

# Production of Bio-Mimetic Nanoparticles for the Detection of Ascorbic Acid



MS Thesis  
By  
Nazar Ali

CIIT/SP24-RPH-018/LHR

**COMSATS University Islamabad**  
**Pakistan**  
**Fall 2025**



# Production of Bio-Mimetic Nanoparticles for the Detection of Ascorbic Acid

A thesis submitted to  
COMSATS University Islamabad

In partial fulfilment  
of the requirements for the degree of

Master of Science  
in  
Physics  
by

Nazar Ali  
CIIT/SP24-RPH-018/LHR

**COMSATS University Islamabad**  
**Pakistan**  
**Fall 2025**

# Production of Bio-Mimetic Nanoparticles for the Detection of Ascorbic Acid

This thesis is submitted to the Department of Physics in partial fulfilment of the requirements for the award of the degree of Master of Science in Physics.

| Name      | Registration Number   |
|-----------|-----------------------|
| Nazar Ali | CIIT/SP24-RPH-018/LHR |

## Supervisory Committee

### Supervisor

Dr. Muhammad Ashfaq Ahmad  
Tenured Professor  
Department of Physics  
COMSATS University Islamabad (CUI)  
Lahore Campus

### Co-Supervisor

Dr. Muhammad Nasir  
Associate Professor  
Department of Interdisciplinary  
Research Centre in Biomedical Materials  
(IRCBM)  
COMSATS University Islamabad (CUI)  
Lahore Campus

# Certificate of Approval

This thesis titled

## Production of Bio-Mimetic Nanoparticles for the Detection of Ascorbic Acid

By

Nazar Ali

CIIT/SP24-RPH-018/LHR

has been approved

for the Degree of MS Physics

at COMSATS University Islamabad, Lahore Campus

External Examiner:

---

Dr. Abdul Waheed Anwar  
Associate Professor,  
Department of Physics,  
University of Engineering and  
Technology, UET Lahore

Supervisor:

---

Dr. Muhammad Ashfaq Ahmad  
Tenured Professor,  
Department of Physics  
CUI, Lahore Campus

Co-Supervisor:

---

Dr. Muhammad Nasir  
Associate Professor,  
Department of Interdisciplinary Research centre in Biomedical  
Materials (IRCBM)  
CUI, Lahore Campus

Head of Department:

---

Dr. Amir Razaq  
Associate Professor,  
Department of Physics  
CUI, Lahore Campus

## **Author's Declaration**

I Nazar Ali, CIIT/SP24-RPH-018/LHR, hereby declare that I have produced the work presented in this thesis, during the scheduled period of study. I also declare that I have not taken any material from any source except referred to wherever due to that amount of plagiarism is within an acceptable range. If a violation of HEC rules on research has occurred in this thesis, I shall be liable to punishable action under the plagiarism rules of HEC.

Date: \_\_\_\_\_

\_\_\_\_\_

Nazar Ali

CIIT/SP24-RPH-018/LHR

# Certificate

It is certified that Nazar Ali, CIIT/SP24-RPH-018/LHR, has carried out all the work related to this thesis under my supervision at the Department of Physics, COMSATS University Islamabad, Lahore campus, and the work fulfils the requirements for the award of the degree of Master of Science in Physics.

Date: \_\_\_\_\_

Supervisor

\_\_\_\_\_

Dr. Muhammad Ashfaq Ahmad

Tenured Professor,

Department of Physics

COMSATS University Islamabad

Lahore Campus

# **Dedication**

## **TO:**

This thesis is devoted to my beloved parents. Their prayers, love and sacrifices have been with me in my studies all the time. I am highly thankful to my supervisors and teachers who have been very helpful and helpful. I also appreciate the colleagues who worked in a team and their useful discussions. My friends gave me a lot of motivation and supported me in some bad days and a special thanks to them. The work is an easy thank you to all the people who assisted, both directly and indirectly, to finalize this work.

## **Acknowledgements**

I am highly thankful to ALLAH as he is the maker and he blessed us. ALLAH sent His Final Prophet, HAZRAT MUHAMMAD (PBUH) to be a guide to us. We Muslims are fond of HAZRAT Muhammad (PBUH) as he teaches us the Islam religion. The noble and ideal teacher of us is him (PBUH). I would like to acknowledge that my project Supervisor, Prof Dr. Muhammad Ashfaq Ahmad and Co-Supervisor, Dr. Muhammad Nasir were of great assistance and help in this project. They taught me a lot. My family has been significant in this project. To my parents, I am grateful because of love, support, and prayers. They are the best strength and motivation to me. M. Ishfaq Ahmad, M. Saad Shbir, Shahid Ali Bhatti, Usman Ali, M. Shahzad Haider and Muhammad Umair Azam also assisted me in my research.

Nazar Ali

CIIT/SP24-RPH-018/LHR

## **Abstract**

# **Production of Bio-Mimetic Nanoparticles for the Detection of Ascorbic Acid**

By

Nazar Ali

An economical, sensitive and new approach based on colorimetric and UV- visible of ascorbic acid (AA) is developed. The mechanism of this method is the oxidation of 3,3',5,5'-Tetramethylbenzidine TMB with the material of the synthesis to create the oxidized TMB of a blue shade (ox-TMB). The ox-TMB is then reduced by the ascorbic acid leading to a decrease in the color intensity which can be both visually and quantitatively determined with the help of a UV Vis scanner. The main experimental parameters of the concentration of materials, TMB concentration, pH and concentration of the analyte were adjusted to improve sensing result. The most favorable conditions were found to be material concentration of 5 $\mu$ M, 0.5 $\mu$ M TMB and an acidic medium with a pH of 3-4. The response had a high reliance on pH indicating a change in color in an acidic conditions and no response at neutral and alkaline conditions (pH 7-11). The UV-vis absorbance at 652 nm under such optimized parameters reduced proportional to the change in AA concentration. High sensitivity was observed in the linear response in the concentration range of 2-5 nM where it had the lowest limit of 1.18 nM detection limit (LOD) and the highest limit of 3.59 nM was the quantification limit (LOQ). The technique proved to be very selective to the AA with minimum signal interference with the normal substances such as dopamine, glucose, cholesterol, urea, uric acid, amino acids, and the metal ions. The feasibility of the sensor was shown by using samples of diluted lemon and orange juice that were able to recover diluted samples of 97.98%-101.11%. To conclude, this has created sensing platform is easy, fast, accurate, and cheap, and therefore can be utilized in ascorbic acid detection in food samples and the future portable sensing platform is achievable.

# Table of Contents

## Chapter 1

|                                                                            |          |
|----------------------------------------------------------------------------|----------|
| <b>Introduction .....</b>                                                  | <b>1</b> |
| 1.1 Background .....                                                       | 1        |
| 1.2 The Importance of Ascorbic Acid (Vitamin C) .....                      | 1        |
| 1.3 Redox Chemistry of Ascorbic Acid .....                                 | 2        |
| 1.4 Non-electronic Techniques for Ascorbic Acid Detection.....             | 2        |
| 1.5 Colorimetric Detection.....                                            | 2        |
| 1.6 Nanomaterials in Colorimetric Sensing .....                            | 3        |
| 1.7 MnOOH and Manganese-based nanomaterials .....                          | 3        |
| 1.8 MnOOH Multilayer Nanowires .....                                       | 3        |
| 1.9 Compared to Other Metal Oxide Sensors .....                            | 4        |
| 1.10 Hydrothermal Synthesis and Its Significance .....                     | 4        |
| 1.11 Ascorbic Acid Detection by MnOOH.....                                 | 4        |
| 1.12 Application in Real Samples.....                                      | 4        |
| 1.13 Research Gap and Motivation.....                                      | 4        |
| 1.14 Objectives of Current Research .....                                  | 5        |
| 1.15 Biochemical use of Ascorbic Acid .....                                | 5        |
| 1.16 Ascorbic Acid Degradation and Stability .....                         | 5        |
| 1.17 Analysis issues in Ascorbic Acid Detection .....                      | 6        |
| 1.18 Surface and Crystal Structure of MnOOH.....                           | 6        |
| 1.19 Influence of Morphology on the Sensing Performance.....               | 6        |
| 1.20 UV- visible Behaviour and Optical Properties.....                     | 6        |
| 1.21 Economic and Environmental Considerations.....                        | 7        |
| 1.22 Point-of-Care and On-Site Analysis Relevance .....                    | 7        |
| 1.23 Future of MnOOH-Based Colorimetric Sensors.....                       | 7        |
| 1.24 General importance of the current study .....                         | 7        |
| 1.25 Comprehensive review of the development of colorimetric sensors ..... | 8        |

|                                                                         |    |
|-------------------------------------------------------------------------|----|
| 1.26 Redox Reactions Kinetics in Colorimetric Systems .....             | 8  |
| 1.28 Effects of pH and Reaction Conditions .....                        | 8  |
| 1.29 Mechanism of Selectivity To Ascorbic Acid .....                    | 9  |
| 1.30 Merit Figures for Analysis .....                                   | 9  |
| 1.31 As opposed Enzyme-Based Sensors .....                              | 9  |
| 1.32 Simple Detection Platforms Integration.....                        | 9  |
| 1.33 Societal and Industrial Relevancy .....                            | 10 |
| 1.34 Restriction and Scope of the Study .....                           | 10 |
| 1.35 Final Statements of the Introduction.....                          | 10 |
| 1.36 The Analysis of Ascorbic Acid in history .....                     | 10 |
| 1.37 Development of Colorimetric Sensing Technologies .....             | 11 |
| 1.38 Basic Concept of Nanozyme Catalysis .....                          | 11 |
| 1.39 Thermodynamics Redox reaction in MnOOH .....                       | 11 |
| 1.40 Determination of Surface Defects and Oxygen Vacancies.....         | 11 |
| 1.41 Introduction to Biosensors .....                                   | 12 |
| 1.41.1 Principle of Biosensors .....                                    | 12 |
| 1.41.2 Components of Biosensors .....                                   | 12 |
| 1.41.2.1 Bioreceptor.....                                               | 12 |
| 1.41.2.2 Transducer.....                                                | 12 |
| 1.41.2.3 The Signal Processor and Display .....                         | 13 |
| 1.41.3 Attributes of a perfect Biosensor .....                          | 13 |
| 1.41.4 History of Biosensors Development .....                          | 13 |
| 1.41.5 Types of Biosensors .....                                        | 13 |
| 1.41.6 Classification Based on Bioreceptor .....                        | 13 |
| 1.41.6.1 Classification Based on Transducer .....                       | 14 |
| 1.41.7 Application of Biosensor Concepts to the Current Research.....   | 14 |
| 1.41.8 Making the connection between Biosensor and Nanozyme system..... | 14 |

|                                                                                                                            |           |
|----------------------------------------------------------------------------------------------------------------------------|-----------|
| <b>2 Chapter 2 .....</b>                                                                                                   | <b>15</b> |
| <b>Literature Review .....</b>                                                                                             | <b>15</b> |
| <b>3 Chapter 3 Materials and Methods .....</b>                                                                             | <b>23</b> |
| <b>3 Hydrothermal Method .....</b>                                                                                         | <b>23</b> |
| <b>3.1 Principle of Hydrothermal Method .....</b>                                                                          | <b>24</b> |
| <b>3.2 The Hydrothermal Method has the following benefits .....</b>                                                        | <b>24</b> |
| 3.2.1 Advantages of Hydrothermal Method .....                                                                              | 24        |
| 3.2.2 Types of Hydrothermal Techniques .....                                                                               | 25        |
| <b>3.2 Materials .....</b>                                                                                                 | <b>25</b> |
| <b>3.3 Synthesis of MnOOH Multilayer Nanowires .....</b>                                                                   | <b>25</b> |
| <b>4 Chapter 4 .....</b>                                                                                                   | <b>26</b> |
| <b>Results and Discussions .....</b>                                                                                       | <b>26</b> |
| <b>4.1 XRD Diffraction .....</b>                                                                                           | <b>26</b> |
| <b>4.2 FTIR Spectrum .....</b>                                                                                             | <b>28</b> |
| <b>4.3 Field Emission-Scanning Electron Microscopy (FE-SEM) .....</b>                                                      | <b>29</b> |
| <b>4.4 RAMAN Spectroscopy.....</b>                                                                                         | <b>30</b> |
| <b>4.5 Zeta Potential .....</b>                                                                                            | <b>31</b> |
| <b>4.6 Dynamic Light Scattering (DLS) Particle Size Analysis .....</b>                                                     | <b>32</b> |
| <b>4.7 Optimization and Mechanism of Colorimetric Detection of Ascorbic Acid Based on MnOOH nanowires Biosensors .....</b> | <b>32</b> |
| <b>4.8 Preparation of Stock solution for Selectivity Studies .....</b>                                                     | <b>33</b> |
| <b>4.9 Colorimetric Sensor Preparation .....</b>                                                                           | <b>33</b> |
| <b>4.10 Colorimetric Detection of Ascorbic Acid .....</b>                                                                  | <b>33</b> |
| 4.10.1 Visual Response of MnOOH-TMB System Visual Colorimetric .....                                                       | 34        |
| 4.10.1.1 Redox Reaction Mechanism .....                                                                                    | 34        |
| 4.10.1.2 Oxidation of TMB .....                                                                                            | 34        |
| 4.10.1.3 Reduction by Ascorbic Acid .....                                                                                  | 35        |
| <b>4.11 The Anti- Interference Performance and selectivity .....</b>                                                       | <b>35</b> |
| <b>4.13 The benefits of Developed Colorimetric Sensor .....</b>                                                            | <b>37</b> |
| <b>4.14 Practical Significance.....</b>                                                                                    | <b>37</b> |

|                                                                                      |           |
|--------------------------------------------------------------------------------------|-----------|
| <b>4.15 Condition Enhancement of Detection .....</b>                                 | <b>38</b> |
| 4.15.1 Optimization Exercise Condition Colorimetrically .....                        | 38        |
| 4.15.2 Improvement of material Potentization .....                                   | 38        |
| 4.15.3 Maximization of TMB Concentration .....                                       | 39        |
| 4.15.4 Optimization of pH .....                                                      | 40        |
| 4.15.5 Ascorbic Acid Analyte Optimization .....                                      | 42        |
| <b>4.16 Parameters Optimization of UV-vis Spectrophotometry .....</b>                | <b>43</b> |
| 4.16.1 Material's Concentration (UV-vis Study) Optimization .....                    | 43        |
| 4.16.2 TMB Concentration (UV-vis Study) Optimization.....                            | 45        |
| 4.16.3 pH (UV-vis Study) Optimization .....                                          | 45        |
| 4.16.4 Optimization of Ascorbic Acid (AA) Analyte Concentration (UV-vis Study) ..... | 46        |
| <b>4.17 Actual Sample Analysis.....</b>                                              | <b>48</b> |
| 4.17.1 Sample Analysis of Lemon juice .....                                          | 48        |
| 4.17.2 Sample Analysis of Orange juice .....                                         | 49        |
| 4.17.3 Discussion of Recovery Result .....                                           | 49        |
| <b>Chapter 5 Conclusion .....</b>                                                    | <b>50</b> |
| <b>References .....</b>                                                              | <b>51</b> |

## List of Figures

|                                                                                       |    |
|---------------------------------------------------------------------------------------|----|
| Figure 1 Hydrothermal Synthesis of MnOOH Wires .....                                  | 26 |
| Figure 2 XRD Spectra of MnOOH .....                                                   | 26 |
| Figure 3 FTIR Spectrum of MnOOH .....                                                 | 28 |
| Figure 4 FE-SEM Image of MnOOH.....                                                   | 29 |
| Figure 5 RAMAN Spectra of MnOOH .....                                                 | 30 |
| Figure 6 Zeta Potential values of MnOOH in DI-water .....                             | 31 |
| Figure 7 DLS Size distribution of MnOOH Nanowires.....                                | 32 |
| Figure 8 Photograph of Oxidation of TMB .....                                         | 35 |
| Figure 9 Reduction by Ascorbic Acid .....                                             | 35 |
| Figure 10 Photograph of Selectivity of Ascorbic Acid against 12 Amino Acids .....     | 37 |
| Figure 11 Photograph of the color change after adding 0.005mg/1mL Material .....      | 39 |
| Figure 12 Photograph of the color change of adding various concentration of TMB ...   | 40 |
| Figure 13 Photograph of the color change at various pH .....                          | 42 |
| Figure 14 Photograph of the color change after adding various concentration of AA ... | 43 |
| Figure 15 UV-vis Spectra of MnOOH .....                                               | 44 |
| Figure 16 Calibration Curve of MnOOH .....                                            | 44 |
| Figure 17 UV-vis absorption spectra of TMB .....                                      | 45 |
| Figure 18 UV-vis absorption spectra at different pH .....                             | 46 |
| Figure 19 UV-vis spectra of Analyte (AA) at 652nm .....                               | 47 |
| Figure 20 Calibration Curve of Analyte (AA) .....                                     | 48 |

# List of Abbreviations

| <b>Abbreviations</b>                              | <b>Full Form</b>                            |
|---------------------------------------------------|---------------------------------------------|
| <b>TMB</b>                                        | 3,3',5,5'-tetramethylbenzidine              |
| <b>AA</b>                                         | Ascorbic Acid                               |
| <b>DA</b>                                         | Dopamine                                    |
| <b>KCl</b>                                        | Potassium Chloride                          |
| <b>Ca(OH)<sub>2</sub></b>                         | Calcium Hydroxide                           |
| <b>DI</b>                                         | Deionized (water)                           |
| <b>Cu- K<math>\alpha</math></b>                   | Copper K-alpha Radiation                    |
| <b>FE-SEM</b>                                     | Field Emission Scanning Electron Microscopy |
| <b>H<sub>2</sub>O<sub>2</sub></b>                 | Hydrogen Peroxide                           |
| <b>NaOH</b>                                       | Sodium Hydroxide                            |
| <b>C<sub>6</sub>H<sub>13</sub>MnO<sub>8</sub></b> | Manganese (III) acetate dihydrate           |
| <b>LOD</b>                                        | Limit of Detection                          |
| <b>LOQ</b>                                        | Limit of Quantization                       |
| <b>nM</b>                                         | Nanomolar                                   |
| <b><math>\mu</math>M</b>                          | Micromolar                                  |