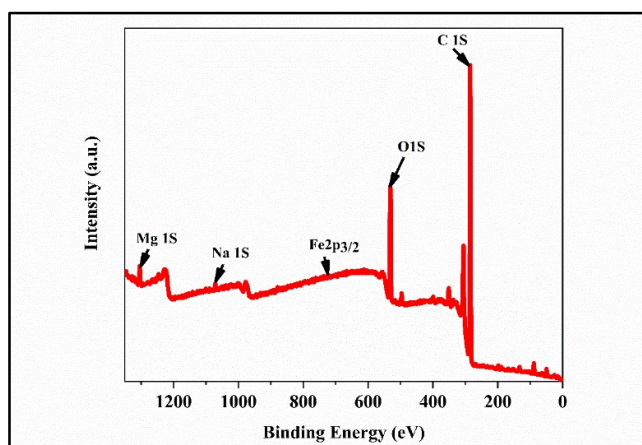


Figure 4.7: shows the survey of XPS spectra of *FBI* over the α -Fe₂O₃/rGO/ITO immunoelectrode

4.3.7 Electrochemical study

Electrochemical characterization studies, including CV, DPV, and EIS, were performed for the fabricated immunoelectrodes—ITO, α -Fe₂O₃/rGO/ITO, ab-*FBI*/ α -Fe₂O₃/rGO/ITO, and BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO to investigate their electrochemical behavior and interfacial electron transfer activity. The measurements were carried out in PBS containing a ferri/ferro redox mediator at a 50 mV/s scan rate using a galvanostat/potentiostat (Autolab).

4.3.7.1 Cyclic Voltammetry study

The CV response signal of fabricated electrode surfaces of (i) ITO, (ii) α -Fe₂O₃/rGO/ITO, (iii) ab-*FBI*/ α -Fe₂O₃/rGO/ITO, and (iv) BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO is depicted in **Figure 4.8a**. It was found that the anodic and cathodic peak currents for nanomaterials deposited onto the ITO surface (α -Fe₂O₃/rGO) were higher (i.e., 331.02 μ A) in comparison to the anodic and cathodic current response (250.05 μ A) after immobilization of antibodies of *FBI* (ab-*FBI*/ α -Fe₂O₃/rGO/ITO). This happened due to the presence of α -Fe₂O₃/rGO nanomaterials over the ITO surface, which provides a large surface-to-volume ratio, improves electro-catalytic activity, and enhances electron conductivity [244]. After immobilization of antibodies of *FBI* onto the α -Fe₂O₃/rGO/ITO surface, the electrochemical current response was reduced owing to the existence of dense, large molecules of antibodies. The current is lower than that of the bare ITO as a result of adding a functional layer on the ITO. Its purpose is to enhance the sensitivity and selective sensing of the *FBI*.

Further, the ab-*FBI*/ α -Fe₂O₃/rGO/ITO immunoelectrode was treated with BSA, which acts as a blocking agent to prevent non-specific binding of antibodies to the electrode surface, and the current response increased (301.31 μ A), indicating that BSA helps in the fast movement of electrons [261].

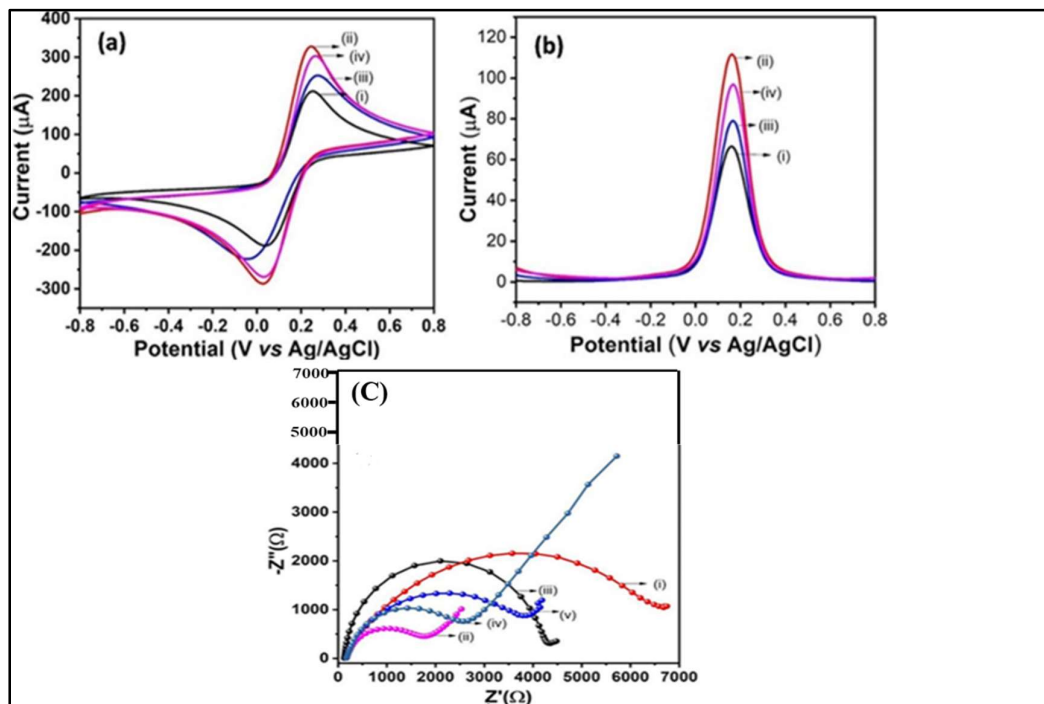


Figure 4.8: (a) CV response study of the (i) ITO, (ii) α -Fe₂O₃/rGO/ITO, (iii) ab-FBI/ α -Fe₂O₃/rGO/ITO, (iv) BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO electrode surfaces, (b) DPV responses obtained from four modified electrode surfaces: (i) ITO (ii) α -Fe₂O₃/rGO/ITO, (iii) ab-FBI/ α -Fe₂O₃/rGO/ITO, (iv) BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO electrode surface, (c) EIS response of (i) ITO, (ii) α -Fe₂O₃/rGO/ITO, (iii) ab-FBI/ α -Fe₂O₃/rGO/ITO, (iv) BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO, (v) FBI/BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO

4.3.7.2 Differential pulse voltammetry study

Similarly, the DPV response signal of fabricated electrode surfaces of (i) ITO, (ii) α -Fe₂O₃/rGO/ITO, (iii) ab-FBI/ α -Fe₂O₃/rGO/ITO, and (iv) BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO are depicted in **Figure 4.8b**. DPV measurements were performed at the applied potential values varying from -0.8 to +0.8 V in PBS buffer solution having [Fe(CN)₆]^{3-/4-} at pH 7.4. It was found that the peak current for nanomaterials deposited onto the ITO surface (α -Fe₂O₃/rGO) was higher, that is, 112.2 μ A, in comparison to the current response (78.38 μ A) after immobilization of antibodies of FBI (ab-FBI/ α -Fe₂O₃/rGO/ITO). This happens due to the presence of α -Fe₂O₃/rGO over the ITO surface, which provides a large surface-to-volume ratio, improves

electro-catalytic activity, and enhances electron conductivity. After immobilization of antibodies of *FBI* onto the α -Fe₂O₃/rGO/ITO surface, the electrochemical current response is decreased owing to the occurrence of denser biomolecules of antibodies. After that, the fabricated electrode surface was then washed with BSA (**Figure 4.8b[iv]**) to remove any non-binding active sites of the surface; therefore, the magnitude of the current was increased to 96.84 μ A.

4.3.7.3 Electrochemical impedance spectroscopy study

EIS is a powerful and sensitive technique to investigate the physicochemical processes occurring at the electrode-electrolyte interface. The EIS potentiometric Nyquist plot presents a frequency-dependent electrochemical behavior from the high-frequency to lower-frequency region; the semicircle diameter reveals the charge transfer process, and a straight line presents the diffusion process. The Nyquist plot, fitted using the Randles equivalent circuit, provides in-depth electrochemical interfacial analysis, including charge transfer resistance value, double layer capacitance, Warburg impedance, and so forth. The progressive change in such parameters with each step modification provides clear evidence for successful electrode surface modification and binding with *FBI* analyte. Therefore, **Figure 4.8c** shows the EIS Nyquist plot results of stepwise modified ITO electrodes in PBS (pH 7.0) containing 5 mM [Fe(CN)₆]^{3-/4-}, as a function of frequency in the range of 0.01–100 kHz with an AC amplitude of 10 mV. The EIS study of (i) ITO, (ii) α -Fe₂O₃/rGO/ITO, (iii) ab-*FBI*/ α -Fe₂O₃/rGO/ITO, and (iv) BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO electrode surfaces. The EIS response of (i) ITO, (ii) α -Fe₂O₃/rGO/ITO, (iii) ab-*FBI*/ α -Fe₂O₃/rGO/ITO, (iv) BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO, and (v) *FBI*/BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO is depicted in **Figure 4.8c**. **Figure 4.8c(i)** shows the EIS spectrum of ITO, which has an R_{CT} value of 3.98 k Ω . After EPD thin film deposition of α -Fe₂O₃/rGO onto ITO (**Figure 4.8c (ii)**), the R_{CT} decreased to 1.26 k Ω due to its high intrinsic electrical conductivity, which provides an efficient charge transfer pathway. Therefore, it facilitates fast electron transfer between the electrode and electrolyte, reduces the energy barrier for charge transfer, and consequently lowers the R_{CT} value. The charge transfer resistance value increased to 3.60 k Ω after the immobilization of anti-*FBI* over the α -Fe₂O₃/rGO/ITO

electrode (**Figure 4.8c[iii]**). This happens due to the loading of heavier, non-conducting protein biomolecules over the $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$ surface, which form an insulating biolayer between the electrode and the electrolyte, thereby creating resistance to electron transfer between them. Therefore, the efficient charge transfer process gets affected; hence, the R_{CT} value increased. When the immobilized electrode $\text{ab-FBI}/\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$ was treated with BSA, the R_{CT} value was found to be 1.80 k Ω , which is lower than the R_{CT} value obtained after immobilization of $\text{ab-FBI}/\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$ (**Figure 4.8c[iv]**). This happens because BSA is a blocking agent that passivates the electrode surface and removes unbound or weakly adsorbed antibodies, leaving behind a more stable and oriented antibody layer, thus reducing the resistance and improving electron transfer kinetics. The fabricated immunoelectrode $\text{BSA}/\text{ab-FBI}/\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$ was tested with a 100 ng/mL concentration of *FBI* (**Figure 4.8c[v]**); the R_{CT} value increased to 2.54 k Ω due to the formation of an immunocomplex between ab-FBI and *FBI*, which creates resistance in the transfer of electrons between the electrode and the electrolyte; therefore, it impedes the electron transfer pathway.

4.3.7.4 A study of the electrokinetic scan rate

Using the cyclic voltammetry technique, the electrokinetic interface characteristics were studied using the CV technique for electrodes $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$, $\text{ab-FBI}/\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$, and $\text{BSA}/\text{ab-FBI}/\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$ with a change in scan rate values from 10 to 100 mV/s, as illustrated [**Figure 4.9 (a-c)**]. The peak anodic (I_a) and cathodic (I_c) redox currents for the immunoelectrodes $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$, $\text{ab-FBI}/\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$, and $\text{BSA}/\text{ab-FBI}/\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$ were computed at a scan rate of 50 mV/s.

In **Figure 4.9 (a-c)**, where the anodic (I_a) and cathodic (I_c) currents are plotted against the square root of the scan rate, respectively, the observed linear relationship suggests that the electrochemical processes occurring at the electrode surface are diffusion-controlled. The peak current ratios (I_a/I_c) for $\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$, $\text{ab-FBI}/\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$, and $\text{BSA}/\text{ab-FBI}/\alpha\text{-Fe}_2\text{O}_3/\text{rGO}/\text{ITO}$ were found to be 1.18, 1.17, and 1.16, respectively. These calculated results represent a quasi-reversible process for

electron transfer. Moreover, the value of ΔE_p for the BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO electrode was calculated to be 0.25 V by applying the formula $\Delta E_p = E_{pa} - E_{pc}$, where E_{pc} and E_{pa} denote the anodic and cathodic potentials. The computed potential, which was greater than that of the ab-FBI/ α -Fe₂O₃/rGO/ITO electrode, indicated that the immunoelectrode and the redox solution had effective electron transfer.

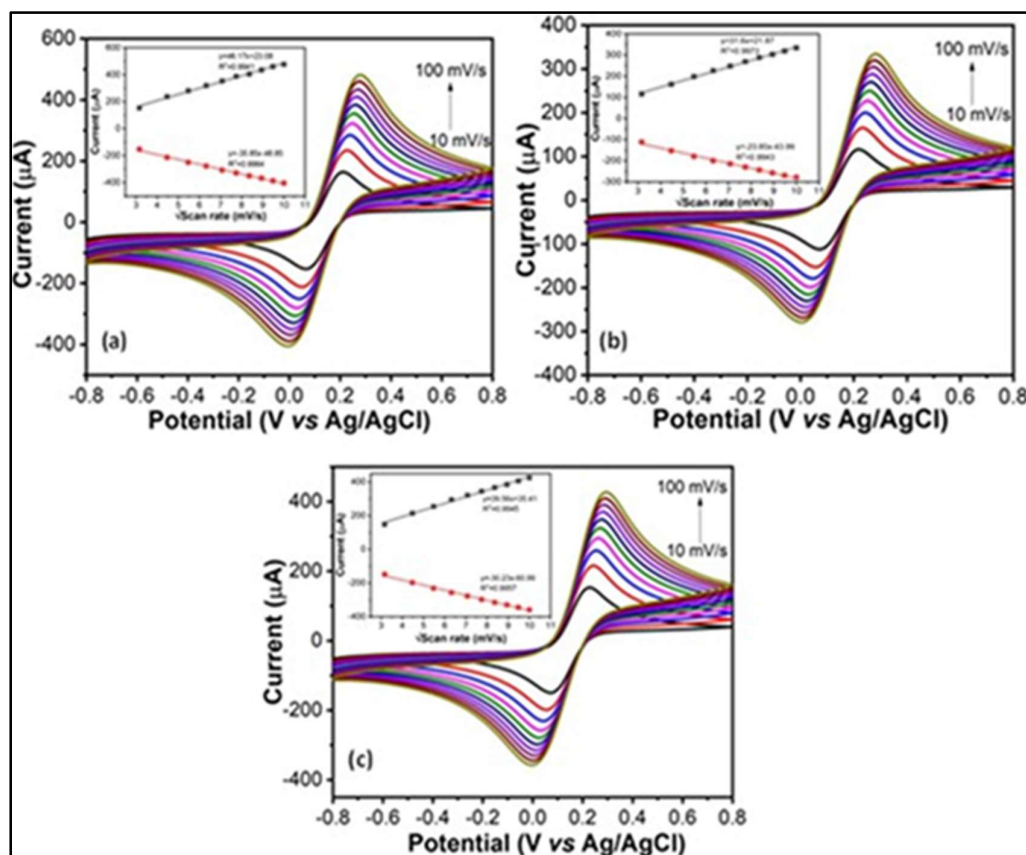


Figure 4.9: Scan rate study conducted across a range of 10 to 100 mV/s and presents linear plots between the square root of the scan rate and the peak current for three different immunoelectrodes: (a) α -Fe₂O₃/rGO/ITO, (b) ab-FBI/ α -Fe₂O₃/rGO/ITO, and (c) BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO, respectively

As the scan rate increases, peak separation widens because the electrode reaction has less time to occur at the peak potentials, leading to a quasi-reversible or irreversible process. Peak current increases due to faster diffusion of more species to the electrode surface in the shortened time frame; the slower kinetics of the electron transfer prevent the peaks from staying close together, increasing the separation.

Figure (4.9) shows the linear response of the anodic and cathodic peaks current varied

linearly with the square root of scan rate, and the linear response (**equations 4.3**) for α -Fe₂O₃/rGO/ITO, (**equations 4.4**) for ab-FBI/ α -Fe₂O₃/rGO/ITO, and BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO fabricated electrodes linear response (**equations 4.5**) are as follows:

$$\begin{aligned} I_a &= 23.08 \mu A + 46.17 \mu A/[mV/s]^{1/2} \times \text{scan rate } [mV/s]^{-1/2}, R^2=0.9941 \\ I_c &= 35.85 \mu A - 48.85 \mu A/[mV/s]^{1/2} \times \text{scan rate } [mV/s]^{-1/2}, R^2=0.9964 \end{aligned} \quad [4.3]$$

$$\begin{aligned} I_a &= 21.87 \mu A + 31.6 \mu A/[mV/s]^{1/2} \times \text{scan rate } [mV/s]^{-1/2}, R^2=0.9973 \\ I_c &= 23.85 \mu A - 43.99 \mu A/[mV/s]^{1/2} \times \text{scan rate } [mV/s]^{-1/2}, R^2=0.9943 \end{aligned} \quad [4.4]$$

$$\begin{aligned} I_a &= 35.41 \mu A + 39.56 \mu A/[mV/s]^{1/2} \times \text{scan rate } [mV/s]^{-1/2}, R^2=0.9945 \\ I_c &= 30.23 \mu A - 60.99 \mu A/[mV/s]^{1/2} \times \text{scan rate } [mV/s]^{-1/2}, R^2=0.9957 \end{aligned} \quad [4.5]$$

Equation [4.6] (the Randles-Sevcik equation) will be used to calculate the diffusivity (D) of the immunoelectrode.

$$I_p = (2.69 \times 10^5) C n^{3/2} D^{1/2} \nu^{1/2} A \quad [4.6]$$

Where I_p is the electrode's current, C is the electrolyte concentration, n is the number of electrons, A represents the working electrode's surface area, and ν is the scan rate (mV/s). The diffusivity for BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO immunoelectrode was found to be $3.94 \times 10^{-8} cm^2 s^{-1}$. This suggests that there was a faster rate of electron transport at the electro-analytical interface.

To determine the accessibility of binding sites existing at the immunoelectrode, the Randles-Sevcik equation, as described below in (**equation 4.7**), was used to calculate the effective electro-active surface area (A_e). The effective surface area was essential for understanding the density of available binding sites for antigens.

$$A_e = \frac{S}{(2.69 \times 10^5) n^3 C D^{1/2}} \quad [4.7]$$

Where S denotes the slope of the line as determined by plotting I_{pa} versus scan rate linearly. An effective electro-active surface area (A_e) of $4.11 mm^2$ suggested that there were numerous binding sites per unit volume on the immunoelectrode surface. This

finding implied that the immunoelectrode possesses a high density of binding sites for antigens.

Furthermore, the following Brown-Anson equation (**equation 4.8**) was used to calculate the concentration of ionic species (I^*) at the surface:

$$I_p = \frac{n^2 F^2 I^* A V}{4RT} \quad [4.8]$$

where T denotes temperature (300 K), R denotes the gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), and F stands for the Faraday constant ($96,485 \text{ C mol}^{-1}$). The other symbols have the same meanings as in the preceding equation. The calculated result of $2.7 \times 10^{-8} \text{ mol cm}^{-2}$ of BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO immunoelectrode indicated a high density of reactive species at the interface.

Moreover, findings of electrochemical parameters of the fabricated immunoelectrode BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO, such as the anodic peak current (I_{pa}), diffusion coefficient, concentration of ionic species (I^*), and effective electro-active surface area (A_e), are summarized in **Table 4.1**.

Table 4.1: Electrochemical characteristics of the BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO immunoelectrode for FBI detection

Immunoelectrode	ΔE_p	$I_{pa} (\mu A)$	$D (\text{cm}^2 \text{ s}^{-1})$	$A_e (\text{mm}^2)$	$I^* (\text{mol cm}^{-2})$
BSA/ab-FBI/ α -Fe ₂ O ₃ /rGO/ITO	0.25 V	98.64	3.94×10^{-8}	4.11 mm ²	2.7×10^{-8}

4.3.7.5 pH Optimization study

The BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO immunoelectrode is subjected to optimization studies to evaluate the effect of buffer pH solution used as an electrolyte to monitor the effect of pH on the electrode surface. The study of pH value is essential as it provides the sensitivity and stability of the biomolecules immobilized on the electrode surface. **Figure 4.10** illustrates the results of the pH investigation conducted on the immunoelectrode, displaying the electrochemical responses monitored using DPV. The pH values were adjusted from 6.0 to 8.0 to study the conformational changes in

ab-*FBI* and BSA within the potential range from -2.0 to $+0.4$ V in an electrolytic solution containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$. At pH 7.4, the maximum current of $77.9 \mu\text{A}$ was attained within the pH range of 6.0 to 8.0. The BSA/ab-*FBI*/ α - Fe_2O_3 /rGO/ITO immunoelectrode was therefore made using pH 7.4 PBS containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$, as the composition of ab-*FBI* may be verified by acidic or basic media.

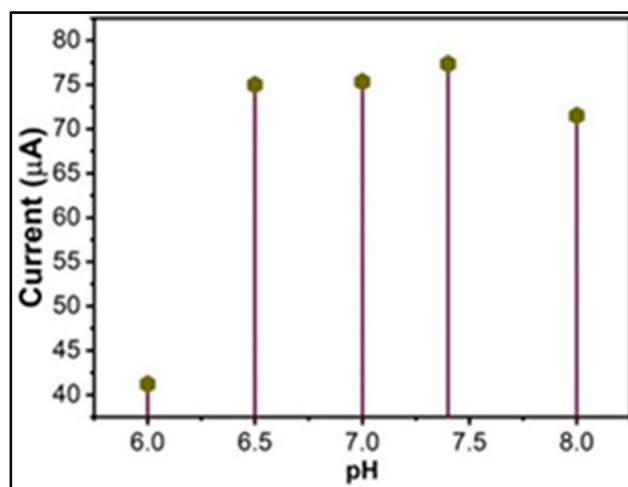


Figure 4.10. pH effect on BSA/ab-*FBI*/ α - Fe_2O_3 /rGO/ITO immunoelectrode in PBS containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$

4.3.7.6 Response study

To study the electrochemical response, the DPV technique was employed. Here, a three-electrode assembly containing a counter (Pt), reference (Ag/AgCl), and working electrode (BSA/ab-*FBI*/ α - Fe_2O_3 /rGO/ITO) in PBS having $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in the -0.2 to 0.4 V potential range was used. The DPV technology is affordable, easy to use, and requires a relatively short analysis time, in which the potential (ΔE) in pulse form is applied in a linear potential range with set increments. The electrochemical response observed in DPV can resemble the first derivative of a linear voltammogram, where the peak originates from the Faradaic response and is located close to $E_{1/2}$. DPV is a valuable technique for investigating redox properties at extremely low concentrations of analytes due to its ability to reduce capacitive currents, improve signal-to-noise ratio, enhance sensitivity, and provide enhanced potential control. DPV's higher sensitivity and selective measurement of faradaic currents enable a more precise and controlled study of electrode reactions compared to linear scanning methods. α - Fe_2O_3 nanostructures serve

as catalytic centers that lower the overpotential required for electrochemical reactions. The interaction between α -Fe₂O₃ and rGO improves detection performance by more than two times compared to standard materials, achieving ultra-low detection limits and increasing sensitivity. The crystalline structure of α -Fe₂O₃ offers numerous bioactive sites for target molecules. Hematite nanoparticles are preferred in biosensing due to their inherent non-toxicity and high stability in biological matrices, which preserves the orientation and integrity of the immobilized antibody layer. It improves biocompatibility and antibody immobilization.

To improve the resolution, sensitivity, and limit of detection for the sensing response study, FBI was analyzed at various levels using the DPV technique (**Figure 4.11**). As a result, FBI concentrations varied from 1×10^{-3} –700 ng mL⁻¹ and were used in electrochemical biosensing investigations, with results displayed in **Figure 4.11a,b** demonstrates a decrease in current response as the concentration of FBI rises from 1×10^{-3} –700 ng mL⁻¹ on the BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO immunoelectrode. This observation was consistent with the expected behavior in an immunoelectrode system due to the formation of an immunocomplex between the immobilized ab-FBI and FBI molecules that prevents electron transfer between the electrode and electrolyte and, as a result, lowers the signal response. The formation of an immunocomplex between antigen and antibody, which has an electrically insulating nature, thus prevents the electron transfer process that takes place between the immunoelectrode and redox species [Fe(CN)₆]^{3-/4-}. **Figure 4.11b** illustrates the calibration curve of FBI concentration plotted against the corresponding current response. This calibration plot demonstrated a linear correlation between the concentration of FBI and the measured current response within the measured range from 0.001 to 700 ng mL⁻¹. By increasing the FBI concentration, no measurable change in current response was recorded. Therefore, linear discrimination in the current signal was found to be in the detection range of 1×10^{-3} –700 ng mL⁻¹. Assuming that the area of the film was 0.25 cm², the sensitivity of the fabricated immunoelectrode can be calculated using the provided formula: sensitivity = slope/area of the film. The immunoelectrode sensitivity was calculated as 6.36 μ A mL ng⁻¹ cm⁻², with a regression coefficient (R^2) of 0.9883. The signal-to-noise ratio (S/N)

of 3 was attained when the experiments were run in triplicate sets with identical experimental conditions. LOD was derived based on the standard formula as $3\sigma/m$ (m is the slope and σ is the standard deviation), and its value is calculated as $0.0052 \text{ ng mL}^{-1}$. The present work shows high sensitivity, selectivity, and specificity, with a wide detection range and a low limit of detection for the *FBI* analyte of mycotoxin using the BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO immunoelectrode. This performance is attributed to the α -Fe₂O₃/rGO composite, which improves the surface-to-volume ratio and provides a larger area for antibody immobilization, increases the catalytic activity of the electrode, offers an easy path for electron transfer, and improves conductivity. In addition, the presence of the functional group of –COOH in the rGO on the ITO surface conjugates with the present –NH₂ groups of *FBI* antibodies, forming strong and stable amide bonds (–CO–NH) bonds, thereby enhancing the stability of the immunoelectrode. The mechanism is driven by the efficient movement of charge carriers across the heterojunction interface. rGO provides high conductivity, which serves as a fast channel for electron movement. α -Fe₂O₃ nanoparticles act as catalytic centers that facilitate redox reactions in specific biosensing applications. The rGO layer introduces new interface states within the band gap of α -Fe₂O₃, effectively reducing the band gap and enhancing overall electronic conductivity. The α -Fe₂O₃/rGO composite enhances electrochemical and sensing signals. The α -Fe₂O₃ nanoparticles on rGO prevent restacking and nanoparticle agglomeration, resulting in a significantly larger electrochemically active surface area for target molecule adsorption.

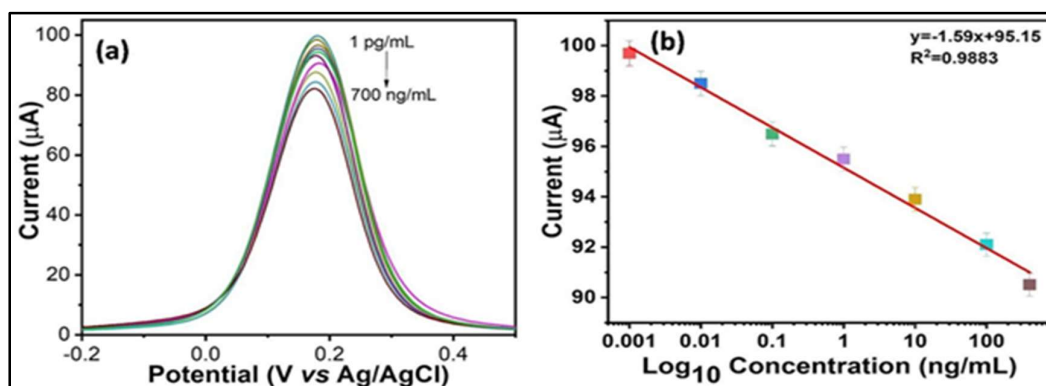


Figure 4.11: (a) DPV biosensing response, (b) a linear plot in the concentration range from 1×10^{-3} to 400 ng mL^{-1} against *FBI* of the BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO immunoelectrode.

Moreover, electrochemical biosensor-based research articles are published for *FBI* detection using nanomaterials. The findings of these articles are summarized in Table 2 and present the results of the current work for *FBI* detection. It can be concluded that the use of an α -Fe₂O₃/rGO composite matrix over the ITO-coated glass electrode surface for immobilization and detection of *FBI* analyte using an electrochemical biosensor improves the biosensing characteristics, such as sensitivity, detection range, and detection limit of the sensor.

Table 4.2: Evaluation of the present study findings with earlier published research articles for FBI detection.

Electrode/ Method	Technique	Detection Range (ng mL ⁻¹)	Sensitivity (μA mL ng ⁻¹ cm ⁻²)	LOD (ng mL ⁻¹)	Ref.
<i>FBI</i> /ss-HSDNA/Au NPs/SPCE	CV	0.5-500	-	0.14	[228]
<i>FBI</i> /GO-CHA	FRET	0.032-500	-	0.0121	[229]
GO/Fe ₃ O ₄	FRET	0.05-300		0.0162	[230]
Ag/CeO ₂	CV	0.01-100	7.33	0.0015	[231]
SWCNT/chitosan	DPV	0.01-1000	-	5	[232]
SPGE	CV	1-1000	-	5	[221]
GCE/PT-PDMA-MWCNT/anti-Fms-BSA	EIS	0.007- 0.049	-	0.00046	[233]
Mn ₃ O ₄ /GO	CV	0.001-800	10.08	0.000195	[234]
DBH/AuNPs/CS@cMWCNTS/GCE	DPV	0.01- 10 ⁶	-	0.0046	[235]
GO	SPCE	200-4500	-	4.2	[236]
Au/GO	CV	0.01-10 ⁵	-	0.01	[237]
Fe ₂ O ₃ @Cu@Cu ₂ O	PEC	0.001-10	-	0.00028	[238]
α-Fe₂O₃/ rGO	DPV	1x10⁻³-700	6.36	0.0052	This work

ss-HSDNA: Single-stranded DNA; DNA: Deoxyribonucleic acid; SPCE: Screen-printed carbon electrode; dcNLISA: Direct competitive nanozyme-linked immunosorbent assay; MPA: Mercaptopropionic acid; CdTe: Cadmium telluride; SPGE: Screen-printed gold electrode; CHA: Catalytic hairpin assembly; PDMA: Poly(2,5-dimethoxyaniline); MWCNT: Multiwalled carbon nanotubes; PT: Palladium telluride; GCE: Glassy carbon electrode; UCNPs: Upconversion nanoparticles; DBH:

Dopamine β -hydroxylase; CS@cMWCNTs: Chitosan-functionalized carboxylated multi-walled carbon nanotubes

4.3.7.7 Reproducibility, repeatability, interferent, and stability study

To study the reproducibility of the BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO immunoelectrode, six different immunoelectrodes were used, and their electrochemical response was monitored using the DPV technique. **Figure 4.12a,b** displays the electrochemical responses obtained from six immunoelectrodes, illustrating significant agreement between specific immunoelectrodes in a similar region, with a standard deviation (RSD) of 0.336%. It was found that all immunoelectrodes show nearly identical responses with very slight variations obtained from six different immunoelectrodes, indicating the stability and reproducibility of immunoelectrodes.

The DPV method was used to test the repeatability of the immunoelectrode BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO; the results obtained are presented in **Figure 4.12c**. Six consecutive readings (2-7 number in X-axis) were taken with *FBI* concentration, showing nearly a stable current value with an RSD of 3.667%. This repeatable response further confirms the presence of ab-*FBI* onto the α -Fe₂O₃/rGO/ITO electrode.

To assess the sensitivity and selectivity of the biosensor alone for *FBI* antigen, it is important to investigate the performance of *FBI* with other interfering analytes over the fabricated immunoelectrode. Therefore, to assess the effect of other analytes on the BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO electrode surface, an interference test was carried out using various interfering analytes, including *AFBI*, *Fructose*, *OchA*, *Starch*, and *DON*, which are generally present in food samples. **Figure 4.12d** displays the DPV response. To determine selectivity, 20 μ L of the interferents was added to an electrolytic solution containing 100 ng/mL of *FBI* mycotoxin. The response was then recorded with the DPV technique. **Figure 4.12e** results show that the developed biosensor, as BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO, shows the same current signals with all other interfering analytes, such as *AFBI*, *Fructose*, *OchA*, and *Starch* in food samples, but the current signal decreased in the presence of *FBI*. Hence, these results show that the developed biosensor is highly selective toward *FBI*. The acquired data

demonstrated the extremely selective nature of the fabricated immunoelectrode, and ab-FBI was primarily specific for FBI.

To evaluate the extended stability of the BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO immunoelectrode, its response was also studied over a period of 30 days, with measurements taken at regular intervals of 5 days. As represented in **Figure 4.12f**, no variation in the responses of the as-fabricated BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO immunoelectrode is observed within 25 days, showing that the fabricated biosensor has a great prospect for long-term detection of FBI.

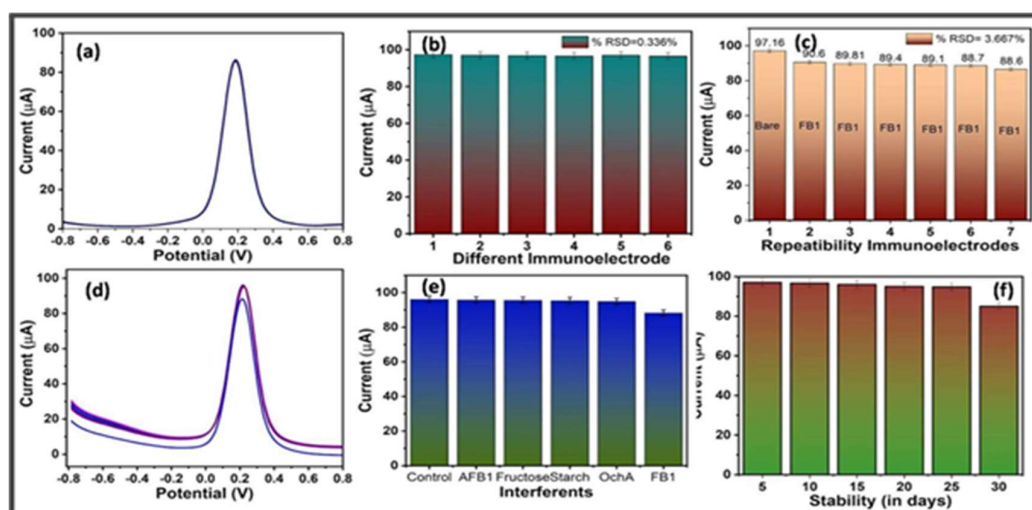


Figure 4.12: (a) DPV response of reproducibility study of six different electrode, (b) column graph of reproducibility study, (c) column graph of repeatability study of six different electrode responses with 0.33% RSD value, (d) DPV response of interference study, (e) column graph of interferent study with different interfering species, and (f) column graph of stability study of BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO immunoelectrode.

4.3.7.8 Real sample study

Validation of the developed electrochemical biosensor is a very important parameter to ensure accurate and reliable measurements. Therefore, the fabricated immunoelectrode was also tested with real food samples, such as sweet corn, for the validation of the biosensor for FBI detection. The preparation steps for real sweet corn samples are mentioned in the materials and methods section. In this study, the BSA/ab-FBI/ α -Fe₂O₃/rGO/ITO immunoelectrode was tested with several prepared spiked corn samples having different concentrations of FBI analyte, and electrochemical responses were recorded using the DPV technique. The analytical

results of spiking *FBI* concentrations are displayed in **Table 4.3**, where it was found that the RSD values varied from 0.37% to 1.99%.

TABLE 4.3: Tested fabricated biosensor BSA/ab-*FBI*/α-Fe₂O₃/rGO/ITO with spiked sweet corn samples using DPV technique.

S.No.	Spiked concentration (ng mL ⁻¹)	Found concentration by sensor (ng mL ⁻¹)	% RSD	Percentage recovery
1.	1	0.99	0.37	99.4
2.	10	9.90	0.68	99
3.	100	98.80	0.85	98.8
4.	400	388	1.99	97.2

Additionally, using spiked *FBI* concentrations, the percentage recovery was computed and determined to be between 97.2% and 99.4%. Therefore, the findings of this study on spiked samples showed that an immunoelectrode for mycotoxin detection had been developed and was able to identify *FBI* in sweet corn samples. Moreover, the performance of the fabricated biosensor BSA/ab-*FBI*/α-Fe₂O₃/rGO/ITO was investigated with spiked corn samples, and results were compared with the UPLC-MS/MS technique. The sample volume and percentage recovery for the concentration of *FBI* for electrochemical sensing by BSA/ab-*FBI*/α-Fe₂O₃/rGO/ITO and UPLC-MS/MS are presented in **Table 4.4**. Also, the UPLC-MS/MS technique was not able to detect lower concentrations of *FBI* ($\sim 1 \times 10^{-3}$ ng/mL), whereas lower concentrations were successfully detected by the fabricated electrochemical biosensors. Moreover, a 400 times greater sample volume was required for UPLC-MS/MS study in comparison to the fabricated electrochemical biosensor. Therefore, the performance and data obtained from BSA/ab-*FBI*/α-Fe₂O₃/rGO/ITO are satisfactory, in accordance with the UPLC-MS/MS data. The recovery percentage for the BSA/ab-*FBI*/α-Fe₂O₃/rGO/ITO and UPLC-MS/MS is consistent with each other, with satisfactory results.

TABLE 4.4: Comparative recovery analysis of *FBI* in spiked samples using UPLC-MS/MS and fabricated DPV-based electrochemical biosensor BSA/ab-*FBI*/α-Fe₂O₃/rGO/ITO.

Technique	Spiked concentration (ng/mL)	Recovery concentration (ng/mL)	Sample volume (μ L)	% recovery
UPLC-MS/MS	83.2	81.7	2000	98.1
DPV (BSA/ab-FBI/ α -Fe ₂ O ₃ /rGO/ITO)	100	98.8	5	98.8

4.3.7.9 Control study

The specificity and selectivity study of the fabricated electrode α -Fe₂O₃/rGO/ITO was carried out by direct interaction of *FBI* onto the nanomaterial (**Figure 4.13** (red-yellow color column)). To perform this study, a control experiment was carried out; that is, the α -Fe₂O₃/rGO/ITO electrode was tested with a series of concentrations of *FBI* from 1×10^{-3} to 700 ng mL^{-1} . The electrochemical response revealed that a negligible change in the variation of current response over the tested concentration was found. This indicates that the absence of ab-*FBI* showed nonspecific adsorption of *FBI* on the α -Fe₂O₃/rGO/ITO surface. This confirms that the nanocomposites itself do not contribute to the *FBI* recognition and clearly demonstrates that the fabricated immunosensor showed selective detection toward *FBI* without any physical adsorption or interference effects from the α -Fe₂O₃/rGO/ITO electrode.

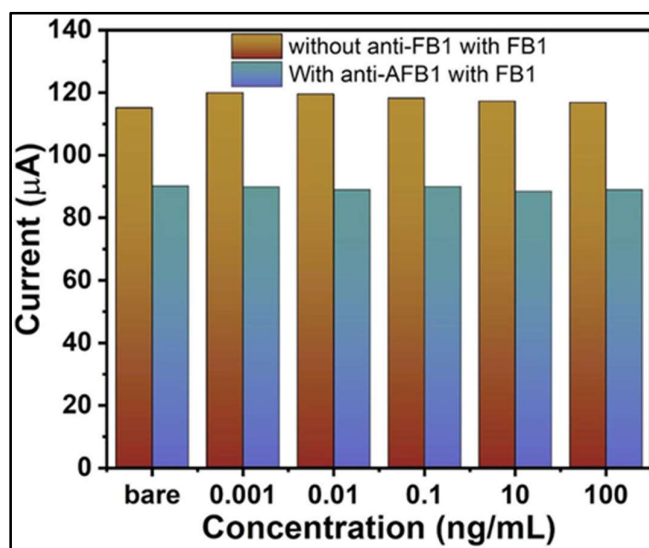


Figure 4.13: Column graph (red-yellow colour) shows a control study of an α -Fe₂O₃/rGO/ITO fabricated electrode with various concentrations of *FBI* without immobilization of anti-*FBI* using the DPV technique; column graph (blue colour) shows the current response of the α -Fe₂O₃/rGO/ITO electrode with immobilization of non-specific antibodies of *AFBI* with varying the concentration of *FBI*. Moreover, a control study was

performed to evaluate the cross-reactivity and specificity of the fabricated immunoelectrode by immobilizing a non-specific antibody of aflatoxin B1 (*AFBI*) instead of a target-specific antibody (*FBI*). The result of a non-specific antibody-based immunoelectrode is depicted in **Figure 4.13** (blue color column) with various concentrations of *FBI* under the same experimental conditions. The DPV current response exhibited negligible changes across *FBI* concentrations, indicating the absence of effective binding between *FBI* antigen and non-specific antibody. This study confirms that *FBI* does not exhibit cross-reactivity with non-specific antibody (*AFBI*) on the α -Fe₂O₃/rGO surface.

4.4 Conclusions

The BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO immunoelectrodes were developed for the detection of FB1 in samples of sweet corn. By using an in-situ co-precipitation process, the α -Fe₂O₃/rGO nanocomposite was synthesized, and its hexagonal phase was confirmed by XRD and HR-TEM characterization techniques. Using an EPD process, an α -Fe₂O₃/rGO thin film was deposited onto the ITO-coated glass substrate. With a sweet corn sample spiked with *FBI*, the α -Fe₂O₃/rGO/ITO immunoelectrodes exhibited an extremely high sensitivity of 6.36 μ A mL ng⁻¹ cm⁻² and R^2 of 0.9883. The detection range obtained is from 1×10^{-3} –700 ng mL⁻¹. The immunoelectrode showed an LOD of 0.0052 ng mL⁻¹. This demonstrated the full applicability of the BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO immunoelectrode system for *FBI* detection in real samples. *AFBI*, ochA, fructose, starch, and other naturally occurring interferences were employed in interference investigations in addition to *FBI* to validate the precise identification of *FBI*. Additionally, the BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO immunoelectrodes with five electrodes and continuous readings were demonstrated to be very reliable as well as repeatable. The BSA/ab-*FBI*/ α -Fe₂O₃/rGO/ITO immunoelectrode verified the particular interaction with *FBI*, but the α -Fe₂O₃/rGO/ITO electrode tended not to detect *FBI* in a controlled study.

4.5 Acknowledgments

For the FESEM image analysis, the authors are grateful to MNIT, Jaipur. The JNU Advanced Instrumentation Research Facility (AIRF) assisted with UPLC-MS/MS, TEM, and Raman characterization, for which the authors are grateful. The authors are very thankful to SAIF, Panjab University, Chandigarh, for the XPS analysis. One of the authors, Hema Bhardwaj, is appreciative of the DBT-RA fellowship from the Department of Biotechnology. Pratima R. Solanki acknowledges the Department of Health Research, Ministry of Health and Family Welfare, Government of India (grant no.: R.11013/46/2021-GIA/HR) for funding support. PR Solanki is also thankful to

the JNU-PAIR network on Science for Sustainable Future project (ANRF/PAIR/2025/000029/PAIR). For FTIR, XRD, and electrochemical data processing, the authors are extremely appreciative of the Special Centre for Nanosciences, JNU. The authors thank Harshulika Singh for writing and drafting the manuscript.

7

Chapter 5

Conclusion, Future Scope, and Social Impact

Chapter 5 : The present thesis work presents the development of a nanomaterial-based sensor for *FBI* detection. In order to detect this mycotoxin, an electrochemical sensor has been developed. Manganese oxide and iron oxide nanomaterials have been fabricated for *FBI* detection. This thesis aims to develop an efficient, reliable, and simple detection tool for FBI at very low concentrations.

5.1 Conclusion

The present work provides a systematic investigation of the synthesis, characterization, and *FBI* sensing performance of nanostructured materials. The key conclusions drawn from this research are elaborated below:

5.1.1. Key Findings

This study highlights the increasing demands for *FBI* detection due to increasing toxicity and safety concerns. It establishes that 2D nanomaterials like Mn_3O_4 , $\alpha\text{-Fe}_2\text{O}_3$ with graphene oxide possess unique properties such as high surface area and enhanced adsorption capability, making them suitable for *FBI* sensing applications. The limitations of conventional sensing techniques further justify the need for such advanced materials.

5.1.2. Structural and Morphological Analysis

The structural analysis confirmed the successful formation of the tetragonal phase of Mn_3O_4 and the hexagonal phase of $\alpha\text{-Fe}_2\text{O}_3$. Morphological studies revealed a uniform distribution of nanosheets, which increases the active surface area. These structural features play a crucial role in enhancing *FBI* adsorption and improving the sensing performance.

5.1.3. FBI Interaction Studies (In-situ XRD)

The in-situ investigation provided valuable insight into the interaction between Mn_3O_4 and $\alpha\text{-Fe}_2\text{O}_3$ with graphene oxide. It was observed that the *FBI* exposure leads to measurable changes in the diffraction patterns, indicating structural modifications within the material. This real-time analysis helped in understanding the dynamic behavior of Mn_3O_4 and $\alpha\text{-Fe}_2\text{O}_3$ with graphene oxide at room temperature.

5.1.4. FBI Sensing Performance

The *FBI* sensing studies demonstrated that the sensor exhibited improved performance with lowering concentration of *FBI*. Optimal sensitivity behavior was observed at room temperature, with rapid response and recovery times, indicating its potential for practical applications.

5.1.5. Performance Limitations

Despite the performance results, certain limitations were identified. The selectivity of the sensor towards specific mycotoxins remains a challenge, as well as the influence of environmental factors such as humidity.

5.1.6. Overall Conclusion

Overall, the study establishes a clear relationship between synthesis conditions, material properties, and sensing performance. The results confirm that nanostructured Mn_3O_4 and $\alpha\text{-Fe}_2\text{O}_3$ with graphene oxide are promising materials for developing *FBI* sensors. The use of advanced characterization techniques has significantly contributed to understanding the sensing mechanism at a deeper level.

The thin films of Mn_3O_4 and $\alpha\text{-Fe}_2\text{O}_3$ with graphene oxide were successfully synthesized using the electrophoretic deposition (EPD) technique. The structural and morphological characterizations confirmed the formation of highly crystalline, uniform, and continuous thin films of Mn_3O_4 and $\alpha\text{-Fe}_2\text{O}_3$ with graphene oxide. Raman spectroscopy, XRD, SEM, EDAX, and FTIR verified the phase purity and nanosheet morphology suitable for *FBI* sensing applications.

FBI sensing investigations demonstrated that nanostructured Mn_3O_4 and $\alpha\text{-Fe}_2\text{O}_3$ with graphene oxide exhibit strong sensitivity towards *FBI*.

5.2 Future Scope of Work

Although this research provides important insights, several areas can be explored further to enhance the performance and applicability of Mn_3O_4 and $\alpha\text{-Fe}_2\text{O}_3$ with graphene oxide-based *FBI* sensors.

5.2.1 Improvement in Selectivity

Future research should focus on improving the selectivity of the sensor towards the specific mycotoxins. This can be achieved using other nanomaterials, surface modifications, or functionalization techniques, which can tailor the interaction between the sensing material and the mycotoxins.

5.2.2 Room Temperature *FBI* Sensing

Achieving mycotoxin sensing at room temperature remains a key objective, thereby making the sensors more energy-efficient and suitable for portable devices.

5.2.3 Environmental Stability Studies

Further studies are required to evaluate the performance of sensors under real-world environmental conditions. The effects of long-term stability and reliability are key factors in the sensors.

5.2.4 Device Miniaturization and Integration

The development of compact and integrated sensing devices is essential for practical applications. Future work can focus on wearable devices and IOT platforms for continuous monitoring.

5.3 Social Impact

The development of *FBI* sensing technologies has a wide range of benefits for society.

5.3.1 Environmental Monitoring

The sensors developed in this work can contribute significantly to monitoring *FBI* sensing.

5.3.2 Public Health Benefits

Early detection of *FBI* can prevent serious health issues. Continuous monitoring systems can reduce exposure to harmful mycotoxins and improve public health outcomes.

5.3.3 Smart Cities and IOT Integration

Integration of *FBI* sensors into smart sensors can enable real-time monitoring of environmental conditions. This supports the development of smart cities with improved food quality management.

5.3.4 Economic and Technological Impact

The use of cost-effective materials and scalable techniques makes these sensors economically viable. This can enhance technological advancements in sensor development.

5.3.5 Scientific Contribution

This research contributes to the scientific understanding of *FBI* sensing mechanisms in 2D materials. It provides a strong foundation for future research in nanotechnology and advanced sensing applications.

In conclusion, this thesis demonstrates the potential of Mn_3O_4 and $\alpha\text{-Fe}_2\text{O}_3$ with graphene oxide as efficient *FBI* sensing materials. The findings of this work not only enhance the understanding of *FBI* sensing mechanisms but also pave the way for future advancements in sensor technology. The research holds significant promise for addressing real-world challenges related to environmental monitoring, industrial safety, and sustainable energy.

REFERENCES

- [1] Paramasivam, G., Palem, V.V., Sundaram, T., Sundaram, V., Kishore, S.C., Bellucci, S., 2021. Nanomaterials: Synthesis and applications in theranostics. *Nanomaterials*, Vol. 11, 3228.
- [2] Lehner, R., Wang, X., Marsch, S., and Hunziker, P., 2013. Intelligent nanomaterials for medicine: Carrier platforms and targeting strategies in the context of clinical application. *Nanomedicine: Nanotechnology, Biology and Medicine*, Vol. 9, Issue 6, pp. 742.
- [3] Chakroborty, S. and Sahoo, S.K., 2021. Carbon-based nanomaterials in drug delivery systems, *Environmental Applications of Carbon Nanomaterials-Based Devices*, pp.375-394.
- [4] Zhang, H., Cheng, H.M. and Ye, P., 2018. 2D nanomaterials: beyond graphene and transition metal dichalcogenides. *Chemical Society Reviews*, Vol 47, Issue 16, pp.6009-6012.
- [5] Li, L.H. and Chen, Y., 2016. Atomically thin boron nitride: unique properties and applications. *Advanced Functional Materials*, 26(16), pp.2594-2608.
- [6] Lu, Q., Yu, Y., Ma, Q., Chen, B. and Zhang, H., 2016. 2D transition-metal-dichalcogenide-nanosheet-based composites for photocatalytic and electrocatalytic hydrogen evolution reactions. *Advanced Materials*, 28(10), pp.1917-1933.
- [7] Deng, L., Xu, Y., Sun, C., Yun, B., Sun, Q., Zhao, C. and Li, Z., 2018. Functionalization of small black phosphorus nanoparticles for targeted imaging and photothermal therapy of cancer. *Science bulletin*, 63(14), pp.917-924.
- [8] Niu, T., Zhou, W., Zhou, D., Hu, X., Zhang, S., Zhang, K., Zhou, M., Fuchs, H. and Zeng, H., 2019. Modulating epitaxial atomic structure of antimonene through interface design. *Advanced Materials*, 31(29), p.1902606.
- [9] Wang, Q. and O'Hare, D., 2012. Recent advances in the synthesis and application of layered double hydroxide (LDH) nanosheets. *Chemical reviews*, 112(7), pp.4124-4155.

-
- [10] Zhang, J., Chen, Y. and Wang, X., 2015. Two-dimensional covalent carbon nitride nanosheets: synthesis, functionalization, and applications. *Energy & Environmental Science*, 8(11), pp.3092-3108.
- [11] Wang, H., Li, C., Fang, P., Zhang, Z. and Zhang, J.Z., 2018. Synthesis, properties, and optoelectronic applications of two-dimensional MoS₂ and MoS₂-based heterostructures. *Chemical Society Reviews*, 47(16), pp.6101-6127.
- [12] Dong, Y., Wu, Z.S., Ren, W., Cheng, H.M. and Bao, X., 2017. Graphene: a promising 2D material for electrochemical energy storage. *Science Bulletin*, 62(10), pp.724-740.
- [13] Shen, S., Liu, M., Li, T., Lin, S. and Mo, R., 2017. Recent progress in nanomedicine-based combination cancer therapy using a site-specific co-delivery strategy. *Biomaterials science*, 5(8), pp.1367-1381.
- [14] Wang L, Xiong Q, Xiao F, and Duan, H., 2017. 2D nanomaterials based electrochemical biosensors for cancer diagnosis. *Biosensors and Bioelectronics*, Vol 9, pp.136–51.
- [15] Peng, X., Peng, L., Wu, C. and Xie, Y., 2014. Two-dimensional nanomaterials for flexible supercapacitors. *Chemical Society Reviews*, 43(10), pp.3303-3323.
- [16] Wang, X., Weng, Q., Yang, Y., Bando, Y. and Golberg, D., 2016. Hybrid two-dimensional materials in rechargeable battery applications and their microscopic mechanisms. *Chemical Society Reviews*, 45(15), pp.4042-4073.
- [17] Nicolosi, V., Chhowalla, M., Kanatzidis, M.G., Strano, M.S. and Coleman, J.N., 2013. Liquid exfoliation of layered materials. *Science*, 340(6139), p.1226419.
- [18] Liu, Y., Duan, X., Huang, Y. and Duan, X., 2018. Two-dimensional transistors beyond graphene and TMDCs. *Chemical Society Reviews*, 47(16), pp.6388-6409.
- [19] Bitounis, D., Ali-Boucetta, H., Hong, B.H., Min, D.H. and Kostarelos, K., 2013. Prospects and challenges of graphene in biomedical applications. *Advanced materials*, 25(16), pp.2258-2268.

-
- [20] Thévenot, D.R., Toth, K., Durst, R.A. and Wilson, G.S., 2001. Electrochemical biosensors: recommended definitions and classification. *Biosensors and bioelectronics*, 16(1-2), pp.121-131.
- [21] Clark Jr, L.C. and Lyons, C., 1962. *Annals. New York Academy of Sciences*, 102, pp.29-145.
- [22] Turner, A., Karube, I. and Wilson, G.S., 1987. *Biosensors: fundamentals and applications*. Oxford university press.
- [23] Gronow, M., 1984. Biosensors. *Trends in Biochemical sciences*, 9(8), pp.336-340.
- [24] Clarke, D.J., 1987. Biosensors in process control. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, 316(1176), pp.169-181.
- [25] Clark Jr, L.C., 1979. [41] The hydrogen peroxide sensing platinum anode as an analytical enzyme electrode. In *Methods in Enzymology* (Vol. 56, pp. 448-479). Academic Press.
- [26] Luong, J.H.T., Mulchandani, A. and Guilbault, G.G., 1988. Developments and applications of biosensors. *Trends in Biotechnology*, 6(12), pp.310-316.
- [27] Guilbault, G.G., 1989. Gas phase biosensors. In *Ion-Selective Electrodes* (pp. 101-112). Pergamon.
- [28] Adley, C.C. and Ryan, M.P., 2015. Conductometric biosensors for high throughput screening of pathogens in food. In *high throughput screening for food safety assessment* (pp. 315-326). Woodhead Publishing.
- [29] Verma, A., Arqam, M. and Fraiwan, A., 2025. Advances in Impedimetric Biosensors: Current Applications and Future Directions. *Micromachines*, 16(11), p.1244.
- [30] Borisov, S.M. and Wolfbeis, O.S., 2008. Optical biosensors. *Chemical reviews*, 108(2), pp.423-461.
- [31] Hoa, X.D., Kirk, A.G. and Tabrizian, M., 2007. Towards integrated and sensitive surface plasmon resonance biosensors: A review of recent progress. *Biosensors and bioelectronics*, 23(2), pp.151-160.

-
- [32] Vasuki, S., Varsha, V., Mithra, R., Dharshni, R.A., Abinaya, S., Dharshini, R.D. and Sivarajasekar, N., 2019. Thermal biosensors and their applications. *Am. Int. J. Res. Sci. Technol. Eng. Math*, 1, pp.262-264.
- [33] Rocchitta, G., Spanu, A., Babudieri, S., Latte, G., Madeddu, G., Galleri, G., Nuvoli, S., Bagella, P., Demartis, M.I., Fiore, V. and Manetti, R., 2016. Enzyme biosensors for biomedical applications: Strategies for safeguarding analytical performances in biological fluids. *Sensors*, 16(6), p.780.
- [34] Moina, C. and Ybarra, G., 2012. Fundamentals and applications of immunosensors. *Advances in immunoassay technology*, 66.
- [35] Slagle, K.M. and Ghosn, S.J., 1996. Immunoassays: tools for sensitive, specific, and accurate test results. *Laboratory medicine*, 27(3), pp.177-183.
- [36] Darwish, I.A., 2006. Immunoassay methods and their applications in pharmaceutical analysis: basic methodology and recent advances. *International journal of biomedical science: IJBS*, 2(3), p.217.
- [37] Fischer, M.J., 2010. Amine coupling through EDC/NHS: a practical approach. In *Surface plasmon resonance: methods and protocols* (pp. 55-73). Totowa, NJ: Humana Press.
- [38] Zhou, W., Huang, P.J.J., Ding, J. and Liu, J., 2014. Aptamer-based biosensors for biomedical diagnostics. *Analyst*, 139(11), pp.2627-2640.
- [39] Zhai, J., Cui, H. and Yang, R., 1997. DNA based biosensors. *Biotechnology advances*, 15(1), pp.43-58.
- [40] Pancrazio, J.J., Whelan, J.P., Borkholder, D.A., Ma, W. and Stenger, D.A., 1999. Development and application of cell-based biosensors. *Annals of biomedical engineering*, 27(6), pp.697-711.
- [41] Arnold, M.A. and Rechnitz, G.A., 1981. Tissue-based membrane electrode with high biocatalytic activity for measurement of adenosine 5'-monophosphate. *Analytical Chemistry*, 53(12), pp.1837-1842.
- [42] Chen, Y., Kistler, H.C. and Ma, Z., 2019. Fusarium graminearum trichothecene mycotoxins: biosynthesis, regulation, and management. *Annual review of phytopathology*, 57(1), pp.15-39.

-
- [43] Richard, J.L., 2007. Some major mycotoxins and their mycotoxicoses—An overview. *International journal of food microbiology*, 119(1-2), pp.3-10.
- [44] Milićević, D.R., Škrinjar, M. and Baltić, T., 2010. Real and perceived risks for mycotoxin contamination in foods and feeds: challenges for food safety control. *Toxins*, 2(4), pp.572-592.
- [45] Nordkvist, E. and Häggblom, P., 2014. Fusarium mycotoxin contamination of cereals and bedding straw at Swedish pig farms. *Animal Feed Science and Technology*, 198, pp.231-237.
- [46] Kumar, P., Mahato, D.K., Kamle, M., Mohanta, T.K. and Kang, S.G., 2017. Aflatoxins: A global concern for food safety, human health and their management. *Frontiers in microbiology*, 7, p.235289.
- [47] Ráduly, Z., Szabó, L., Madar, A., Pócsi, I. and Csernoch, L., 2020. Toxicological and medical aspects of Aspergillus-derived mycotoxins entering the feed and food chain. *Frontiers in microbiology*, 10, p.2908.
- [48] Calderone, R., 2001. *Fungal pathogenesis: principles and clinical applications*. CRC Press.
- [49] Dixon, D.M., McNeil, M.M., Cohen, M.L., Gellin, B.G. and La Montagne, J.R., 1996. Fungal infections: a growing threat. *Public health reports*, 111(3), p.226.
- [50] El-Nezami, H., Kankaanpää, P., Salminen, S. and Ahokas, J., 1998. Physicochemical alterations enhance the ability of dairy strains of lactic acid bacteria to remove aflatoxin from contaminated media. *Journal of food protection*, 61(4), pp.466-468.
- [51] El-Nezami, H., Polychronaki, N., Salminen, S. and Mykkänen, H., 2002. Binding rather than metabolism may explain the interaction of two food-grade Lactobacillus strains with zearalenone and its derivative α -zearalenol. *Applied and environmental microbiology*, 68(7), pp.3545-3549.
- [52] Fink-Grenmels, J., 1999. Mycotoxins: their implications for human and animal health. *Veterinary Quarterly*, 21(4), pp.115-120.

-
- [53] Gajęcka, M., Stopa, E., Tarasiuk, M., Zielonka, Ł. and Gajęcki, M., 2013. The expression of type-1 and type-2 nitric oxide synthase in selected tissues of the gastrointestinal tract during mixed mycotoxicosis. *Toxins*, 5(11), pp.2281-2292.
- [54] Pickova, D., Ostry, V., Toman, J. and Malir, F., 2021. Aflatoxins: History, significant milestones, recent data on their toxicity and ways to mitigation. *Toxins*, 13(6), p.399.
- [55] Forgacs, J. and Carll, W.T., 1962. Mycotoxicoses.
- [56] Lubulwa, G. and Davis, J., Completed-project Economic Assessment of Two ACIAR Projects on Fungi and Aflatoxins. *Mycotoxin Contamination in Grains*, p.66.
- [57] Moss, 1998. Recent studies of mycotoxins. *Journal of Applied Microbiology*, 84(S1), pp.62S-76S.
- [58] Frisvad, J.C., Smedsgaard, J., Samson, R.A., Larsen, T.O. and Thrane, U., 2007. Fumonisin B2 production by *Aspergillus niger*. *Journal of agricultural and food chemistry*, 55(23), pp.9727-9732.
- [59] Dall'Asta, C. and Battilani, P., 2016. Fumonisin and their modified forms, a matter of concern in future scenario?. *World Mycotoxin Journal*, 9(5), pp.727-740.
- [60] Cendoya, E., Chiotta, M.L., Zachetti, V., Chulze, S.N. and Ramirez, M.L., 2018. Fumonisin and fumonisin-producing *Fusarium* occurrence in wheat and wheat by products: A review. *Journal of cereal science*, 80, pp.158-166.
- [61] Braun, M.S. and Wink, M., 2018. Exposure, occurrence, and chemistry of fumonisin and their cryptic derivatives. *Comprehensive Reviews in Food Science and Food Safety*, 17(3), pp.769-791.
- [62] Rheeder, J.P., Marasas, W.F., Thiel, P.G., Sydenham, E.W., Shephard, G.S. and Van Schalkwyk, D.J., 1992. *Fusarium moniliforme* and fumonisin in corn in relation to human esophageal cancer in Transkei.
- [63] Abbas, H.K., Duke, S.O., Shier, W.T. and Duke, M.V., 2002. Inhibition of ceramide synthesis in plants by phytotoxins. In *Advances in Microbial Toxin Research and Its Biotechnological Exploitation* (pp. 211-229). Boston, MA: Springer US.

-
- [64] Damiani, T., Righetti, L., Suman, M., Galaverna, G. and Dall'Asta, C., 2019. Analytical issue related to fumonisins: A matter of sample comminution?. *Food Control*, 95, pp.1-5.
- [65] Pitt, J.I. and Hocking, A.D., 2009. *Fungi and food spoilage* (Vol. 519, p. 388). New York: Springer.
- [66] Riley, R.T., Voss, K.A., Norred, W.P., Sharma, R.P., Wang, E. and Merrill, A.H., 1998. Fumonisins: mechanism of mycotoxicity. *Rev. Med. Vet*, 149(6), pp.617-626.
- [67] Bose, R., Verheij, M., Haimovitz-Friedman, A., Scotto, K., Fuks, Z. and Kolesnick, R., 1995. Ceramide synthase mediates daunorubicin-induced apoptosis: an alternative mechanism for generating death signals. *Cell*, 82(3), pp.405-414.
- [68] Bucci, T.J., Hansen, D.K. and Laborde, J.B., 1996. Leukoencephalomalacia and hemorrhage in the brain of rabbits gavaged with mycotoxin fumonisin B1. *Natural toxins*, 4(1), pp.51-52.
- [69] Soriano, J.M., Gonzalez, L. and Catala, A.I., 2005. Mechanism of action of sphingolipids and their metabolites in the toxicity of fumonisin B1. *Progress in lipid research*, 44(6), pp.345-356.
- [70] Marasas, W.F.O., Kellerman, T.S., Gelderblom, W.C.A., Thiel, P.G., Van der Lugt, J.J. and Coetzer, J.A., 1988. Leukoencephalomalacia in a horse induced by fumonisin B isolated from *Fusarium moniliforme*.
- [71] Gelderblom, W.C., Abel, S., Smuts, C.M., Marnewick, J., Marasas, W.F., Lemmer, E.R. and Ramljak, D., 2001. Fumonisin-induced hepatocarcinogenesis: mechanisms related to cancer initiation and promotion. *Environmental Health Perspectives*, 109(Suppl 2), p.291.
- [72] Abarca, M.L., Bragulat, M.R., Castella, G. and Cabanes, F., 1994. Ochratoxin A production by strains of *Aspergillus niger* var. *niger*. *Applied and environmental microbiology*, 60(7), pp.2650-2652.
- [73] Freire, L., Passamani, F.R.F., Thomas, A.B., Nassur, R.D.C.M.R., Silva, L.M., Paschoal, F.N., Pereira, G.E., Prado, G. and Batista, L.R., 2017. Influence of physical and chemical characteristics of wine grapes on the incidence of *Penicillium* and *Aspergillus* fungi in grapes and ochratoxin A in wines. *International journal of food microbiology*, 241, pp.181-190.

-
- [74] Duarte, S.C., Pena, A. and Lino, C.M., 2010. A review on ochratoxin A occurrence and effects of processing of cereal and cereal derived food products. *Food microbiology*, 27(2), pp.187-198.
- [75] Sun, X.D., Su, P. and Shan, H., 2017. Mycotoxin contamination of maize in China. *Comprehensive Reviews in Food Science and Food Safety*, 16(5), pp.835-849.
- [76] Janik, E., Niemcewicz, M., Podogrocki, M., Ceremuga, M., Gorniak, L., Stela, M. and Bijak, M., 2021. The existing methods and novel approaches in mycotoxins' detection. *Molecules*, 26(13), p.3981.
- [77] Wang, X. and Liu, X., 2003. Study on the polymerase chain reaction methods for the detection of fumonisin-producing strains of *Fusarium moniliforme*. *Wei Sheng yan jiu = Journal of Hygiene Research*, 32(3), pp.228-232.
- [78] Anyakudo, F., Adams, E. and Van Schepdael, A., 2020. Thin-layer chromatography–flame ionization detection. *Chromatographia*, 83(2), pp.149-157.
- [79] Zhang, J., Zhang, X., Zhang, Y., Yang, X., Guo, L., Man, C., Jiang, Y., Zhang, W. and Zhang, X., 2025. Emerging biosensors integrated with microfluidic devices: a promising analytical tool for on-site detection of mycotoxins. *npj Science of Food*, 9(1), p.84.
- [80] Lei, H., Chen, C. and Yan, H., 2022. A portable optical fiber tip facet LSPR aptasensor for detection of fumonisin B1. *IEEE Sensors Journal*, 22(18), pp.17838-17844.
- [81] Sun, Q., Chen, X., Lei, X., Ran, X., Yin, Y., Li, J. and Le, T., 2025. Molecularly imprinted polymers for mycotoxin biosensing: Rational design, advanced applications, and future horizons. *Biosensors and Bioelectronics*, p.118111.
- [82] Monbaliu, S., Van Poucke, C., Detavernier, C.L., Dumoulin, F., Van De Velde, M., Schoeters, E., Van Dyck, S., Averkieva, O., Van Peteghem, C. and De Saeger, S., 2010. Occurrence of mycotoxins in feed as analyzed by a multi-mycotoxin LC-MS/MS method. *Journal of Agricultural and Food chemistry*, 58(1), pp.66-71.

-
- [83] Garg, K., Villavicencio-Aguilar, F., Solano-Rivera, F. and Gilbert, L., 2022. Analytical validation of a direct competitive ELISA for multiple mycotoxin detection in human serum. *Toxins*, 14(11), p.727.
- [84] Futamura, R., Iiyama, T., Ueda, T., Bonnaud, P.A., Coudert, F.X., Furuse, A., Tanaka, H., Pellenq, R.J.M. and Kaneko, K., 2024. Staggered structural dynamic-mediated selective adsorption of H₂O/D₂O on flexible graphene oxide nanosheets. *Nature Communications*, 15(1), p.3585.
- [85] Dreyer, D.R., Park, S., Bielawski, C.W. and Ruoff, R.S., 2010. The chemistry of graphene oxide. *Chemical society reviews*, 39(1), pp.228-240.
- [86] Pekola, J.P., Vartiainen, J.J., Möttönen, M., Saira, O.P., Meschke, M. and Averin, D.V., 2008. Hybrid single-electron transistor as a source of quantized electric current. *Nature Physics*, 4(2), pp.120-124.
- [87] Walker, A., 1991. Introduction to carbon science: Edited by H. Marsh; published by Butterworths, London, 1989; 321 pp., price£ 35.00, ISBN 0-408-03837-3.
- [88] Siqueira Jr, J.R. and Oliveira Jr, O.N., 2017. Carbon-based nanomaterials. In *Nanostructures* (pp. 233-249). William Andrew Publishing.
- [89] Mostofizadeh, A., Li, Y., Song, B. and Huang, Y., 2011. Synthesis, properties, and applications of low-dimensional carbon-related nanomaterials. *Journal of nanomaterials*, 2011(1), p.685081.
- [90] Siqueira Jr, J.R. and Oliveira Jr, O.N., 2017. Carbon-based nanomaterials. In *Nanostructures* (pp. 233-249). William Andrew Publishing.
- [91] N. Rao, R. Singh, and L. Bashambu, Mater. Today Proc. **44**, 608 (2021).
- [92] Chen, D., Feng, H. and Li, J., 2012. Graphene oxide: preparation, functionalization, and electrochemical applications. *Chemical reviews*, 112(11), pp.6027-6053.
- [93] Sengunthar, P., Patel, S., Thankachen, N. and Joshi, U.S., 2021. Core-shell hybrid structured rGO decorated ZnO nanorods synthesized via a facile chemical route with photosensitive properties. *New Journal of Chemistry*, 45(40), pp.18853-18862.

- [94] Staudenmaier, L., 1898. Method for the preparation of graphitic acid. *Ber Dtsch Chem Ges*, 31, pp.1481-1487.
- [95] Hummers, W.S. and Offeman, R.E., 1958. J. Am. Chem. Soc. *J. Am. Chem. Soc*, 80, p.1339.
- [96] Gao, W., 2015. Synthesis, structure, and characterizations. In *Graphene Oxide: Reduction Recipes, Spectroscopy, and Applications* (pp. 1-28). Cham: Springer International Publishing.
- [97] Yu, W., Sisi, L., Haiyan, Y. and Jie, L., 2020. Progress in the functional modification of graphene/graphene oxide: A review. *RSC advances*, 10(26), pp.15328-15345.
- [98] Eda, G. and Chhowalla, M., 2010. Chemically derived graphene oxide: towards large-area thin-film electronics and optoelectronics. *Advanced materials*, 22(22), pp.2392-2415.
- [99] Eda, G., Fanchini, G. and Chhowalla, M., 2008. Large-area ultrathin films of reduced graphene oxide as a transparent and flexible electronic material. *Nature nanotechnology*, 3(5), pp.270-274.
- [100] Stankovich, S., Dikin, D.A., Piner, R.D., Kohlhaas, K.A., Kleinhammes, A., Jia, Y., Wu, Y., Nguyen, S.T. and Ruoff, R.S., 2007. Synthesis of graphene-based nanosheets via chemical reduction of exfoliated graphite oxide. *carbon*, 45(7), pp.1558-1565.
- [101] Morales-Narváez, E. and Merkoçi, A., 2012. Graphene oxide as an optical biosensing platform. *Advanced materials*, 24(25), pp.3298-3308.
- [102] Veeramani, H., Aruguete, D., Monsegue, N., Murayama, M., Dippon, U., Kappler, A. and Hochella, M.F., 2013. Low-temperature green synthesis of multivalent manganese oxide nanowires. *ACS Sustainable Chemistry & Engineering*, 1(9), pp.1070-1074.
- [103] Asaikkutti, A., Bhavan, P.S., Vimala, K., Karthik, M. and Cheruparambath, P., 2016. Dietary supplementation of green synthesized manganese-oxide nanoparticles and its effect on growth performance, muscle composition and digestive enzyme activities of the giant freshwater prawn *Macrobrachium rosenbergii*. *Journal of Trace Elements in Medicine and Biology*, 35, pp.7-17.

- [104] Hoseinpour, V. and Ghaemi, N., 2018. Green synthesis of manganese nanoparticles: Applications and future perspective—A review. *Journal of Photochemistry and Photobiology B: Biology*, 189, pp.234-243.
- [105] Prasad, A.S., 2017. Green synthesis of nanocrystalline manganese (II, III) oxide. *Materials Science in Semiconductor Processing*, 71, pp.342-347.
- [106] Biswas, S., Kar, S. and Chaudhuri, S., 2005. Optical and magnetic properties of manganese-incorporated zinc sulfide nanorods synthesized by a solvothermal process. *The Journal of Physical Chemistry B*, 109(37), pp.17526-17530.
- [107] Qiu, Y., Xu, G.L., Yan, K., Sun, H., Xiao, J., Yang, S., Sun, S.G., Jin, L. and Deng, H., 2011. Morphology-conserved transformation: synthesis of hierarchical mesoporous nanostructures of Mn₂O₃ and the nanostructural effects on Li-ion insertion/deinsertion properties. *Journal of Materials Chemistry*, 21(17), pp.6346-6353.
- [108] Liu, X., Chen, C., Zhao, Y. and Jia, B., 2013. A review on the synthesis of manganese oxide nanomaterials and their applications on lithium-ion batteries. *Journal of Nanomaterials*, 2013(1), p.736375.
- [109] Moon, S.A., Salunke, B.K., Alkotaini, B., Sathiyamoorthi, E. and Kim, B.S., 2015. Biological synthesis of manganese dioxide nanoparticles by *Kalopanax pictus* plant extract. *IET nanobiotechnology*, 9(4), pp.220-225.
- [110] Kuo, W.S., Chen, H.H., Chen, S.Y., Chang, C.Y., Chen, P.C., Hou, Y.I., Shao, Y.T., Kao, H.F., Hsu, C.L.L., Chen, Y.C. and Chen, S.J., 2017. Graphene quantum dots with nitrogen-doped content dependence for highly efficient dual-modality photodynamic antimicrobial therapy and bioimaging. *Biomaterials*, 120, pp.185-194.
- [111] Xu, B., Fell, C.R., Chi, M. and Meng, Y.S., 2011. Identifying surface structural changes in layered Li-excess nickel manganese oxides in high voltage lithium ion batteries: A joint experimental and theoretical study. *Energy & Environmental Science*, 4(6), pp.2223-2233.
- [112] Hu, C.C. and Tsou, T.W., 2002. Ideal capacitive behavior of hydrous manganese oxide prepared by anodic deposition. *Electrochemistry Communications*, 4(2), pp.105-109.

- [113] Giraldo, O., Brock, S.L., Willis, W.S., Marquez, M., Suib, S.L. and Ching, S., 2000. Manganese oxide thin films with fast ion-exchange properties. *Journal of the American Chemical Society*, 122(38), pp.9330-9331.
- [114] Cheng, F., Zhao, J., Song, W., Li, C., Ma, H., Chen, J. and Shen, P., 2006. Facile controlled synthesis of MnO₂ nanostructures of novel shapes and their application in batteries. *Inorganic chemistry*, 45(5), pp.2038-2044.
- [115] Wei, W., Cui, X., Chen, W. and Ivey, D.G., 2011. Manganese oxide-based materials as electrochemical supercapacitor electrodes. *Chemical society reviews*, 40(3), pp.1697-1721.
- [116] Fei, J.B., Cui, Y., Yan, X.H., Qi, W., Yang, Y., Wang, K.W., He, Q. and Li, J.B., 2008. Controlled preparation of MnO₂ hierarchical hollow nanostructures and their application in water treatment. *Advanced Materials*, 20(3), pp.452-456.
- [117] Hosny, N.M. and Dahshan, A., 2012. Facile synthesis and optical band gap calculation of Mn₃O₄ nanoparticles. *Materials Chemistry and Physics*, 137(2), pp.637-643.
- [118] Wang, G., Huang, B., Lou, Z., Wang, Z., Qin, X., Zhang, X. and Dai, Y., 2016. Valence state heterojunction Mn₃O₄/MnCO₃: Photo and thermal synergistic catalyst. *Applied Catalysis B: Environmental*, 180, pp.6-12.
- [119] Chen, D., Yang, B., Jiang, Y. and Zhang, Y.Z., 2018. Synthesis of Mn₃O₄ nanoparticles for catalytic application via ultrasound-assisted ball milling. *ChemistrySelect*, 3(14), pp.3904-3908.
- [120] Chen, D., Zhang, Y. and Kang, Z., 2013. A low temperature synthesis of MnFe₂O₄ nanocrystals by microwave-assisted ball-milling. *Chemical Engineering Journal*, 215, pp.235-239.
- [121] Peng, J., Gao, W., Gupta, B.K., Liu, Z., Romero-Aburto, R., Ge, L., Song, L., Alemany, L.B., Zhan, X., Gao, G. and Vithayathil, S.A., 2012. Graphene quantum dots derived from carbon fibers. *Nano letters*, 12(2), pp.844-849.
- [122] Gao, J., Lowe, M.A. and Abruna, H.D., 2011. Spongelike nanosized Mn₃O₄ as a high-capacity anode material for rechargeable lithium batteries. *Chemistry of Materials*, 23(13), pp.3223-3227.

- [123] Gao, J., Lowe, M.A. and Abruna, H.D., 2011. Spongelike nanosized Mn_3O_4 as a high-capacity anode material for rechargeable lithium batteries. *Chemistry of Materials*, 23(13), pp.3223-3227.
- [124] Chowdhury, A.N., Azam, M.S., Aktaruzzaman, M. and Rahim, A., 2009. Oxidative and antibacterial activity of Mn_3O_4 . *Journal of hazardous materials*, 172(2-3), pp.1229-1235.
- [125] Stobbe, E.D., De Boer, B.A. and Geus, J.W., 1999. The reduction and oxidation behaviour of manganese oxides. *Catalysis Today*, 47(1-4), pp.161-167.
- [126] Chen, L., Horiuchi, T. and Mori, T., 2001. Catalytic reduction of NO over a mechanical mixture of NiGa_2O_4 spinel with manganese oxide: influence of catalyst preparation method. *Applied Catalysis A: General*, 209(1-2), pp.97-105.
- [127] Rui, S.O.N.G., Hong-jun, W. and Shou-hua, F., 2012. Solvothermal preparation of Mn_3O_4 nanoparticles and effect of temperature on particle size. *Chemical Research in Chinese Universities*, 28(4), pp.577-580.
- [128] Zwinkels, M.F., Järås, S.G., Menon, P.G. and Griffin, T.A., 1993. Catalytic materials for high-temperature combustion. *Catalysis Reviews—Science and Engineering*, 35(3), pp.319-358.
- [129] Chang, Y.Q., Xu, X.Y., Luo, X.H., Chen, C.P. and Yu, D.P., 2004. Synthesis and characterization of Mn_3O_4 nanoparticles. *Journal of crystal growth*, 264(1-3), pp.232-236.
- [130] Wu, W., Wu, Z., Yu, T., Jiang, C. and Kim, W.S., 2015. Recent progress on magnetic iron oxide nanoparticles: synthesis, surface functional strategies and biomedical applications. *Science and technology of advanced materials*, 16(2), p.023501.
- [131] Ali, A., Zafar, H., Zia, M., ul Haq, I., Phull, A.R., Ali, J.S. and Hussain, A., 2016. Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. *Nanotechnology, science and applications*, pp.49-67.
- [132] Ahmad, W.R.W., Mamat, M.H., Zoolfakar, A.S., Khusaimi, Z. and Rusop, M., 2016, December. A review on hematite $\alpha\text{-Fe}_2\text{O}_3$ focusing on nanostructures, synthesis methods and applications. In *2016 IEEE Student Conference on Research and Development (SCOReD)* (pp. 1-6). IEEE.

- [133] Rabenau, A., 1985. The role of hydrothermal synthesis in preparative chemistry. *Angewandte Chemie International Edition in English*, 24(12), pp.1026-1040.
- [134] Laudise, R.A., Bridenbaugh, P.M. and Iradi, T., 1994. Pressure balance under hydrothermal conditions. *Journal of crystal growth*, 140(1-2), pp.51-56.
- [135] Lobachev, A.N. ed., 2013. *Crystallization processes under hydrothermal conditions*. Springer Science & Business Media.
- [136] Roy, R., 1994. Accelerating the kinetics of low-temperature inorganic syntheses. *Journal of Solid State Chemistry*, 111(1), pp.11-17.
- [137] K. Byrappa (Ed.), *Pergamon Press*, Oxford, UK, (1992).
- [138] Yoshimura, M., Sujaridworakun, P., Koh, F., Fujiwara, T., Pongkao, D. and Ahniyaz, A., 2004. Hydrothermal conversion of calcite crystals to hydroxyapatite. *Materials Science and Engineering: C*, 24(4), pp.521-525.
- [139] Khan, M.M., 2025. Precipitation and coprecipitation synthesis methods. *Photocatalysts: synthesis and characterization methods*, pp.11-25.
- [140] Gutierrez, F.V., Lima, I.S., De Falco, A., Ereias, B.M., Baffa, O., de Abreu Lima, C.D., Sinimbu, L.I.M., de la Presa, P., Luz-Lima, C. and Araujo, J.F.D.F., 2024. The effect of temperature on the synthesis of magnetite nanoparticles by the coprecipitation method. *Heliyon*, 10(4).
- [141] Hargreaves, J.S.J., 2016. Some considerations related to the use of the Scherrer equation in powder X-ray diffraction as applied to heterogeneous catalysts. *Catalysis, Structure & Reactivity*, 2(1-4), pp.33-37.
- [142] Postek, M.T., Villarrubia, J.S. and Vladár, A.E., 2005. Advanced electron microscopy needs for nanotechnology and nanomanufacturing. *Journal of Vacuum Science & Technology B: Microelectronics and Nanometer Structures Processing, Measurement, and Phenomena*, 23(6), pp.3015-3022.
- [143] Bozzola, J.J. and Russell, L.D., 1999. *Electron microscopy: principles and techniques for biologists*. Jones & Bartlett Learning.
- [144] Schatten, H., 2012. biology: SEM basics, past accomplishments, and new frontiers. *Scanning Electron Microscopy for the Life Sciences*, p.1.

-
- [145] Joy, D.C., 1991. The theory and practice of high-resolution scanning electron Microscopy. *Ultramicroscopy*, 37(1-4), pp.216-233.
- [146] Ul-Hamid, A., 2018. Components of the SEM. In *A Beginners' Guide to Scanning Electron Microscopy* (pp. 15-76). Cham: Springer International Publishing.
- [147] Colle, R. and Simonucci, S., 2005. Deep Level Spectroscopy and Auger Spectra. In *Encyclopedia of Condensed Matter Physics Volume One*, (pp. 369-378). ELSEVIER, Academic Press.
- [148] Goodhew, P., 2011. General introduction to transmission electron microscopy (TEM). *Aberration-corrected analytical transmission electron microscopy*, pp.1-19.
- [149] Raman, C.V. and Krishnan, K.S., 1928. A new type of secondary radiation. *Nature*, 121(3048), pp.501-502.
- [150] Weber, W.H. and Merlin, R. eds., 2013. *Raman scattering in materials science* (Vol. 42). Springer Science & Business Media.
- [151] Stevie, F.A. and Donley, C.L., 2020. Introduction to x-ray photoelectron spectroscopy. *Journal of Vacuum Science & Technology A*, 38(6).
- [152] Klopogge, J.T. and Wood, B.J., 2020. *Handbook of Mineral Spectroscopy: Volume 1: X-ray Photoelectron Spectra*. Elsevier.
- [153] Khoshhesab, Z.M., 2012. Reflectance IR spectroscopy. *Infrared spectroscopy-Materials science, engineering and technology*, 11, pp.233-244.
- [154] Shukla, U., 2025. Fourier transform infrared spectroscopy: A powerful method for creating fingerprint of molecules of nanomaterials. *Journal of Molecular Structure*, 1322, p.140454.
- [155] Tamtam, F., Mercier, F., Eurin, J., Chevreuil, M. and Le Bot, B., 2009. Ultra performance liquid chromatography tandem mass spectrometry performance evaluation for analysis of antibiotics in natural waters. *Analytical and bioanalytical chemistry*, 393(6), pp.1709-1718.
- [156] Heinze, J., 1984. Cyclic voltammetry—"electrochemical spectroscopy". New analytical methods (25). *Angewandte Chemie International Edition in English*, 23(11), pp.831-847.

- [157] Weltin, A. and Kieninger, J., 2021. Electrochemical methods for neural interface electrodes. *Journal of neural engineering*, 18(5), p.052001.
- [158] Kashyap, B. and Kumar, R., 2022. A novel multi-set differential pulse voltammetry technique for improving precision in electrochemical sensing. *Biosensors and Bioelectronics*, 216, p.114628.
- [159] Dhillon, S. and Kant, R., 2017. Theory for electrochemical impedance spectroscopy of heterogeneous electrode with distributed capacitance and charge transfer resistance. *Journal of chemical sciences*, 129(8), pp.1277-1292.
- [160] Christwardana, M., Chung, Y. and Kwon, Y., 2017. A correlation of results measured by cyclic voltammogram and impedance spectroscopy in glucose oxidase based biocatalysts. *Korean Journal of Chemical Engineering*, 34(11), pp.3009-3016.
- [161] Oswald, I.P., Desautels, C., Laffitte, J., Fournout, S., Peres, S.Y., Odin, M., Le Bars, P., Le Bars, J. and Fairbrother, J.M., 2003. Mycotoxin fumonisin B1 increases intestinal colonization by pathogenic *Escherichia coli* in pigs. *Applied and environmental microbiology*, 69(10), pp.5870-5874.
- [162] Bennett, J.W., 2003. Klich. M. Mycotoxins. *Clinical Microbiology Reviews*, 16(3), pp.497-516.
- [163] Singh, A.K., Lakshmi, G.B.V.S., Fernandes, M., Sarkar, T., Gulati, P., Singh, R.P. and Solanki, P.R., 2021. A simple detection platform based on molecularly imprinted polymer for AFB1 and FuB1 mycotoxins. *Microchemical Journal*, 171, p.106730.
- [164] Rheeder, J.P., Marasas, W.F., Thiel, P.G., Sydenham, E.W., Shephard, G.S. and Van Schalkwyk, D.J., 1992. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. The American phytopathological Society. 82, p.353-7.
- [165] Plattner, R.D., Norred, W.P., Bacon, C.W., Voss, K.A., Peterson, R., Shackelford, D.D. and Weisleder, D., 1990. A method of detection of fumonisins in corn samples associated with field cases of equine leukoencephalomalacia. *Mycologia*, 82(6), pp.698-702.

- [166] Wang, S., Quan, Y., Lee, N. and Kennedy, I.R., 2006. Rapid determination of fumonisin B1 in food samples by enzyme-linked immunosorbent assay and colloidal gold immunoassay. *Journal of agricultural and food chemistry*, 54(7), pp.2491-2495.
- [167] Shephard, G.S., Thiel, P.G. and Sydenham, E.W., 1992. Determination of fumonisin B1 in plasma and urine by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications*, 574(2), pp.299-304.
- [168] Hartl, M. and Humpf, H.U., 1999. Simultaneous determination of fumonisin B1 and hydrolyzed fumonisin B1 in corn products by liquid chromatography/electrospray ionization mass spectrometry. *Journal of agricultural and food chemistry*, 47(12), pp.5078-5083.
- [169] Peña-Bahamonde, J., Nguyen, H.N., Fanourakis, S.K. and Rodrigues, D.F., 2018. Recent advances in graphene-based biosensor technology with applications in life sciences. *Journal of nanobiotechnology*, 16(1), p.75.
- [170] Li, D., Zhang, W., Yu, X., Wang, Z., Su, Z. and Wei, G., 2016. When biomolecules meet graphene: from molecular level interactions to material design and applications. *Nanoscale*, 8(47), pp.19491-19509.
- [171] Gagrani, A., Ding, B., Wang, Y. and Tsuzuki, T., 2019. pH dependent catalytic redox properties of Mn₃O₄ nanoparticles. *Materials Chemistry and Physics*, 231, pp.41-47.
- [172] Hosny, N.M. and Dahshan, A., 2012. Facile synthesis and optical band gap calculation of Mn₃O₄ nanoparticles. *Materials Chemistry and Physics*, 137(2), pp.637-643.
- [173] Şahin, B., Aydin, R. and Cetin, H., 2020. Tuning the morphological, structural, optical and dielectric properties of hausmannite (Mn₃O₄) films by doping heavy metal lead. *Superlattices and Microstructures*, 143, p.106546.
- [174] Gao, J., Lowe, M.A. and Abruna, H.D., 2011. Spongelike nanosized Mn₃O₄ as a high-capacity anode material for rechargeable lithium batteries. *Chemistry of Materials*, 23(13), pp.3223-3227.

- [175] Reza, K.K., Singh, N., Yadav, S.K., Singh, M.K. and Biradar, A.M., 2014. Pearl shaped highly sensitive Mn_3O_4 nanocomposite interface for biosensor applications. *Biosensors and Bioelectronics*, 62, pp.47-51..
- [176] Gund, G.S., Dubal, D.P., Patil, B.H., Shinde, S.S. and Lokhande, C.D., 2013. Enhanced activity of chemically synthesized hybrid graphene oxide/ Mn_3O_4 composite for high performance supercapacitors. *Electrochimica Acta*, 92, pp.205-215.
- [177] Khan, H.A., Lee, Y.K., Shaik, M.R., Alrashood, S.T. and Ekhzaimy, A.A., 2022. Nanocomposites of nitrogen-doped graphene oxide and manganese oxide for photodynamic therapy and magnetic resonance imaging. *International Journal of Molecular Sciences*, 23(23), p.15087.
- [178] Chen, J., Bai, Z., Li, X., Wang, Q., Du, J., Lu, R. and Liu, X., 2022. In-situ synthesis of reduced graphene oxide wrapped Mn_3O_4 nanocomposite as anode materials for high-performance lithium-ion batteries. *Ceramics International*, 48(21), pp.31923-31930.
- [179] Ahmed, S., Ansari, A., Haidyrah, A.S., Chaudhary, A.A., Imran, M. and Khan, A., 2022. Hierarchical molecularly imprinted inverse opal-based platforms for highly selective and sensitive determination of histamine. *ACS Applied Polymer Materials*, 4(4), pp.2783-2793.
- [180] Dhiman, T.K., Lakshmi, G.B.V.S., Dave, K., Roychoudhury, A., Dalal, N., Jha, S.K., Kumar, A., Han, K.H. and Solanki, P.R., 2021. Rapid and label-free electrochemical detection of fumonisin-B1 using microfluidic biosensing platform based on Ag-CeO₂ nanocomposite. *Journal of The Electrochemical Society*, 168(7), p.077510.
- [181] Alsulami, T., Nath, N., Flemming, R., Wang, H., Zhou, W. and Yu, J.H., 2021. Development of a novel homogeneous immunoassay using the engineered luminescent enzyme NanoLuc for the quantification of the mycotoxin fumonisin B1. *Biosensors and Bioelectronics*, 177, p.112939.
- [182] Zheng, Y., Shi, Z., Wu, W., He, C. and Zhang, H., 2021. Label-free DNA electrochemical aptasensor for fumonisin B1 detection in maize based on graphene and gold nanocomposite. *Journal of Analytical Chemistry*, 76(2), pp.252-257.

- [183] Mao, L., Ji, K., Yao, L., Xue, X., Wen, W., Zhang, X. and Wang, S., 2019. Molecularly imprinted photoelectrochemical sensor for fumonisin B1 based on GO-CdS heterojunction. *Biosensors and Bioelectronics*, 127, pp.57-63.
- [184] Wu, S., Duan, N., Li, X., Tan, G., Ma, X., Xia, Y., Wang, Z. and Wang, H., 2013. Homogenous detection of fumonisin B1 with a molecular beacon based on fluorescence resonance energy transfer between NaYF₄: Yb, Ho upconversion nanoparticles and gold nanoparticles. *Talanta*, 116, pp.611-618.
- [185] Jodra, A., López, M.Á. and Escarpa, A., 2015. Disposable and reliable electrochemical magnetosensor for Fumonisin simplified determination in maize-based foodstuffs. *Biosensors and Bioelectronics*, 64, pp.633-638.
- [186] Lu, T., Zhan, S., Zhou, Y., Chen, X., Huang, X., Leng, Y., Xiong, Y. and Xu, Y., 2018. Fluorescence ELISA based on CAT-regulated fluorescence quenching of CdTe QDs for sensitive detection of FB1. *Analytical Methods*, 10(48), pp.5797-5802.
- [187] Yang, X., Zhou, X., Zhang, X., Qing, Y., Luo, M., Liu, X., Li, C., Li, Y., Xia, H. and Qiu, J., 2015. A highly sensitive electrochemical immunosensor for fumonisin B1 detection in corn using single-walled carbon nanotubes/chitosan. *Electroanalysis*, 27(11), pp.2679-2687.
- [188] Chen, X., Miao, X., Ma, T., Leng, Y., Hao, L., Duan, H., Yuan, J., Li, Y., Huang, X. and Xiong, Y., 2021. Gold nanobeads with enhanced absorbance for improved sensitivity in competitive lateral flow immunoassays. *Foods*, 10(7), p.1488.
- [189] Qu, C., Zhao, L., He, X., Yu, S. and Wei, M., 2021. Magnetic beads-assisted fluorescence aptasensing approach based on dual DNA tweezers for detection of ochratoxin A and fumonisin B1 in wine and corn: Qu C. et al. *Analytical and bioanalytical chemistry*, 413(26), pp.6677-6685.
- [190] Zaaba, N.I., Foo, K.L., Hashim, U., Tan, S.J., Liu, W.W. and Voon, C.H., 2017. Synthesis of graphene oxide using modified hummers method: solvent influence. *Procedia engineering*, 184, pp.469-477.

- [191] Singh, A.K., Dhiman, T.K., Lakshmi, G.B.V.S., Raj, R., Jha, S.K. and Solanki, P.R., 2022. Rapid and label-free detection of Aflatoxin-B1 via microfluidic electrochemical biosensor based on manganese (III) oxide (Mn_3O_4) synthesized by co-precipitation route at room temperature. *Nanotechnology*, 33(28), p.285501.
- [192] Nam, I., Kim, N.D., Kim, G.P., Park, J. and Yi, J., 2013. One step preparation of Mn_3O_4 /graphene composites for use as an anode in Li ion batteries. *Journal of power sources*, 244, pp.56-62.
- [193] Ata, M.S., Liu, Y. and Zhitomirsky, I., 2014. A review of new methods of surface chemical modification, dispersion and electrophoretic deposition of metal oxide particles. *Rsc Advances*, 4(43), pp.22716-22732.
- [194] Ain, Q.T., Haq, S.H., Alshammari, A., Al-Mutlaq, M.A. and Anjum, M.N., 2019. The systemic effect of PEG-nGO-induced oxidative stress in vivo in a rodent model. *Beilstein journal of nanotechnology*, 10(1), pp.901-911.
- [195] Kanwal, A., Sajjad, S., Leghari, S.A.K. and Khan, M.N., 2021. Strong interfacial charge transfer between hausmannite manganese oxide and alumina for efficient photocatalysis. *Chinese Journal of Chemical Engineering*, 33, pp.147-159.
- [196] Rani, B.J., Ravina, M., Ravi, G., Ravichandran, S., Ganesh, V. and Yuvakkumar, R., 2018. Synthesis and characterization of hausmannite (Mn_3O_4) nanostructures. *Surfaces and Interfaces*, 11, pp.28-36.
- [197] Zawrah, M.F., El Fadaly, E.A., Khattab, R.M., Aly, M.H. and El Shafei, H., 2020. Synthesis and characterization of nano Mn_3O_4 and LiMn_2O_4 spinel from manganese ore and pure materials. *Ceramics International*, 46(11), pp.17514-17522.
- [198] Bose, V.C., Maniammal, K., Madhu, G., Veenas, C.L., Raj, A.A. and Biju, V., 2015, February. DC electrical conductivity of nanocrystalline Mn_3O_4 synthesized through a novel sol-gel route. In *IOP conference series: materials science and engineering* (Vol. 73, No. 1, p. 012084).
- [199] hu, L., Zhang, S., Cui, Y., Song, H. and Chen, X., 2013. One step synthesis and capacitive performance of graphene nanosheets/ Mn_3O_4 composite. *Electrochimica Acta*, 89, pp.18-23.

- [200] Zhang, X., Sun, X., Chen, Y., Zhang, D. and Ma, Y., 2012. One-step solvothermal synthesis of graphene/Mn₃O₄ nanocomposites and their electrochemical properties for supercapacitors. *Materials Letters*, 68, pp.336-339.
- [201] Li, Y., Qu, J., Gao, F., Lv, S., Shi, L., He, C. and Sun, J., 2015. In situ fabrication of Mn₃O₄ decorated graphene oxide as a synergistic catalyst for degradation of methylene blue. *Applied Catalysis B: Environmental*, 162, pp.268-274.
- [202] Jiangying, Q., Feng, G., Quan, Z., Zhiyu, W., Han, H., Beibei, L., Wubo, W., Xuzhen, W. and Jieshan, Q., 2013. Highly atom-economic synthesis of graphene/Mn₃O₄ hybrid composites for electrochemical supercapacitors. *Nanoscale*, 5(7), pp.2999-3005.
- [203] Wang, L., Li, Y., Han, Z., Chen, L., Qian, B., Jiang, X., Pinto, J. and Yang, G., 2013. Composite structure and properties of Mn₃O₄/graphene oxide and Mn₃O₄/graphene. *Journal of Materials Chemistry A*, 1(29), pp.8385-8397.
- [204] Khan, A., Habib, M.R., Jingkun, C., Xu, M., Yang, D. and Yu, X., 2021. New insight into the metal-catalyst-free direct chemical vapor deposition growth of graphene on silicon substrates. *The Journal of Physical Chemistry C*, 125(3), pp.1774-1783.
- [205] Khan, A., Cong, J., Kumar, R.R., Ahmed, S., Yang, D. and Yu, X., 2022. Chemical vapor deposition of graphene on self-limited SiC interfacial layers formed on silicon substrates for heterojunction devices. *ACS Applied Nano Materials*, 5(12), pp.17544-17555.
- [206] Buciuman, F., Patcas, F., Craciun, R. and RT Zahn, D., 1999. Vibrational spectroscopy of bulk and supported manganese oxides. *Physical Chemistry Chemical Physics*, 1(1), pp.185-190.
- [207] Deljoo, B., Tan, H., Suib, S.L. and Aindow, M., 2020. Thermally activated structural transformations in manganese oxide nanoparticles under air and argon atmospheres. *Journal of Materials Science*, 55(17), pp.7247-7258.
- [208] Shah, H.U., Wang, F., Toufiq, A.M., Ali, S., Khan, Z.U.H., Li, Y., Hu, J. and He, K., 2018. Electrochemical properties of controlled size Mn₃O₄ nanoparticles for supercapacitor applications. *Journal of nanoscience and nanotechnology*, 18(1), pp.719-724.

- [209] Krishnan, B. and Mahalingam, S., 2017. Synthesis and characterization of $\text{Mn}_3\text{O}_4/\text{BC}$ nanocomposite and its antimicrobial activity. *Journal of Inorganic and Organometallic Polymers and Materials*, 27(1), pp.275-284.
- [210] Wang, Y., Hou, C., Lin, X., Jiang, H., Zhang, C. and Liu, G., 2021. Dye degradation studies of hausmannite manganese oxide (Mn_3O_4) nanoparticles synthesized by chemical method. *Applied Physics A*, 127(4), p.277.
- [211] Bhardwaj, H., Pandey, M.K., Rajesh, N. and Sumana, G., 2019. Electrochemical Aflatoxin B1 immunosensor based on the use of graphene quantum dots and gold nanoparticles. *Microchimica Acta*, 186(8), p.592.
- [212] Bhardwaj, H., Marquette, C.A., Dutta, P., Rajesh and Sumana, G., 2020. Integrated graphene quantum dot decorated functionalized nanosheet biosensor for mycotoxin detection: Bhardwaj H. et al. *Analytical and bioanalytical chemistry*, 412(25), pp.7029-7041.
- [213] Karimi, M. and Eshraghi, M.J., 2017. One-pot and green synthesis of Mn_3O_4 nanoparticles using an all-in-one system (solvent, reactant and template) based on ethaline deep eutectic solvent. *Journal of Alloys and Compounds*, 696, pp.171-176.
- [214] Wang L, Li Y, Han Z, Chen L, Qian B, Jiang X, Pinto J, Yang G. Composite structure and properties of $\text{Mn}_3\text{O}_4/\text{graphene oxide}$ and $\text{Mn}_3\text{O}_4/\text{graphene}$. *Journal of Materials Chemistry A*. 2013 Jul 2;1(29):8385-97.
- [215] Ashiq, S., 2015. Natural occurrence of mycotoxins in food and feed: Pakistan perspective. *Comprehensive reviews in food science and food safety*, 14(2), pp.159-175.
- [216] Peivasteh-Roudsari, L., Pirhadi, M., Shahbazi, R., Eghbaljoo-Gharehgheshlaghi, H., Sepahi, M., Mirza Alizadeh, A., Tajdar-Oranj, B. and Jazaeri, S., 2022. Mycotoxins: Impact on health and strategies for prevention and detoxification in the food chain. *Food reviews international*, 38(sup1), pp.193-224.
- [217] El-Sayed, R.A., Jebur, A.B., Kang, W. and El-Demerdash, F.M., 2022. An overview on the major mycotoxins in food products: Characteristics, toxicity, and analysis. *Journal of Future Foods*, 2(2), pp.91-102.

- [218] Gao, Z., Luo, K., Zhu, Q., Peng, J., Liu, C., Wang, X., Li, S. and Zhang, H., 2023. The natural occurrence, toxicity mechanisms and management strategies of Fumonisin B1: A review. *Environmental Pollution*, 320, p.121065.
- [219] Zentai, A., Szeitzné-Szabó, M., Mihucz, G., Szeli, N., Szabó, A. and Kovács, M., 2019. Occurrence and risk assessment of fumonisin B1 and B2 mycotoxins in maize-based food products in Hungary. *Toxins*, 11(12), p.709.
- [220] Corrêa, J.A.F., Orso, P.B., Bordin, K., Hara, R.V. and Luciano, F.B., 2018. Toxicological effects of fumonisin B1 in combination with other Fusarium toxins. *Food and Chemical Toxicology*, 121, pp.483-494.
- [221] Kadir, M.K.A. and Tothill, I.E., 2010. Development of an electrochemical immunosensor for fumonisins detection in foods. *Toxins*, 2(4), pp.382-398.
- [222] Mirón Mérida, V.A., Gong, Y.Y. and Goycoolea, F., 2020. Aptamer-based detection of fumonisin B1: A systematic comparison with conventional and other novel methods.
- [223] Ramya, M., Kumar, P.S., Rangasamy, G., Rajesh, G., Nirmala, K., Saravanan, A. and Krishnapandi, A., 2022. A recent advancement on the applications of nanomaterials in electrochemical sensors and biosensors. *Chemosphere*, 308, p.136416.
- [224] Bhardwaj, H., Singh, C., Kotnala, R.K. and Sumana, G., 2018. Graphene quantum dots-based nano-biointerface platform for food toxin detection: Bhardwaj H. et al. *Analytical and bioanalytical chemistry*, 410(28), pp.7313-7323.
- [225] Patel, M.S., M, S., Rahman Faisal, A., B, A., BW, S., Chaudhary, V. and C, M., 2022. Functionalized iron oxide nanostructures: Recent advances in the synthesis, characterization, and electrochemical biosensor applications. *Electrochemical Society Transactions*, 107(1), pp.15477-15486.
- [226] Yin, Y., Zhang, X., Li, L., Spiccia, L. and Sun, C., 2017. Experimental and computational investigation of the optical, electronic, and electrochemical properties of hydrogenated α -Fe₂O₃. *The Journal of Physical Chemistry C*, 121(30), pp.16059-16065.

- [227] Priyavrat, Khan, A., Kunal, Sadasivuni, K.K., Singh, A.K. and Ansari, J.R., 2024. Fe₂O₃ decorated reduced graphene oxide sheets for enhanced sensing applications for dopamine. *Emergent Materials*, 7(6), pp.2973-2984.
- [228] Naghshbandi, B., Adabi, M., Pooshang Bagheri, K. and Tavakolipour, H., 2022. Design of a new electrochemical aptasensor based on screen printed carbon electrode modified with gold nanoparticles for the detection of fumonisin B1 in maize flour. *Journal of nanobiotechnology*, 20(1), p.534.
- [229] Qin, Y., Li, S., Wang, Y., Peng, Y., Han, D., Zhou, H., Bai, J., Ren, S., Li, S., Chen, R. and Han, T., 2022. A highly sensitive fluorometric biosensor for Fumonisin B1 detection based on upconversion nanoparticles-graphene oxide and catalytic hairpin assembly. *Analytica Chimica Acta*, 1207, p.339811.
- [230] Wang, C., Huang, X., Tian, X., Zhang, X., Yu, S., Chang, X., Ren, Y. and Qian, J., 2019. A multiplexed FRET aptasensor for the simultaneous detection of mycotoxins with magnetically controlled graphene oxide/Fe₃O₄ as a single energy acceptor. *Analyst*, 144(20), pp.6004-6010.
- [231] Dhiman, T.K., Lakshmi, G.B.V.S., Dave, K., Roychoudhury, A., Dalal, N., Jha, S.K., Kumar, A., Han, K.H. and Solanki, P.R., 2021. Rapid and label-free electrochemical detection of fumonisin-B1 using microfluidic biosensing platform based on Ag-CeO₂ nanocomposite. *Journal of The Electrochemical Society*, 168(7), p.077510.
- [232] Yang, X., Zhou, X., Zhang, X., Qing, Y., Luo, M., Liu, X., Li, C., Li, Y., Xia, H. and Qiu, J., 2015. A highly sensitive electrochemical immunosensor for fumonisin B1 detection in corn using single-walled carbon nanotubes/chitosan. *Electroanalysis*, 27(11), pp.2679-2687.
- [233] Masikini, M., Mailu, S.N., Tsegaye, A., Njomo, N., Molapo, K.M., Ikpo, C.O., Sunday, C.E., Rassie, C., Wilson, L., Baker, P.G. and Iwuoha, E.I., 2014. A fumonisins immunosensor based on polyanilino-carbon nanotubes doped with palladium telluride quantum dots. *Sensors*, 15(1), pp.529-546.
- [234] Sarpal, S., Singh, A.K., Bhardwaj, H., Puri, N.K. and Solanki, P.R., 2023. Graphene oxide-Mn₃O₄ nanocomposites for advanced electrochemical biosensor for fumonisin B1 detection. *Nanotechnology*, 34(46), p.465708.

- [235] Dang, Q., Sun, B., Shi, H., Ma, Q., Liu, Y., Zou, H., Wang, X., Da, X., Zhou, M., Lv, Y. and Yang, L., 2024. A highly sensitive and rapid enzyme biosensor based on AuNPs/CS@ cMWCNTS for detecting fusarium acid. *Journal of The Electrochemical Society*, 171(8), p.087521.
- [236] Lu, L., Seenivasan, R., Wang, Y.C., Yu, J.H. and Gunasekaran, S., 2016. An electrochemical immunosensor for rapid and sensitive detection of mycotoxins fumonisin B1 and deoxynivalenol. *Electrochimica Acta*, 213, pp.89-97.
- [237] Zheng, Y., Shi, Z., Wu, W., He, C. and Zhang, H., 2021. Label-free DNA electrochemical aptasensor for fumonisin B1 detection in maize based on graphene and gold nanocomposite. *Journal of Analytical Chemistry*, 76(2), pp.252-257.
- [238] Xue, X., Leng, Y., Li, Y. and Zhang, J., 2024. A magnetic plasma Fe₃O₄@Cu@Cu₂O photoelectrochemical sensor for the detection of fumonisin B1. *Analytical Methods*, 16(27), pp.4534-4538.
- [239] Zhang, R., Zhu, Y., Huang, J., Xu, S., Luo, J. and Liu, X., 2018. Electrochemical sensor coating based on electrophoretic deposition of Au-doped self-assembled nanoparticles. *ACS applied materials & interfaces*, 10(6), pp.5926-5932.
- [240] Pei, J., Zhao, H., Yang, F. and Yan, D., 2021. Graphene oxide/Fe₂O₃ nanocomposite as an efficient catalyst for thermal decomposition of ammonium perchlorate via the vacuum-freeze-drying method. *Langmuir*, 37(20), pp.6132-6138.
- [241] Kang, Y.S., Risbud, S., Rabolt, J.F. and Stroeve, P., 1996. Synthesis and characterization of nanometer-size Fe₃O₄ and γ -Fe₂O₃ particles. *Chemistry of materials*, 8(9), pp.2209-2211.
- [242] Maji, S.K., Mukherjee, N., Mondal, A. and Adhikary, B., 2012. Synthesis, characterization and photocatalytic activity of α -Fe₂O₃ nanoparticles. *Polyhedron*, 33(1), pp.145-149.
- [243] Besra, L. and Liu, M., 2007. A review on fundamentals and applications of electrophoretic deposition (EPD). *Progress in materials science*, 52(1), pp.1-61.

- [244] Boccaccini, A.R., Keim, S., Ma, R., Li, Y. and Zhitomirsky, I., 2010. Electrophoretic deposition of biomaterials. *Journal of the Royal Society Interface*, 7(Suppl 5), p.S581.
- [245] Debnath, A., Deb, K., Das, N.S., Chattopadhyay, K.K. and Saha, B., 2016. Simple chemical route synthesis of Fe_2O_3 nanoparticles and its application for adsorptive removal of Congo red from aqueous media: artificial neural network modeling. *Journal of Dispersion Science and Technology*, 37(6), pp.775-785.
- [246] Liu, Z., Fu, H., Gao, B., Wang, Y., Li, K., Sun, Y., Yin, J. and Kan, J., 2020. In-situ synthesis of $\text{Fe}_2\text{O}_3/\text{rGO}$ using different hydrothermal methods as anode materials for lithium-ion batteries. *Reviews on Advanced Materials Science*, 59(1), pp.477-486.
- [247] Han, S., Hu, L., Liang, Z., Wageh, S., Al-Ghamdi, A.A., Chen, Y. and Fang, X., 2014. One-step hydrothermal synthesis of 2D hexagonal nanoplates of $\alpha\text{-Fe}_2\text{O}_3$ /graphene composites with enhanced photocatalytic activity. *Advanced Functional Materials*, 24(36), pp.5719-5727.
- [248] Verma, D., Ranjan, K.R., Mukherjee, M.D. and Solanki, P.R., 2022. Bioinspired synthesis of hematite nanoparticles-reduced graphene oxide composite for application in bisphenol a detection: A new in-sight. *Biosensors and Bioelectronics: X*, 11, p.100217.
- [249] Sephra, P.J., Baraneedharan, P., Sivakumar, M., Thangadurai, T.D. and Nehru, K., 2018. In situ growth of hexagonal-shaped $\alpha\text{-Fe}_2\text{O}_3$ nanostructures over few layered graphene by hydrothermal method and their electrochemical performance. *Journal of Materials Science: Materials in Electronics*, 29(8), pp.6898-6908.
- [250] Kudin, K.N., Ozbas, B., Schniepp, H.C., Prud'Homme, R.K., Aksay, I.A. and Car, R., 2008. Raman spectra of graphite oxide and functionalized graphene sheets. *Nano letters*, 8(1), pp.36-41.
- [251] Apte, S.K., Naik, S.D., Sonawane, R.S., Kale, B.B. and Baeg, J.O., 2007. Synthesis of nanosize-necked structure α - and $\gamma\text{-Fe}_2\text{O}_3$ and its photocatalytic activity. *Journal of the American Ceramic Society*, 90(2), pp.412-414.

- [252] Wang, F., Qin, X.F., Meng, Y.F., Guo, Z.L., Yang, L.X. and Ming, Y.F., 2013. Hydrothermal synthesis and characterization of α -Fe₂O₃ nanoparticles. *Materials Science in Semiconductor Processing*, 16(3), pp.802-806.
- [253] Andrijanto, E., Shoelarta, S., Subiyanto, G. and Rifki, S., 2016, April. Facile synthesis of graphene from graphite using ascorbic acid as reducing agent. In *AIP Conference Proceedings* (Vol. 1725, No. 1, p. 020003). AIP Publishing LLC.
- [254] Bhardwaj, H., Pandey, M.K., Rajesh, N. and Sumana, G., 2019. Electrochemical Aflatoxin B1 immunosensor based on the use of graphene quantum dots and gold nanoparticles. *Microchimica Acta*, 186(8), p.592.
- [255] Bhardwaj, H., Marquette, C.A., Dutta, P., Rajesh and Sumana, G., 2020. Integrated graphene quantum dot decorated functionalized nanosheet biosensor for mycotoxin detection: Bhardwaj H. et al. *Analytical and bioanalytical chemistry*, 412(25), pp.7029-7041.
- [256] Karimi, M. and Eshraghi, M.J., 2017. One-pot and green synthesis of Mn₃O₄ nanoparticles using an all-in-one system (solvent, reactant and template) based on ethaline deep eutectic solvent. *Journal of Alloys and Compounds*, 696, pp.171-176.
- [257] Ravichandran, S., Bhuvanendran, N., Peng, K., Zhang, W., Xu, Q. and Su, H., 2021. Pt nanoparticles decorated on Fe₂O₃/N, P-doped mesoporous carbon for enhanced oxygen reduction activity and durability. *Journal of Electrochemical Energy Conversion and Storage*, 18(2), p.021006.
- [258] Dolgov, A., Lopaev, D., Lee, C.J., Zoethout, E., Medvedev, V., Yakushev, O. and Bijkerk, F., 2015. Characterization of carbon contamination under ion and hot atom bombardment in a tin-plasma extreme ultraviolet light source. *Applied surface science*, 353, pp.708-713.
- [259] Jain, S., Shah, J., Negi, N.S., Sharma, C. and Kotnala, R.K., 2019. Significance of interface barrier at electrode of hematite hydroelectric cell for generating ecopower by water splitting. *International Journal of Energy Research*, 43(9), pp.4743-4755.

- [260] Ijaz, A., Asif, M., Kashita, E., El-kott, A.F., AlShehri, M.A., Al-Shahari, E.A., Khan, M.T., Fatima, M., Atamuratova, Z. and Aziz, M.H., 2026. Electrochemical immunosensor based on Au-NPs/Fe₂O₃/CeO₂ nanocomposite for aflatoxin B1 detection. *Results in Physics*, p.108578.
- [261] Ambrosi, A., Chua, C.K., Bonanni, A. and Pumera, M., 2014. Electrochemistry of graphene and related materials. *Chemical reviews*, 114(14), pp.7150-7188.

SANDEEP SARPAL

Department of Applied Physics,
Delhi Technological University, Delhi, India-110042

E-mail: sandeepsarpal@rediffmail.com

Phone: +91-9868343607

Education

🚩 **Ph.D. (Pursuing)** – Delhi Technological University, 2018 – 2026

Thesis: Synthesis of two-dimensional nanomaterials for electrochemical sensing application

🚩 **M. Tech. Instrumentation** –Panjab University, Chandigarh 2000–2002 (65.88%)

🚩 **M.Sc. in Applied Physics** – Guru Nanak Dev University, Amritsar, Panjab, 1997–1999 (63.66%)

🚩 **B.Sc. in Electronic Science-** Guru Nanak Dev University, Amritsar, Panjab, 1994–1997 (57.16%)

🚩 **UGC NET- Electronic Science** Qualified for Assistant Professor, June 2024

Research Focus

- 🚩 Development of electrochemical sensors with superior sensitivity and selectivity.
- 🚩 Exploration of 2D nanomaterials for various electronic properties.

Research Publications

- [1] **Sandeep Sarpal**, Avinash Kumar Singh, Hema Bhardwaj, Nitin Kumar Puri, and Pratima R Solanki, “Graphene oxide-Mn₃O₄ nanocomposites for advanced electrochemical biosensor for fumonisin B1 detection” *Nanotechnology*, 2023, 34(46), p.465708.
- [2] **Sandeep Sarpal**, Hema Bhardwaj, Nitin Kumar Puri, Vinod Singh, and Pratima R Solanki, “Hematite-reduced graphene oxide Nanocomposite-Based Electrochemical Biosensing Platform for Rapid Detection of Fumonisin B1 in Corn Samples” *Chemistry Select*, 2026,
- [3] S. Sreekesh, Meenakshi, Sreerama Naik, Pankaj Kumar, **Sandeep Sarpal**, 2025. Mineralogical and geochemical proxies from sediment cores to understand paleoclimate and sea level changes along the Southwest coast of India. *Applied Geochemistry*, p.106532.
- [4] Uzma Parveen, S Sreekesh, **Sandeep Sarpal**, 2020. Reconstruction of the paleoenvironmental condition using geochemical proxies in the Lower Baitarani Basin, Coastal Odisha, India. *Journal of Climate Change*, 6(1), pp.37-46.
- [5] Uzma Parveen, S Sreekesh, **Sandeep Sarpal**, 2023. Paleo-Reconstruction of

Heavy Metal Accumulation during the Holocene in Coastal Odisha, India. *Geochemistry International*, 61(13), pp.1426-1441.

Technical Experience

 **Technical Officer (2008–till date), J.N.U, Delhi**

Technical Skills

 Data Analysis: Origin, MS Office, Excel

 Operating Systems: Windows

Conferences, Seminars, and Workshops

 **Sandeep Sarpal**, A.S.Rao, Nitin K. Puri, Hema Bhardwaj, Pratima Solanki, “Mn₃O₄/GO Nanocomposites for Advanced Electrochemical Biosensor for detection of Fumonisin B1”, Recent Trends in Applied Physics & Material Science (RAM 2024) at Bikaner Technical University, Bikaner, 15th – 16th Nov 2024. (POSTER)

 **Sandeep Sarpal**, A.S.Rao, Nitin K. Puri, Hema Bhardwaj, Pratima Solanki, “Ferric Oxide EOGO nanocomposite for mycotoxin detection”, International Conference on Materials & Applied Science for Society (MASS 2024), Govt Engineering College, Bikaner, 30th – 31st Dec 2024. (POSTER)

 Attended a one-day national seminar on “Sensors for Society” organized by The Electrochemical Society at Jawaharlal Nehru University, New Delhi, on 27th April 2022.

 Participated in a six-day course on “Radiation Biology & Its Clinical Applications” in Global Initiative of Academic Networks (GIAN) from 26th March to 31st March 2022.

 Participated in a six-day course on “Nanotechnology in Water and Wastewater Treatment” in Global Initiative of Academic Networks (GIAN) from 04th April to 09th April 2022.

References

 **Prof. Vinod Singh** – Professor (*Ph.D. Advisor*)
Department of Applied Physics, DTU, New Delhi, India (110042) |
✉ vinodsingh@dtu.ac.in

 **Prof. Nitin Kumar Puri** – Professor
Department of Applied Physics, DTU, New Delhi, India (110042) |
✉ vinodsingh@dtu.ac.in

 **Dr. Partima Solanki**- Asstt. Professor
Special centre of Nanoscience, Jawaharlal Nehru University, New Delhi