

**Development of Molecularly Imprinted Polymer  
Based Electrodes and Electrochemical Measurement  
System for Quantifying Key Biomolecules in Selected  
Indian Spices**

***Thesis Submitted By***

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**2025**

**JADAVPUR UNIVERSITY**

**KOLKATA- 700032, INDIA**

INDEX NO.89/22/E

**Title of the Thesis**     *Development of Molecularly Imprinted  
Polymer Based Electrodes and  
Electrochemical Measurement System for  
Quantifying Key Biomolecules in Selected  
Indian Spices*

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### **Paper published in International Journals**

- [1] **Sanjoy Banerjee**, Milan Dhara, Hemanta Naskar, Barnali Ghatak, Sk. Babar Ali, Nityananda Das, K. Chezyian, Deepak Kumar Das, Bhimsen Tudu, Arpitam Chatterjee, Bidya Mondal, Dipankar Mandal, Santanu Ghorai, Rajib Bandyopadhyay, Bipan Tudu, “Detection of piperine content in black pepper using a molecular imprinted poly(N,N-dimethylacrylamide) embedded graphite electrode: A machine learning based prediction approach”, *Microchemical Journal*, vol. 207, no. 111914, 2024.
- [2] **Sanjoy Banerjee**, Santanu Ghorai, Milan Dhara, Hemanta Naska, SK. Babar Ali, Nityananda Das, Pradip Saha, Bhimsen Tudu, Arpitam Chatterjee, Rajib Bandyopadhyay, Bipan Tudu, “Accurate prediction of piperine content in black pepper using combined CNN and regression modelling with PDMAM@G electrode and cyclic voltammetry”, *Journal of Food Composition and Analysis*, vol. 141, no. 107355, 2025.
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### **Paper published in International and National Conference proceeding**

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## Statement of Originality

I, Sanjoy Banerjee, registered on 06th May, 2022 do hereby declare that this thesis entitled “Development of Molecularly Imprinted Polymer Based Electrodes and Electrochemical Measurement System for Quantifying Key Biomolecules in Selected Indian Spices” contains literature survey and original research work done by the undersigned candidate as part of Doctoral studies.

All information in this thesis have been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by these rules and conduct, I have fully cited and referred all materials and results that are not original to this work.

I also declare that I have checked this thesis as per the “Policy on Anti Plagiarism, Jadavpur University, 2019”, and the level of similarity as checked by iThenticate software is 9%.

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## Certificate from the Supervisor

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This is to certify that the thesis entitled "Development of Molecularly Imprinted Polymer Based Electrodes and Electrochemical Measurement System for Quantifying Key Biomolecules in Selected Indian Spices", submitted by Shri Sanjoy Banerjee, who got his name registered on 06th May, 2022 for the award of Ph.D. (Engineering) degree of Jadavpur University, is absolutely based upon his own work under the supervision of Prof. Bipan Tudu and that neither his thesis nor any part of the thesis has been submitted for any degree/diploma or any other academic award anywhere before.

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***Dedicated***

**To my parents**

***Mr. Siddhanath Banerjee***  
***and***  
***Mrs. Ira Banerjee***

**My wife and my son**

***Mrs. Anuradha Banerjee***  
***and***  
***Syamantak Banerjee***

# *Acknowledgement*

The joy that accompanies the successful completion of any task is incomplete without acknowledging the individuals instrumental in making it possible. Their unwavering guidance, invaluable suggestions, and ceaseless encouragement are the foundation on which every successful endeavour rests. PhD itself is an adventurous journey. In accordance with established conventions, I hereby extend my heartfelt gratitude to those persons who have played a crucial role in making my journey a success.

I would like to express my deepest sense of indebtedness to Prof. Bipan Tudu, my supervisor at Jadavpur University's Department of Instrumentation and Electronics Engineering, for his continuous guidance in achieving the objective of the thesis. I am deeply grateful to Prof. Rajib Bandyopadhyay for his unwavering support and valuable suggestions throughout the research program, and I will need his blessings for my future goal.

I am thankful to the Head of the Department of the Instrumentation and Electronics Engineering Department, Jadavpur University, for his support and providing all the facilities during this journey.

I owe a great deal to Dr. Santanu Ghorai, Professor, Department of Applied Electronics and Instrumentation Engineering, Heritage Institute of Technology, Kolkata, West Bengal for his effective advice during the vital stages of the thesis work.

I am indebted to Dr. Satish Chandra Bera, Retired Professor, Department of Applied Physics, Instrumentation Engineering Section, University of Calcutta, Kolkata, India, for his continuous encouragement and guidance since my Post Graduation days. During my PhD journey, a visit to him was enough to boost up my energy level whenever needed.

I want to express my thanks to my lab mates namely Mr. Milan Dhara, Miss Barnali Ghatak, Mr. Hemanta Naskar, Mr. A.H.M. Toufique Ahmed, Miss Ipshita Bhattacharjee, all faculty, technical and non-technical staff in the Department of the Instrumentation and Electronics Engineering at Jadavpur University.

Finally, I would like to take this opportunity to dedicate this thesis work to all members of my loving family. I would like to express immense gratitude to my father Mr. Siddhanath Banerjee and mother Mrs. Ira Banerjee who inculcated within me the urge of doing higher research since my Graduation days and used to stand by me in the thick and thin of life. I am grateful to my wife, Mrs. Anuradha Banerjee and my son Master Syamantak Banerjee for their continuous support throughout this journey.

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## Abstract

The increasing global emphasis on alternative and complementary medicine has elevated the importance of medicinal plants and their bioactive constituents, known as phytochemicals. However, the efficacy and safety of herbal products remain constrained by the lack of standardized and cost-effective analytical techniques for quantifying these compounds. This thesis addresses this critical gap by developing electrochemical sensing platforms based on Molecularly Imprinted Polymers (MIPs) for the selective detection of key phytochemicals—specifically piperine (PIP) and thymoquinone (TQ), present in Indian spices such as *Piper nigrum* (black pepper) and *Nigella sativa* (black cumin) respectively. The molecular dimensions, auxiliary variations, and morphological modifications of the imprinted electrodes were analyzed using Fourier Transform Infrared (FTIR) spectroscopy, Field Emission Scanning Electron Microscopy (FESEM), and Ultraviolet–Visible (UV–Vis) spectroscopy, respectively.

Firstly a novel molecularly imprinted poly (N,N-dimethylacrylamide)-based graphite electrode (PDMAM-MIP@G) was fabricated for sensitive and selective detection of piperine from black pepper. The sensor was synthesized via polymerization using N,N-DMAM as monomer, piperine as the template molecule, and EGDMA as the cross-linker. The fabricated sensor showed high sensitivity ( $12.92 \mu\text{A}/\mu\text{M}$ ), wide linear range ( $0.001\text{--}200 \mu\text{M}$ ), and low detection limit ( $0.6 \text{ nM}$ ), with excellent stability, reproducibility, and selectivity. A CNN-driven model, validated against RP-HPLC, predicted piperine from CV data with outstanding accuracy ( $R^2 \approx 0.9999$ , MAE =  $0.0339\%$ ). Next two novel electrochemical sensors were developed for detecting thymoquinone (TQ) in black cumin: a titanium oxide-modified graphite paste electrode (GPE/TiO<sub>2</sub>) and a thymoquinone-imprinted polymer electrode (TQIP/G). Both sensors showed excellent sensitivity, low detection limits ( $0.02 \mu\text{M}$  and  $0.03 \mu\text{M}$  respectively), and broad linear ranges. They demonstrated high selectivity, reproducibility, and stability. Principal Component Analysis (PCA) effectively distinguished cumin brands based on TQ content. For enhanced prediction accuracy, a 1D convolutional neural network (CNN) model was trained using DPV signals, achieving near-perfect agreement with reference RP-HPLC data ( $R^2 \approx 0.9996$ ).

The thesis also includes the development of a low-cost potentiostat (LC-PSTAT) designed using Arduino microcontroller, LF347 OPAMPs, and integrated with Python-based software which

supports both cyclic voltammetry (CV) and differential pulse voltammetry (DPV). It showed a detection limit of 0.03  $\mu\text{M}$  and a linear detection range of 1–200  $\mu\text{M}$  for thymoquinone. The sensor maintained high repeatability (RSD 3.23%), reproducibility, and stability. Real-sample analysis of four branded black cumin samples using DPV and Partial Least Squares Regression (PLSR) showed a prediction accuracy of ~94% when compared with RP-HPLC.

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## *List of Abbreviations*

|         |                                                     |
|---------|-----------------------------------------------------|
| ABA     | 4- aminobenzoic acid                                |
| AG      | Silver nanoparticle                                 |
| Ag/AgCl | Silver/Silver chloride                              |
| AHNSA   | 4-amino-3-hydroxy-1-napthaline sulfonic acid        |
| AIBN 2  | 2'-Azobisisobutyronitrile                           |
| AM      | Acryl amide                                         |
| ASV     | Anodic stripping voltammetry                        |
| Au      | Gold                                                |
| BP      | Benzoyl peroxide                                    |
| CA      | Control amplifier                                   |
| CE      | Counter electrode                                   |
| CF      | Current follower                                    |
| CP      | Capsaicin                                           |
| CPE     | Chlorpyris                                          |
| CSV     | Catodic stripping voltammetry                       |
| CuO     | Copper oxide                                        |
| CUR     | Curcumin                                            |
| CUR-MIP | Curcumin – molecular imprinted polymer<br>electrode |
| CV      | Cyclic voltammetry                                  |
| DPV     | Differential pulse voltammetry                      |
| EGDE    | Ethylene glycol diglycidyl ether                    |
| EGDMA   | Ethylene glycol dimethacrylate                      |
| EU      | Eugenol                                             |
| FESEM   | Field emission scanning electron microscope         |
| FMMA    | Ferrocenyl methyl methacrylate                      |
| FTIR    | Fourier transforms infra-red                        |
| G@AuNPs | Graphene/Au mamoparticles                           |



|                    |                                                                 |
|--------------------|-----------------------------------------------------------------|
| GCEs               | Glassy carbon electrodes                                        |
| GCMS               | Gas chromatography mass spectrometry                            |
| GNPs               | Gold nanoparticles                                              |
| GO/AgNPs-MIPs      | Silver nanoparticles- molecular imprinted polymer               |
| GR                 | Graphene                                                        |
| HPLC               | High performance liquid chromatography                          |
| L-Dopa             | L-dopamine                                                      |
| LOD                | Limit of detection                                              |
| LOQ                | Limit of quantitation                                           |
| LSV                | Linear sweep voltammetry                                        |
| MAA                | Methacrylic acid                                                |
| MAAP-Fe(III)       | Methacrylamidoantipyrine-iron (III)                             |
| MAT                | N- methacryloyl-L-tyrosine                                      |
| MIM                | Molecularly imprinted membrane                                  |
| MIP                | Molecular imprinted polymer                                     |
| MIP-CPE            | Molecular imprinted – carbon paste electrode                    |
| MIP- $\beta$ -CD@G | Molecular imprinted poly beta cyclodextrine                     |
| MM                 | Melamine                                                        |
| mPD                | Meta-phenylene                                                  |
| MWCNT              | Multiwalled carbon nanotube                                     |
| N, N-MBA N         | N-methylenebisacryl amide                                       |
| NIP                | Non imprinted polymer                                           |
| NPV                | Normal pulse voltammetry                                        |
| PAM@G              | Polyacrylamide embedded graphite                                |
| PAN/MIP/G          | Polyacrylonitrile molecular imprinted polymer embedded graphite |
| PBS                | Phosphate buffer solution                                       |
| PDDAG              | Poly diallyl dimethylammonium chloride functionalized graphene  |
| PEC                | Photo electrochemical                                           |

|         |                                       |
|---------|---------------------------------------|
| PGE     | Pencil graphite electrode             |
| PV      | Pulse voltammetry                     |
| QCM     | Quartz crystal microbalances          |
| RE      | Reference electrode                   |
| ROS     | Responsive oxygen species             |
| RT      | Rutin                                 |
| SCE     | Saturated calomel reference electrode |
| SV      | Stripping voltammetry                 |
| SWV     | Square wave voltammetry               |
| TAA     | Thiophene acetic acid                 |
| THY     | Thymol                                |
| Tio2NRs | Titanium dioxide nanorods             |
| Tyr     | Tyrosine                              |
| UV-Vis. | Ultra- violet visible spectroscopy    |
| VOCs    | Volatile compounds                    |
| WE      | Working electrode                     |
| WHO     | World health organization             |
| β-CD    | Beta- cyclodextrin                    |

# **Chapter 1**

## **Introduction and scope of the thesis**

This chapter explains the objectives and purpose of this thesis in a simple way. It provides a brief overview of different electrochemical techniques and describes various methods used for the development of Molecularly Imprinted Polymers (MIPs). A detailed literature survey on previous studies related to the use of MIP sensors for detecting various molecules and the development of potentiostats has been thoroughly discussed. Finally, the objectives and scope of the thesis are presented, along with a brief outline of the chapter.

### **List of Sections**

- 1.1. Introduction**
- 1.2. Electrochemical**
- 1.3. Molecular imprinted polymer (MIP) technique**
- 1.4. Literature survey on MIP based sensors**
- 1.5. Literature survey on Potentiostat**
- 1.6. Objectives and scope of the research**
- References**



### 1.1. Introduction

The medicinal use of plants traces back to the earliest days of human civilization, forming the backbone of traditional healing practices across cultures and continents. As modern societies face growing challenges—including rapidly increasing population, limited accessibility to pharmaceutical drugs, soaring healthcare costs, and the adverse effects associated with synthetic medications—the appeal of plant-based medicine has resurged with renewed interest. Furthermore, the global rise in drug-resistant pathogens has further emphasized the need for alternative and complementary therapeutic approaches. The World Health Organization (WHO) estimates that around 80% of the global population relies on herbal medicine as a primary source of healthcare. This widespread reliance underscores the enduring relevance of medicinal plants in the global healthcare landscape.

Botanical sources harbour a remarkable pharmacological reservoir; studies suggest that nearly 21,000 plant species hold the potential to produce therapeutic agents [1,2]. Herbal medicine is often regarded as a safer and more holistic alternative, largely due to its natural origin, biocompatibility, and relatively low incidence of side effects. These treatments are also generally free from the constraints of age or gender, making them universally applicable. Despite the promising prospects, scientific exploration into these resources has been surprisingly limited—only about 20% of medicinal plants have been rigorously studied for their pharmacological efficacy and bioactive compounds [3-4]. This highlights a significant gap between traditional knowledge and modern scientific validation.

Plants naturally synthesize an extensive array of phytochemicals—bioactive compounds that help them defend against environmental stressors like pathogens, Ultra Violet (UV) radiation, and drought [5]. These same compounds, when ingested by humans, offer a wide spectrum of health benefits. Among the most studied phytochemicals are vitamins A, C, and E, along with phenolic acids, flavonoids, tannins, lignans, terpenoids, saponins, and alkaloids [6]. A significant number of these compounds act as potent antioxidants, helping to counteract harmful free radicals, which in turn reduces oxidative stress and decreases the likelihood of developing chronic illnesses like heart disease, cancer, diabetes, and neurodegenerative diseases. In addition to antioxidant activity, phytochemicals exhibit antimicrobial, anti-inflammatory, hormone-modulating, and immune-enhancing properties. Their broad therapeutic potential makes them invaluable to both the treatment and prevention of various diseases.

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Particularly in India, a nation rich in traditional plant-based remedies, spices serve as a potent sources of medicinal phytochemicals [7]. For example, turmeric contains curcumin, a strong anti-inflammatory and antioxidant compound [8]. Black pepper is rich in piperine, which possesses anti-inflammatory, analgesic, anti-carcinogenic, antioxidant activity and also helps to absorb nutrient [9]. Cloves are packed with eugenol, known for its antimicrobial properties [10], while cinnamon is valued for its high content of terpenoids and flavonoids [11]. Other common spices such as ginger, garlic, cardamom, cumin, fenugreek, and asafoetida each contain unique bioactive compounds that contribute to their traditional use in Ayurvedic and folk medicine. These phytochemicals collectively help manage inflammation, infections, metabolic disorders, and even hormone-related imbalances. Table 1.1 shows different bioactive compounds present in different Indian spices and their uses in traditional medicine.

**Table 1.1. Different bioactive compounds present in different Indian spices.**

| Spice        | Key Bioactive(s)                    | Main Traditional / Medicinal Uses                | Ref. |
|--------------|-------------------------------------|--------------------------------------------------|------|
| Turmeric     | Curcumin, Turmerones                | Anti-inflammatory, skin/cardiac health           | [8]  |
| Ginger       | Gingerol, Zingiberene               | Digestion, anti-nausea, anti-inflam              | [12] |
| Garlic       | Allicin, Ajoene                     | Antimicrobial, cardio-protective, anticance      | [13] |
| Black pepper | Piperine, Carvacrol                 | Bioavailability enhancer, antioxidant            | [9]  |
| Cumin        | Cuminaldehyde, Monoterpenes         | Digestion, antimicrobial, antidiabeti            | [14] |
| Fenugreek    | Trigonelline, Diosgenin             | Lowers glucose/cholesterol, lactation            | [15] |
| Cardamom     | Cineole, Tterpenyl Acetate          | Digestive, anti-inflam, cardio-protect           | [16] |
| Cinnamon     | Cinnamaldehyde, Eugenol             | Controls blood sugar, antimicrobial              | [11] |
| Clove        | Eugenol                             | Dental pain relief, antimicrobial                | [10] |
| Asafoetida   | Ferulic Acid, Organosulfides        | Digestive, antispasmodic, respiratory            | [17] |
| Ajwain       | Thymol, Terpinene                   | Eases indigestion, antimicrobial                 | [18] |
| Coriander    | Linalool, Monoterpenes              | Digestive, anti-rheumatic                        | [19] |
| Mustard      | Glucosinolates, Omega-3 Fatty Acids | Lower risk of ardiovascular diseases and cancer. | [20] |

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|              |                          |                                   |      |
|--------------|--------------------------|-----------------------------------|------|
| Red Pepper   | Capsaicin                | Metabolism booster, analgesic     | [21] |
| Pippali      | Piperine,<br>Thermogenic | Respiratory & digestive stimulant | [22] |
| Fennel       | Anethole,<br>Flavonoids  | Soothes GI, diuretic              | [23] |
| Curry Leaves | Alkaloids,               | Lowers blood sugar, antioxidant   | [24] |
| Saffron      | Crocin,<br>Safranal,     | Mood enhancer, antioxidant        | [25] |
| Star Anise   | Anethole                 | Digestive, antimicrobial          | [26] |

Despite the growing recognition of the benefits of phytochemicals, the herbal industry faces critical challenges related to product quality and standardization. Raw plant materials are often processed without stringent testing or established quality control protocols, resulting in inconsistencies in efficacy and safety. The lack of robust, affordable, and practical analytical techniques makes it difficult to routinely monitor the presence and concentration of bioactive compounds in herbal formulations. While advanced analytical tools like gas chromatography-mass spectrometry (GC-MS), gas chromatography (GC) [27], high-performance liquid chromatography (HPLC) [28,29], reverse-phase HPLC (RP-HPLC) [30], thin-layer chromatography (TLC) [31], high-performance thin-layer chromatography (HPTLC) [32], ultra-performance liquid chromatography (UPLC) [33,34], and near-infrared spectroscopy (NIR) [35] offer accurate quantitative data, these methods are time-consuming, and require efficient technicians. Consequently, their application in everyday phytochemical analysis remains limited.

The present thesis titled “Development of Molecularly Imprinted Polymer Based Electrodes and Electrochemical Measurement System for Quantifying Key Biomolecules in Selected Indian Spices” describes the development of two electrodes for different phytochemicals. Piperine (PIP) was considered first for this study. Detection of Thymoquinone (TQ) was taken up next. PIP (285.34 g/mol) is the primary alkaloid responsible for the pungency of black pepper (*Piper nigrum*) and long pepper (*Piper longum*). It is widely recognized for its therapeutic properties and is also used as a bioenhancer in herbal and pharmaceutical formulations. Piperine (C<sub>17</sub>H<sub>19</sub>NO<sub>3</sub>) is poorly soluble in water, but soluble in various organic solvents like Ethanol, Chloroform, benzene etc. Piperine is a nitrogen-containing alkaloid with a piperidine ring linked to a methylenedioxyphenyl group via a conjugated dienone

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chain. Its structure gives it lipophilic properties and contributes to its bioactivity. Piperine possesses diverse pharmacological effects including anticancer, anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, neuroprotective, cardioprotective, and antidiabetic activities [36]. Piperine can also be used as good bioenhancer. Piperine enhances the bioavailability of various drugs and phytochemicals by inhibiting drug-metabolizing enzymes (e.g., CYP3A4) and P-glycoprotein. It significantly improves the absorption of curcumin, resveratrol, and other poorly soluble compounds [37]. Piperine is generally safe at dietary levels. However, high doses may cause gastrointestinal irritation and interfere with drug metabolism, necessitating caution in therapeutic use or supplementation [38]. It is used for the treatment and alleviation of pain, muscular pains, chills, rheumatism, influenza, fever, migraine headaches, strep throat, cardiac, digestive disorders, and even coma [39,40].

Thymoquinone (TQ) (164.20 g/mol) is the principal bioactive constituent of the volatile oil extracted from *Nigella sativa* seeds, commonly known as black cumin or black seeds. For over two thousand years, TQ has attracted considerable interest due to its potent pharmacological properties and widely used in Ayurveda and Unani, particularly across Middle Eastern, South Asian, and North African regions. Thymoquinone ( $C_{10}H_{12}O_2$ ) is poorly soluble in water, but soluble in various organic solvents like Ethanol, Methanol, Chloroform. Due to its poor aqueous solubility researchers often use nano formulations, to improve bioactivity. Thymoquinone belongs to the quinone family and features a benzoquinone core with methyl and isopropyl substituents. Its conjugated ring structure is responsible for its redox activity, which underlies many of its biological effects. Thymoquinone possesses diverse pharmacological effects including anticancer, anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, neuroprotective, cardioprotective, and antidiabetic activities [41-45]. Thymoquinone is generally regarded as safe at low doses. However, elevated doses may lead to hepatotoxicity or nephrotoxicity in animal models. Therefore, dose optimization is crucial for therapeutic applications.

In response to these challenges, the present research aims to develop efficient, cost-effective, and rapid techniques for detecting specific phytochemicals that can serve as quality indicators in medicinal plants. This study focuses on the fabrication of distinct electrochemical sensors using Molecularly Imprinted Polymer (MIP) technology. MIPs are artificial materials, designed to find a specific molecules, like a lock fits a certain key. During the polymerization process, a template molecule (such as a specific phytochemical) is embedded within the



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polymer matrix. Once the template is removed, uniquely shaped cavities remain that are structurally and functionally matched to the original molecule, allowing for highly selective detection [46].

The fabricated MIP-based sensors were meticulously characterized using a suite of analytical techniques. Fourier Transform Infrared (FTIR) Spectroscopy was employed for identifying molecular structures, Field Emission Scanning Electron Microscopy (FESEM) provided detailed imaging of surface morphology, and UV-Visible Spectroscopy was used to assess the optical characteristics of the sensor materials. The sensors were then applied to plant extracts using voltammetric techniques, which enabled real-time detection and quantification of the target phytochemicals. These electrochemical methods offer a practical alternative to conventional analysis techniques, promising faster results with reduced operational complexity and cost.

A key innovation in this study lies in the use of voltammetric techniques for real-time detection and quantification of phytochemicals in plant extracts. In voltammetric techniques, the current produced by an analyte is measured in response to varying applied voltage to generate highly specific electrical signals based on the compound's redox properties. Voltammetric sensors, especially when combined with molecularly imprinted polymers, enhance selectivity by allowing only the target phytochemical to interact with imprinted binding sites. Common voltammetric methods used in phytochemical sensing include cyclic voltammetry (CV), and differential pulse voltammetry (DPV), each offering distinct advantages in terms of resolution, selectivity, sensitivity and low detection limits.

For electrochemical measurement, potentiostats are essential devices that control and record sensor responses, but commercial potentiostats are expensive, bulky, and dependent on external data processing systems. Recent research focuses on developing portable, low-cost potentiostats using affordable microcontrollers and operational amplifiers to enable real-time, on-site analysis. This work addresses the need for an economical, standalone electrochemical system by integrating a custom low-power potentiostat with an MIP-based sensor, reducing cost while maintaining stability, sensitivity, and industrial applicability.

The rest of this chapter is structured as follows: it begins with a brief overview of the electrochemical sensing principles, with a particular focus on voltammetric techniques. This is followed by a discussion on the fabrication of MIP-based sensors, including various

molecular imprinting methods. A comprehensive review of the relevant literature is then presented, concluding with the objectives and scope of the thesis.

### **1.2. Electrochemical**

Electrochemical transduction relies on electrochemical principles to detect and measure both volatile and non-volatile substances. It operates primarily through redox reactions, where the target analyte generates a measurable electrical signal corresponds to the potential difference between electrodes. This signal enables precise quantification of the sample. Due to advantages such as low fabrication cost, high stability, good sensitivity, easy signal recovery, and minimal interference [47], [48], electrochemical methods have become widely adopted in practical applications.

#### ***1.2.1. Different electrochemical techniques***

Several key parameters govern electrochemical reactions, including electrode potential, electric current, charge transfer, and time. The unique characteristics that define electrochemical methods are outlined as follows:

- i) Besides standard oxidation and reduction reactions, the analyte may undergo additional chemical processes.
- ii) The magnitude of the current reflects the rate at which the analyte undergoes redox transformations.
- iii) It is not feasible to control both the current and potential of an electrode simultaneously.

Electrochemical methods can be classified based on the type of excitation signal applied and the nature of the response measured.

##### ***1.2.1.1. Coulometry***

Coulometry is an electroanalytical technique that measures the total electric charge (in coulombs) required to drive a complete redox reaction of a target analyte. This technique is based on Faraday's First Law of Electrolysis [49], which asserts that the amount of material altered at an electrode during electrolysis is directly related to the electric charge transmitted through the system.

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The mass (**w**) of a substance produced or consumed during the electrochemical reaction can be calculated using the following equation (1.1):

$$w = \frac{MQ}{96487n} \quad (1.1)$$

Where: M is the molecular mass of the substance, Q is the total charge passed (in coulombs), n represents the number of electrons involved in the reaction, 96487 is the Faraday constant (in C/mol).

Coulometric techniques are broadly classified into two categories: Potentiostatic (controlled potential) Coulometry, where the working electrode is maintained at a constant potential, enabling selective oxidation or reduction of the analyte. This approach minimizes interference from less reactive components present in the solution, such as impurities or solvent molecules. In the case of Amperostatic (controlled current) Coulometry a constant current is applied, and the reaction is allowed to proceed until completion. The total charge transferred is equal to the product of the constant current and the duration for which it flows, providing a direct measurement of the analyte concentration.

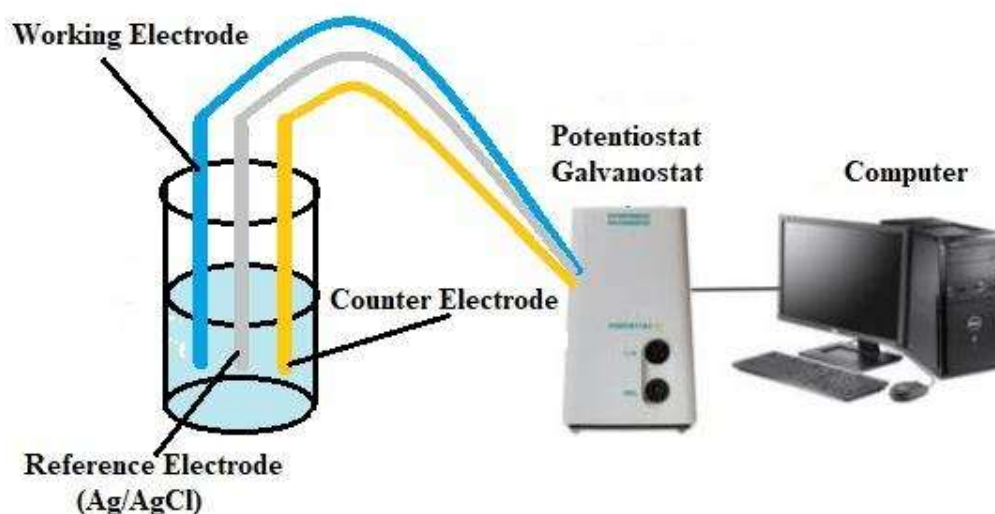
### ***1.2.1.2. Potentiometry***

Potentiometry is an electroanalytical method that focuses on two-electrode system comprising a measuring (indicator) electrode and a reference electrode. The indicator electrode is immersed in the solution containing the analyte, and the resulting potential is measured relative to the stable reference electrode.

The key principle of potentiometry is that the observed cell potential reflects the concentration of specific ions or electroactive species in the solution. Based on its mode of operation, potentiometry is classified into two main types: In Direct Potentiometry the electrode potential is directly related to the concentration of the target analyte, allowing quantitative analysis through established electrochemical relationships (such as the Nernst equation). In Potentiometric Titration the cell potential is monitored as a function of the titrant volume added. Potentiometry offers high selectivity and is widely used in pH measurements, ion-selective electrode applications [49].

### 1.2.1.3. Voltammetry

Voltammetry is widely used electrochemical technique that involves measuring the current response of an electrochemical cell while the potential applied to the working electrode is systematically varied over a specified range [49], [50]. This varying potential facilitates redox reactions present near the electrode surface. The resulting current, especially the peak current, serves as a quantitative indicator of the analyte concentration and provides insights into the electrochemical behaviour of the system. A typical voltammetric experiment uses a three-electrode configuration, as shown in Fig. 1.1.



*Fig. 1.1. Voltammetric experiment uses a three-electrode configuration.*

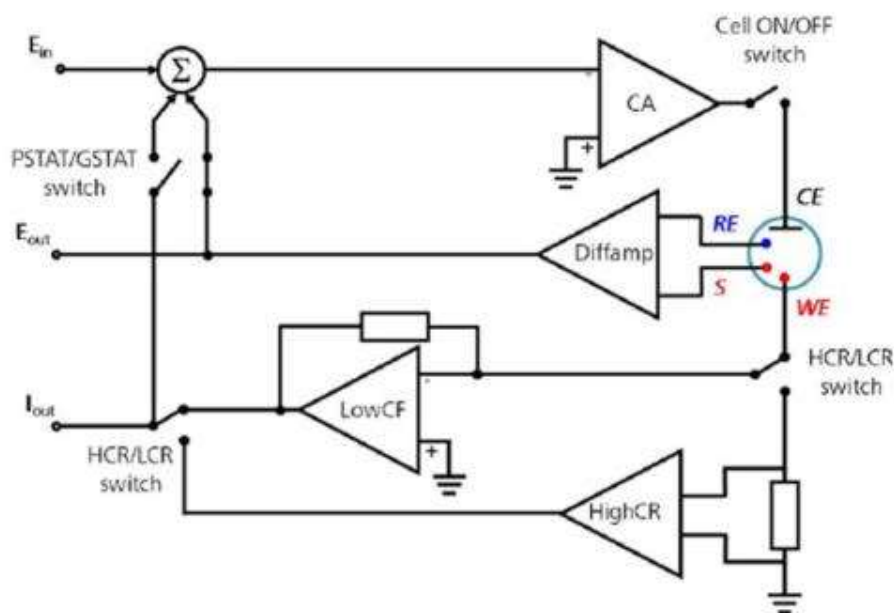
This setup consists of: A Working Electrode (WE), where the electrochemical reaction occurs. A Reference Electrode (RE), which is generally an Ag/AgCl electrode, used to maintain a stable and known reference potential. A Counter Electrode (CE) (auxiliary electrode), which completes the circuit by allowing current to flow.

Unlike potentiometry, voltammetry requires a reference electrode to maintain a fixed voltage across the working electrode, typically controlled by a potentiostat. The potentiostat acts similarly to a controlled voltage source, regulating the potential applied to the WE to trigger redox reactions. As these reactions occur, electrons are transferred, generating a measurable current that flows between the WE and CE. This current provides real-time information on reaction kinetics, concentration, and other electrochemical properties.

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In the electrode configuration current flows between the CE and WE, while the potential is measured between the RE and WE. This close proximity helps minimize solution resistance, ensuring more accurate potential control.

The basic electronic layout of a potentiostat is shown in Fig. 1.2 [50]. A Control Amplifier (CA) connects to the CE and prevents direct current flow through the RE. The WE connects to a Current Follower circuit, which measures the current using a low-resistance shunt. A Differential Amplifier measures the potential between RE and the sense electrode (sometimes WE), and this signal, helps to control the overall cell voltage. The outputs,  $E_{out}$  and  $I_{out}$ , represent the system's potential and current responses, respectively.

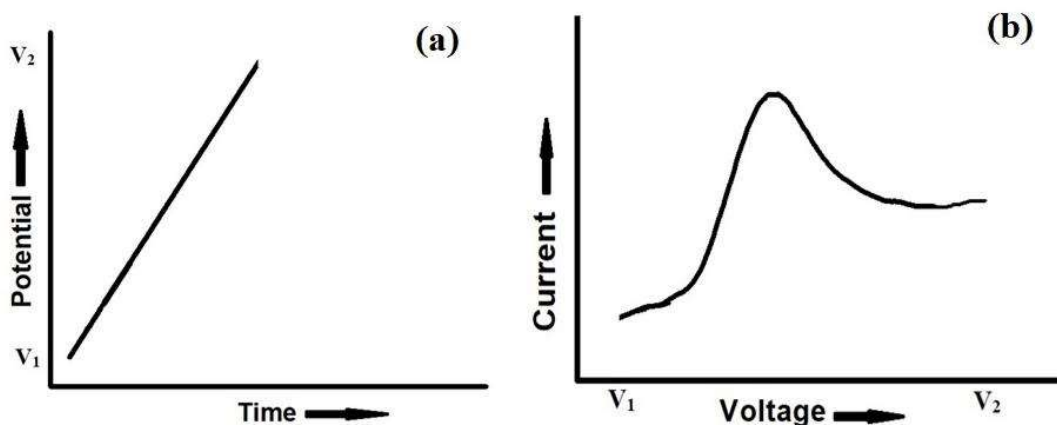


**Fig. 1.2. Basic electronic layout of a potentiostat.**

Traditional Types of Voltammetric Techniques are follows:

### ***a) Linear sweep voltammetry (LSV)***

In Linear Sweep Voltammetry, the potential applied between the working electrode (WE) and reference electrode (RE) increases linearly with time. The resulting current is recorded as a function of this changing potential. As the scan rate increases, capacitive current also increases, which can surpass the limit of electrochemical compensation. Despite this, LSV allows for detection limits in the mg/L range, making it suitable for moderately sensitive applications [51]. Fig. 1.3. shows basic excitation signal and its voltammogram of LSV.

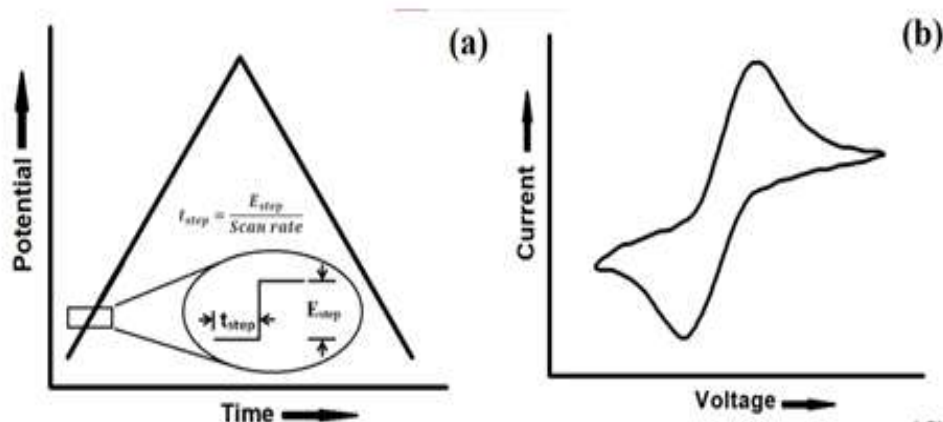


*Fig. 1.3. (a) LSV excitation signal potential and (b) it's voltammogram.*

**b) Cyclic voltammetry (CV)**

CV is most commonly used techniques in electrochemical research. The potential of the WE is swept linearly up to a set upper limit, then reversed to return to the starting potential—producing a triangular waveform. During the forward scan, current increases as oxidation occurs due to the availability of electroactive species.

In the reverse scan, the previously oxidized species undergo reduction, resulting in a cathodic peak. CV provides vital data on reaction kinetics, redox mechanisms, reversibility, and electrochemical stability of analytes [51]. Fig. 1.4. shows basic excitation signal and it's voltammogram of CV.



*Fig. 1.4. Basic excitation signal and it's voltammogram of CV.*

### *c) Pulse voltammetry (PV)*

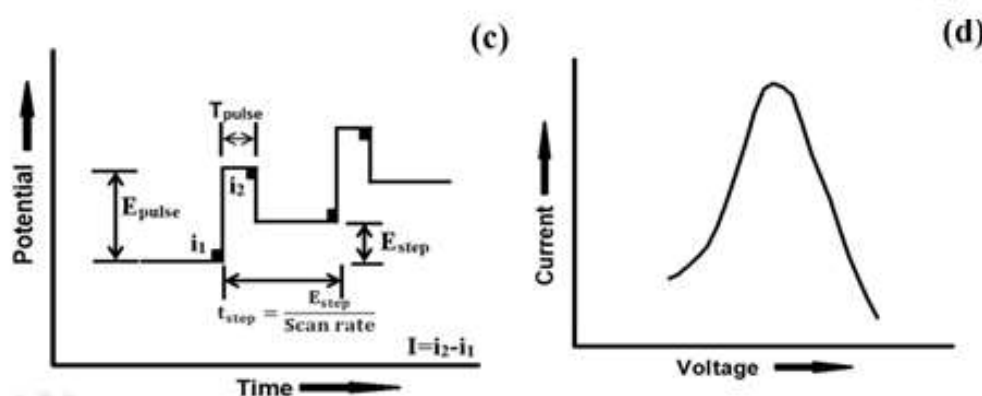
Pulse Voltammetry uses potential pulses to differentiate between the charging current (which decays rapidly) and the Faradaic current (which decays more slowly) [52]. The technique is highly sensitive and capable of detecting analytes down to  $10^{-8}$  M concentrations. Two key variants of PV include:

#### *i) Normal pulse voltammetry (NPV):*

In Normal Pulse Voltammetry (NPV), a sequence of progressively higher potential pulses is applied, with the current recorded at the conclusion of each pulse, allowing sufficient time for the charging current to decay. Pulse duration typically ranges from 1–100 ms, with intervals between 0.1–5 s. The voltammogram presents stepped potential pulses on the x-axis and corresponding sampled currents on the y-axis.

#### *ii) Differential pulse voltammetry (DPV):*

DPV enhances sensitivity by applying incremental potential pulses on top of a constant baseline potential. The current is measured both prior to and at the end of each pulse, and the difference between these two values is documented, offering precise quantification of the analyte. Among all pulse techniques, DPV is the most sensitive, providing extremely low detection limits due to effective minimization of background noise [53]. Fig. 1.5. shows basic excitation signal and it's voltammogram of DPV.



*Fig. 1.5. Basic excitation signal and it's voltammogram of DPV.*

### *d) Square wave voltammetry (SWV)*

SWV utilizes symmetrical square wave pulses superimposed on a staircase waveform. The key feature of this technique is the differential measurement: the current is measured during both the forward and reverse pulses, and the net current is determined by calculating the difference between the two. The peak current appears around the redox potential, and is linearly proportional to analyte concentration. SWV offers high sensitivity, with detection limits reaching as low as 0.1 nM, making it highly effective for trace analysis [54].

### *e) Stripping voltammetry (SV)*

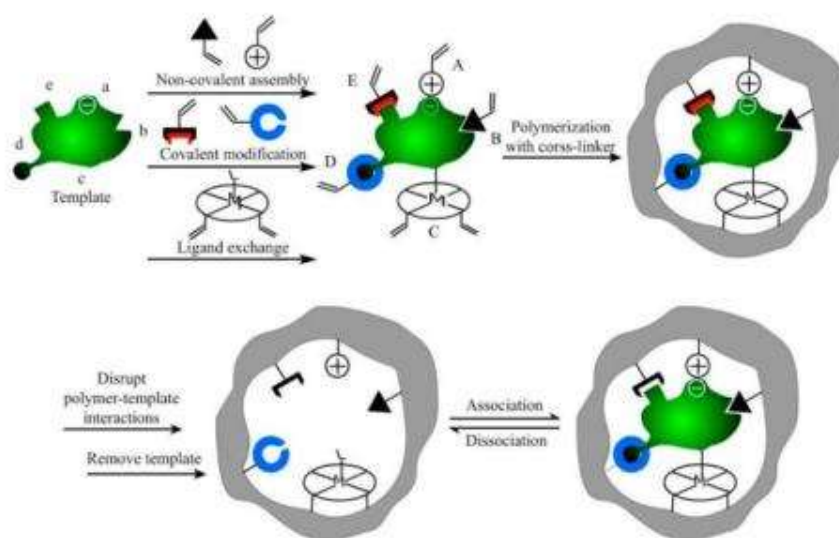
Stripping voltammetry is particularly useful for detecting trace metals in solution. It consists of two main phases: In Deposition Phase, a potential is applied to reduce metal ions in the solution, causing them to deposit on the electrode surface while the solution is stirred. In Stripping Phase, the applied potential is scanned, oxidizing (or reducing) the deposited metal back into the solution, producing a current proportional to the metal concentration. This method offers excellent sensitivity for trace-level detection [55].

## **1.3. Molecularly imprinted polymer (MIP) technique**

An effective strategy for constructing highly selective and sensitive sensors involves the use of Molecularly Imprinted Polymers (MIPs). These materials are synthesized by assembling a template molecule with functional monomers, which are then cross-linked to form a rigid structure around the template. Functional monomers interact with the template through various binding mechanisms, and polymerization around this structure leads to the formation of a matrix. Once polymerized, the template is removed under specific conditions, leaving behind a cavity that matches the template in shape and functional group orientation. This mimics an enzyme's active site, which is geometrically designed to recognize specific substrates. Thus, only molecules that fit the cavity precisely will bind, while mismatched ones are excluded. Early studies on molecular imprinting were conducted by Dickey in the mid-20th century [56], [57], drawing inspiration from Linus Pauling's theoretical concepts [58]. The basic mechanism [59] is illustrated in Fig. 1.6.



The MIP synthesis process consists of three key stages [60], (i) **Association**, in which Functional monomers and template molecules associate through covalent or non-covalent bonds. (ii) **Polymerization**, where polymer networks form through different polymerization techniques, encapsulating the template molecules, and (iii) **Elution**, where suitable solvents are used to extract the template from the polymer, creating precise recognition cavities. MIPs are beneficial due to their versatility, robustness, and mechanical durability. Their compatibility with a wide range of functional monomers allows for sensor fabrication tailored to virtually any analyte. Moreover, MIPs offer a synthetic alternative to unstable biological recognition elements like enzymes and antibodies.



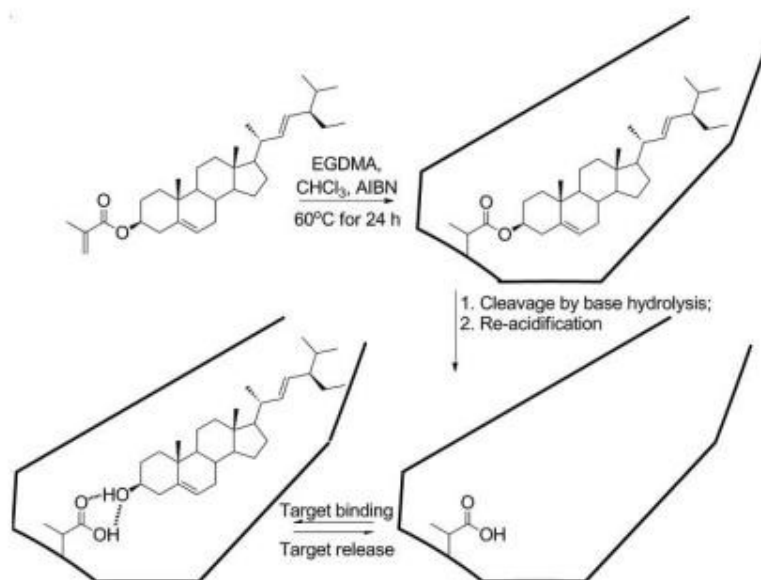
*Fig.1.6. The schematic of a basic MIP process [59].*

### **1.3.1. Types of imprinting techniques**

#### **1.3.1.1. Covalent imprinting**

Covalent imprinting refers to a technique in which the template molecule and at least one functional monomer are connected through reversible covalent bonds prior to the polymerization stage. This bond formation occurs independently of the polymerization process itself. Initially, a pre-polymerization complex is chemically synthesized by reacting the template with the monomer. Polymerization is then carried out with this complex. After

the polymer is formed, the template is removed, breaking the covalent bonds that linked it to the polymer. When the target molecule later rebinds to the imprinted polymer, the same type of covalent interaction is re-established. A notable feature of this method is the precise and selective removal of the template molecule after polymerization. A visual representation of this process [61] is provided in Fig. 1.7.



**Fig. 1.7. Schematic explaining the covalent imprinting process [61].**

### **1.3.1.1.1. Imprinting using readily reversible covalent bonds**

This method utilizes covalent interactions that can be easily reversed to form stable template–monomer complexes. These interactions often involve reactions like boronate ester formation, ketal or acetal linkage, and Schiff base (imine) formation. Such chemical bonds are particularly useful due to their reversible nature, enabling template removal through mild hydrolysis. However, this approach is limited in scope, as it primarily applies to templates containing functional groups such as 1,2- or 1,3-diols (for boronate esters and ketals/acetals), aldehydes (for acetals and Schiff bases), ketones (for ketals), and amines (for Schiff bases).

A significant benefit of this technique is the uniformity of binding sites within the polymer matrix, as each site closely resembles the others in structure and function. Additionally, using fewer monomers can reduce non-specific binding. Nonetheless, a major drawback is the

necessity to synthesize specific functional monomers that can reversibly bind the template. Moreover, such systems may be sensitive to water and may face steric challenges within the imprinted cavities, which can interfere with effective rebinding of the target molecule.

### ***1.3.1.1.2. Covalent imprinting via boronate esters***

Among reversible covalent techniques, boronate ester-based imprinting is considered especially effective for molecules containing diol groups, such as sugars. Originally introduced by Günter Wulff and his team, this approach has been utilized to imprint a range of compounds, including glyceric acid, mannose and its derivatives, sialic acid, L-DOPA, galactose, castasterone, fructose, and several nucleotides [62]–[66]."

The boronate ester strategy has been successfully incorporated into various applications, including fluorescent sensors [67]–[69], imprinted polyelectrolyte hydrogels [70], polyaniline-based coatings for microplates [71], and the chiral resolution of amino acids. Furthermore, boron-containing monomers such as boronophthalide derivatives [72], [73] have expanded this technique to imprint monoalcohols and compounds with multiple spatially separated hydroxyl groups. In addition, sterols have been selectively modified using polymers prepared via this method [74].

### ***1.3.1.1.3. Covalent imprinting via Schiff base formation***

The Schiff base approach involves the condensation reaction between a primary amine and a carbonyl-containing compound, typically an aldehyde, resulting in the formation of an imine bond. This strategy is particularly suitable for templates that contain either amine or aldehyde functional groups. Schiff base-based imprinting has also been employed for amino acid derivatives [75]. However, despite its potential, the slow kinetics of template rebinding often limits its use in high-throughput applications such as chromatographic separations.

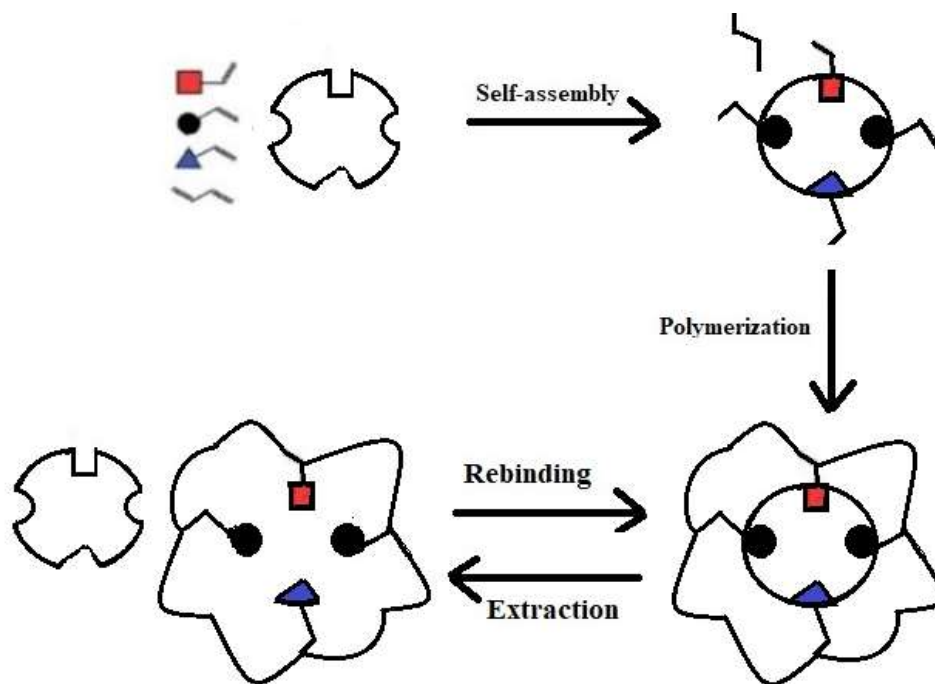
### ***1.3.1.1.4. Covalent imprinting with acetals and ketals***

Shea and colleagues [76]–[78] conducted extensive investigations using mono- and diketone templates for covalent imprinting. In these studies, diols with polymerizable groups were used to form ketal or acetal linkages with the template. Additionally, a range of cyclic hemi-acetals was examined for their suitability as binding groups, especially in the recognition of monoalcohol templates [79]. This approach provides a promising route for designing

imprinted polymers targeting alcohol-containing compounds, though template stability and hydrolysis sensitivity remain considerations.

### 1.3.1.2. *Non-covalent imprinting*

Non-covalent imprinting overcomes many limitations associated with covalent methods. This technique utilizes reversible, non-covalent interactions—such as hydrogen bonding, van der Waals forces, and dipole–dipole interactions—to form template–monomer complexes in an appropriate solvent. Upon polymerization and subsequent removal of the template, the polymer retains recognition sites that can rebind the template using the same interactive forces. The method gained significant attention in the 1980s through the work of Mosbach's group and has since become one of the most widely adopted imprinting strategies [80, 81]. A general representation of this process is illustrated in Fig. 1.8.



**Fig. 1.8.** Schematic explaining the mechanism of non covalent imprinting.

A key advantage of this approach is its flexibility, allowing the imprinting of a wide range of functional groups and target molecules. During polymerization, the monomers arrange themselves around the template molecule, guided by multiple weak interactions. As polymer chains grow, these interactions stabilize and shape the recognition sites, resulting in high-

affinity binding once the template is removed. This process can be carried out using either a single monomer or a combination of monomers.

### ***1.3.1.2.1. Imprinting with a single functional monomer***

Using a single monomer is the simplest form of non-covalent imprinting and is well documented. However, challenges may arise due to monomer self-association, which can hinder effective template binding. For instance, monomers like carboxylic acids often dimerize, interfering with imprint site formation. Additionally, the interactions between monomers and initiators need to be considered, as they can affect binding site specificity.

Some commonly used monomers include vinylbenzoic acid, which offers  $\pi$ -electron interactions but reacts poorly with typical crosslinkers. Phosphonates and phosphates are also used for their metal ion complexation abilities. Vinylpyridine, known for strong  $\pi$ - $\pi$  stacking interactions, binds effectively to electron-deficient aromatic templates. However, in aqueous environments, such interactions can lead to non-specific binding, reducing selectivity. Tertiary amines, though occasionally effective, often perform less efficiently due to their flexible structure and distance from the polymer backbone.

### ***1.3.1.2.2. Imprinting with a combination of monomers***

Combining multiple monomers can offer improved selectivity by introducing varied interaction types. However, the effectiveness of this approach depends on maintaining favorable equilibrium conditions in the pre-polymerization mixture. Recent studies, such as those by Spivak [81], have shown that even in the absence of functional monomers, well-designed crosslinkers can support successful imprinting.

To achieve optimal imprinting with mixed monomers, the interactions between the monomers and the template must be stronger than those among the monomers themselves. Computational modeling and virtual imprinting have increasingly been used to predict and optimize monomer combinations before experimental trials, improving the efficiency and selectivity of MIP development.

### 1.3.1.3. *Semi-covalent imprinting*

Combining the strengths of covalent and non-covalent methods, semi-covalent imprinting uses covalent attachment during polymerization and non-covalent interactions for rebinding. For example, a (meth)acrylate ester derivative of the template is polymerized, then removed via hydrolysis. Upon rebinding, the unmodified template interacts through hydrogen bonds with the recognition site. However, differences in hydrogen bonding patterns between esters and alcohols may affect performance.

### 1.3.1.4. *Imprinting with sacrificial spacers*

This method introduces a spacer or linker group between the template and monomer, which is removed during template extraction. The spacer reduces steric hindrance and ensures better site accessibility during rebinding. One notable example involves using salicylate as a spacer between methacrylic acid and an amine-bearing template. This setup preserves proximity through hydrogen bonding and allows easy cleavage of the ester linkage, making it efficient for imprinting applications.

## 1.4. Literature survey on MIP based sensors

This section discusses the latest advancements in the use of Molecularly Imprinted Polymer (MIP) based sensors for target analyte detection of different spices and their varied applications. The transduction mechanism plays a crucial role in influencing the monomer selection and polymerization process development. Table 1.2. outlines recent studies that incorporate electrochemical transduction in MIP methodologies, followed by elaborations on key research efforts.

**Table 1.2. Literature survey indicating the evolution of molecular imprinting methods coupled with electrochemical transducers for enhanced analyte detection.**

| Sl. No. | Target analyte | Components of MIP                                                                         | Polymerization method                     | Ref. |
|---------|----------------|-------------------------------------------------------------------------------------------|-------------------------------------------|------|
| 1       | Piperine       | <b>Monomer:</b> Acrylic Acid<br><b>Crosslinker:</b> EGDMA<br><b>Initiator:</b> 2,2'- AIBN | Non-covalent precipitation polymerization | [82] |
| 2       | Curcumin       | <b>Monomer:</b> 4-pentenoyl - alanyl-chitosan                                             | Composite compensation                    | [83] |

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|    |                                             |                                                                                                        |                                                                    |      |
|----|---------------------------------------------|--------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|------|
|    |                                             | oligosaccharide (PACO)<br><b>Crosslinker:</b> EGDMA                                                    | polymerization<br>process                                          |      |
| 3  | Curcumin                                    | <b>Monomer:</b> Methacrylic<br>Acid (MAA)<br><b>Crosslinker:</b> EGDMA<br><b>Initiator:</b> 2,2'- AIBN | Thermally initiated<br>precipitation<br>polymerization             | [84] |
| 4  | 6-Gingerol,<br>Zingerone                    | <b>Monomer:</b> MAA<br><b>Crosslinker:</b> EGDMA<br><b>Initiator:</b> 2,2'- AIBN                       | Thermally initiated<br>precipitation<br>polymerization             | [85] |
| 5  | Thymol                                      | <b>Monomer:</b> acrylamide<br>(AM)<br><b>Crosslinker:</b> EGDMA<br><b>Initiator:</b> Benzyl Peroxide   | Thermally initiated<br>precipitation<br>polymerization             | [86] |
| 6  | Diosgenin                                   | <b>Monomer:</b> Methacrylic<br>Acid (MAA)<br><b>Crosslinker:</b> EGDMA                                 | Thermally initiated<br>precipitation<br>polymerization             | [87] |
| 7  | Diosgenin                                   | <b>Monomer:</b> para-<br>aminobenzoic acid (pABA)                                                      | Thermally initiated<br>precipitation<br>polymerization             | [88] |
| 8  | Eugenol                                     | <b>Monomer:</b> Polyacrylonitrile<br><b>Crosslinker:</b> EGDMA<br><b>Initiator:</b> Benzyl Peroxide    | Thermally initiated<br>precipitation<br>polymerization             | [89] |
| 9  | Cinnamic Acid                               | <b>Monomer:</b> Dopamine                                                                               | Surface imprinting<br>polymerization                               | [90] |
| 10 | Capsaicin                                   | <b>Monomer:</b> 4-vinylpyridine                                                                        | Multi-walled carbon<br>nanotubes/molecularly<br>imprinted polymers | [91] |
| 11 | Capsaicin                                   | <b>Monomer:</b> Acrylonitrile<br><b>Crosslinker:</b> EGDMA<br><b>Initiator:</b> Benzyl Peroxide        | Thermally initiated<br>precipitation<br>polymerization             | [92] |
| 12 | Quercetin,<br>Kaempferol,<br>Hydroxytyrosol | <b>Monomer:</b> Methacrylic<br>Acid<br><b>Crosslinker:</b> EGDMA                                       | Thermally initiated<br>precipitation<br>polymerization             | [93] |
| 13 | Norephedrine                                | <b>Monomer:</b> Methacrylic<br>Acid<br><b>Crosslinker:</b> EGDMA                                       | Thermally initiated<br>precipitation<br>polymerization             | [94] |
| 14 | Crocin<br>(Gentiobiose)                     | <b>Monomer:</b><br>Methacrylamide<br><b>Crosslinker:</b> EGDMA<br><b>Initiator:</b> Benzyl Peroxide    | Thermally initiated<br>precipitation<br>polymerization             | [95] |
| 15 | Curcumin                                    | <b>Monomer:</b> Methacrylic acid<br><b>Crosslinker:</b> EGDMA<br><b>Initiator:</b> AIBN                | Thermal bulk<br>polymerization                                     | [96] |
| 16 | Curcumin                                    | <b>Monomer:</b> Methacrylic acid<br><b>Crosslinker:</b> EGDMA<br><b>Initiator:</b> Benzyl Peroxide     | Precipitation<br>polymerization                                    | [97] |

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|    |                     |                                                                                          |                                         |       |
|----|---------------------|------------------------------------------------------------------------------------------|-----------------------------------------|-------|
| 17 | Curcumin            | <b>Monomer:</b> Methacrylic acid<br><b>Crosslinker:</b> EGDMA<br><b>Initiator:</b> ABCVA | Bulk polymerization                     | [98]  |
| 18 | Thymol              | <b>Monomer:</b> 4-Vinylpyridine<br><b>Crosslinker:</b> MBA<br><b>Initiator:</b> AIBN     | Thermal bulk polymerization             | [99]  |
| 19 | 6-, 8-, 10-Gingerol | <b>Monomer:</b> Methacrylamide<br><b>Crosslinker:</b> EGDMA<br><b>Initiator:</b> AIBN    | Surface polymerization on magnetic core | [100] |

As an example Roland et al. [82] developed for Piperine using a non-covalent precipitation polymerization method. Piperine served as the template molecule, while acrylic acid was selected as the functional monomer. Ethylene glycol dimethacrylate (EGDMA) acted as the cross-linking agent, with 2,2'-azobisisobutyronitrile (AIBN) as the radical initiator, and acetonitrile was used as the porogenic solvent. Among the synthesized polymers, MIP 3—formulated with a molar ratio of 0.5:3:8 (template: monomer: cross-linker)—demonstrated the highest specific binding capacity (84.94%) in comparison to other MIPs and the non-imprinted control. Furthermore, this optimized MIP showed excellent extraction performance, achieving over 80% recovery of Piperine from spiked urine samples.

Zhou et al. [84] developed a carbon paste electrode modified with a molecularly imprinted polymer (MIP-CPE) for sensitive and selective detection of curcumin (CUR). The curcumin-specific MIP (CUR-MIP) was synthesized via thermally initiated precipitation polymerization, employing curcumin as the template molecule, methacrylic acid (MAA) as the functional monomer, 2,2'-azobisisobutyronitrile (AIBN) as the initiator, and ethylene glycol dimethacrylate (EGDMA) as the cross-linking agent. Electrochemical characterization revealed a clear oxidation peak for curcumin at 0.434 V (vs. SCE) in a phosphate buffer solution (PBS) at pH 3.06. A linear response was observed for curcumin concentrations ranging from 0.1 to 50  $\mu$ M, with a detection limit of 10.1 nM. The proposed method was successfully applied to analyze curcumin in food samples, yielding recovery rates between 90.77% and 105.7%.

Polymer microspheres imprinted with diosgenin (DG-MIPs) were synthesized using the precipitation polymerization method by Hou et al [87]. Diosgenin served as the template molecule, methacrylic acid (MAA) was used as the functional monomer, and ethylene glycol dimethacrylate (EGDMA) acted as the cross-linking agent. The surface morphology and binding performance of microspheres were analyzed using Scanning Electron Microscopy



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(SEM) and High-Performance Liquid Chromatography (HPLC). The optimal formulation was found to be 2 mM diosgenin, 6 mM MAA, 60 mM EGDMA, and 4 mM AIBN. This technique shows promise for large-scale separation and extraction of bioactive compounds from traditional Chinese herbal medicines.

Naskar et al. [89] presents the fabrication of a graphite electrode modified with polyacrylonitrile-based molecularly imprinted polymer (PAN-MIP/G) for the sensitive and selective detection of eugenol (EU) in various food samples. Among several tested polymer compositions pure polyacrylonitrile used as a functional monomer. The PAN-MIP/G sensor exhibits three distinct linear response ranges: 0.0005–1.0  $\mu\text{M}$ , 1–50  $\mu\text{M}$ , and 50–160  $\mu\text{M}$ , with a detection limit as low as 0.0004  $\mu\text{M}$ . Finally, the developed PAN-MIP/G electrode was effectively applied to detect eugenol in real food samples including curry powder, clove powder, and capsule formulations.

Wang et al. [91] developed an electrochemical sensor which was fabricated using MIP integrated with multi-walled carbon nanotubes (MWCNTs-MIP) to specifically detect capsaicin. The MIP layer was synthesized using capsaicin as the template molecule and 4-vinylpyridine as the functional monomer. The electrochemical characteristics of the sensor were assessed using DPV, and the optimal operational parameters were established. The sensor displayed a strong linear response to capsaicin concentrations ranging from  $0.05 \times 10^{-6}$  to  $1 \times 10^{-4}$  mol  $\text{L}^{-1}$ , achieving a detection limit as low as 0.02  $\mu\text{mol L}^{-1}$ . Additionally, the sensor demonstrated high selectivity, good reproducibility, and stable performance in the analysis of real-world samples.

Molecularly Imprinted Polymers (MIPs) for flavonoids (quercetin, kaempferol, and hydroxytyrosol) were synthesized for selective separation, especially relevant in wine and fermentation product analysis [93]. The polymerization was carried out thermally, employing methacrylic acid as the functional monomer and ethylene glycol dimethacrylate as the cross-linking agent. These flavonoid-specific MIPs exhibited high selectivity for their respective templates, even in the presence of complex matrices, underscoring their promise in developing synthetic receptors for targeted biomolecules. This study not only highlights the effectiveness of the MIP approach but also provides insights into the intricate non-covalent interaction mechanisms involved in binding small bioactive compounds.

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MIP was synthesized using (–)-norephedrine as the template molecule, methacrylic acid (MAA) as the functional monomer, ethylene glycol dimethacrylate (EGDMA) as the cross-linking agent, and chloroform as the porogenic solvent [94] for isolating psychoactive phenyl propyl amino alkaloids—namely, norephedrine, cathinone, and cathine, extracted from the leaves of *Catha edulis* (Khat). The MIP demonstrated high selectivity in isolating these alkaloids from aqueous Khat leaf extracts. Method validation yielded satisfactory recoveries ranging between 90.0% and 107%, with relative standard deviations (RSDs) between 2.3% and 3.2%, confirming the method's reliability. The concentrations of the three alkaloids measured using MIP-SPE were consistent with those obtained using a commercial Isolute C18 (EC) cartridge. These results suggest that MIP-SPE is a dependable approach for pre-treatment and purification of Khat alkaloids in plant matrices, with potential application in pharmaceutical, forensic, and biomedical analyses.

A novel MIP was developed for the targeted extraction of crocin from saffron stigmas, using gentiobiose as the template [95]. The binding performance of the gentiobiose-imprinted polymer (Gent-MIP) was assessed against a non-imprinted control polymer (NIP) in aqueous conditions. Scatchard analysis revealed that the maximum number of binding sites in the polymer was 18.4 mmol/g, with a dissociation constant ( $K_d$ ) of 11.2 mM for crocin. The Gent-MIP was subsequently applied in a solid-phase extraction (SPE) protocol to isolate and purify crocin from methanolic extracts of saffron stigmas. The recoveries of crocin, safranal, and picrocrocin were analyzed during the washing and elution phases. Notably, the Gent-MIP showed a marked selectivity for crocin, enabling its efficient recovery (84%) from a complex matrix.

Nguyen et al. [96] reported synthesis of a curcumin-selective molecularly imprinted polymer for extraction and quantification of curcumin in food samples. Curcumin was used as the template molecule, with methacrylic acid (MAA) as the functional monomer to form hydrogen-bond interactions. Ethylene glycol dimethacrylate (EGDMA) acted as the crosslinker, while azobisisobutyronitrile (AIBN) initiated polymerization. The polymer was prepared via thermal bulk polymerization in acetonitrile porogen. After polymerization, the template was removed using methanol–acetic acid, generating selective binding cavities. The sensor exhibits linear response ranges of 2.7 to 6.8  $\mu\text{M}$ , with a LOD and LOQ of 0.138  $\mu\text{M}$  and 0.424  $\mu\text{M}$ . The MIP showed high selectivity and recovery compared to NIP and C18 sorbents.

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Djunaïdi et al. [97] focused the development of a curcumin-imprinted polymer intended for controlled transdermal drug delivery. Curcumin served as the template molecule, methacrylic acid (MAA) as the functional monomer, and EGDMA as the crosslinker. Benzoyl peroxide (BPO) was employed as a free-radical initiator. Unlike bulk grinding approaches, the MIP was synthesized using precipitation polymerization, which produced uniform, fine polymer particles without post-crushing. Polymerization was thermally carried out at 60–70 °C in acetonitrile. The resulting MIP exhibited higher adsorption capacity and slower curcumin release than the NIP, confirming the formation of specific imprinted binding sites.

In another way, a curcumin-selective MIP was synthesized using UV–Vis spectrophotometric for the determination of curcumin in real food samples [98]. Curcumin acted as the template, acrylamide as the functional monomer, and EGDMA as the crosslinker. The radical initiator used was 4,4'-azobis(4-cyanovaleric acid) (ABCVA). The polymer was synthesized via thermal bulk polymerization in acetonitrile under nitrogen atmosphere at 70 °C. After Soxhlet extraction to remove the template, the MIP exhibited higher surface area and adsorption capacity than the NIP. The sensor exhibits a good linear response ranges with a LOD of 1.90 µM. The MIP showed high selectivity and recovery compared to NIP and C18 sorbents. The material demonstrated good selectivity, recovery, and applicability to turmeric-based food matrices.

Ji et al. [99] reported an alternative approach involving the preparation of a dummy molecularly imprinted polymer for selective extraction of gingerols from ginger. Because gingerols are thermally unstable, N-vanillylnonanamide was designed as a dummy template. 4-Vinylpyridine (4-VP) was used as the functional monomer, N,N'-methylenebisacrylamide (MBA) as the crosslinker, and AIBN as the initiator. Polymerization was performed by thermal bulk polymerization. After template removal, the MIP selectively adsorbed 6-, 8-, and 10-gingerol simultaneously. The developed MISPE method produced gingerols with high purity and recovery, demonstrating the advantage of dummy-template bulk MIPs for natural product extraction.

Kasiri et al. [100] presented a nano-sized magnetic molecularly imprinted polymer for selective preconcentration of thymol and carvacrol from plant extracts. Thymol and carvacrol were used as templates, methacrylic acid (MAA) as the functional monomer, and EGDMA as the crosslinker. AIBN initiated the polymerization. The MIP layer was formed via thermal

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polymerization on  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles, producing a magnetic MIP sorbent. The MMIP exhibited high adsorption capacity, very low LOD of 0.28 nM, and excellent selectivity. Magnetic separation enabled rapid and efficient extraction from complex herbal matrices.

### 1.5. Literature survey on potentiostat

This study makes a notable contribution to the development of a standalone, portable electrochemical detection system that eliminates the need for expensive commercial potentiostats and proprietary data processing software. Table 1.3. outlines several studies for the development of low cost portable potentiostat, followed by elaborations on some key research efforts.

*Table 1.3. Literature survey on portable potentiostat.*

| Sl. No. | Type                              | No of working Electrode | Current rating                                                        | Potential range     | Used to detect                       | Special feature (if any)                                     | Ref.  |
|---------|-----------------------------------|-------------------------|-----------------------------------------------------------------------|---------------------|--------------------------------------|--------------------------------------------------------------|-------|
| 1       | Potentiostat to detect CV         | one                     | $\pm 300 \mu\text{A}$                                                 | $\pm 1.5 \text{ V}$ | $\text{K}_4[\text{Fe}(\text{CN})_6]$ | Low cost, ESP 32 based, smart phone controlled via Bluetooth | [101] |
| 2       | Potentiostat to detect DPV        | one                     | ----                                                                  | ----                | Bisphenol A (BPA)                    | Bluetooth-enabled, wirelessly connects to smartphone         | [102] |
| 3       | Portable potentiostat (UnpadStat) | one                     | $\pm 100 \text{ nA}$                                                  | $\pm 3 \text{ V}$   | Ferricyanide                         | Touchscreen, on device plotting, USB port                    | [103] |
| 4       | Potentiostat                      | one                     | $\pm 1.5 \text{ mA}$<br>$\pm 150 \mu\text{A}$<br>$\pm 15 \mu\text{A}$ | $\pm 1.5 \text{ V}$ | Dopamine                             | Bluetooth Low Energy (BLE) wireless interface                | [104] |
| 5       | Multichannel Potentiostat         | Three                   | $\pm 500 \mu\text{A}$                                                 | $\pm 3 \text{ V}$   | ferri/ferrocyanide                   | Bluetooth and USB connectivity, GUI via android App,         | [105] |
| 6       | Portable Potentiostat             | one                     | $\sim 9.95 \mu\text{A}$                                               | $\pm 0.8 \text{ V}$ | Heavy metal ions                     | Smartphone-powered and controlled using USB/OTG              | [106] |

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|   |              |      |                                           |                            |                                                            |                                                                 |       |
|---|--------------|------|-------------------------------------------|----------------------------|------------------------------------------------------------|-----------------------------------------------------------------|-------|
| 7 | Potentiostat | one  | $\pm 2.5$ nA to $\pm 50 \mu\text{A}$      | $\pm 1.5$ V                | Copper in acetic acid soil extracts                        | Fully portable, battery-operated device                         | [107] |
| 8 | Potentiostat | Four | $\pm 62 \mu\text{A}$ to $\pm 7 \text{nA}$ | $\pm 0.6$ V                | Anti-tTG IgA & IgG antibodies for celiac disease diagnosis | Wi-Fi cloud connectivity<br>Low power<br>Fully battery-operated | [108] |
| 9 | Potentiostat | One  | 10.5 mA                                   | $\pm 1.2$ V to $\pm 0.1$ V | Lead and Cadmium ions in soil                              | Portable, cobalt oxide-modified SPE                             | [109] |

Anshori *et al.* [101] present eSTAT, a low-cost, portable potentiostat for CV was built with ESP32 microcontroller and controlled via Bluetooth from a custom Android app. The system covers a voltage sweep of  $-1.5$  V to  $+1.5$  V and measures currents between  $\pm 300 \mu\text{A}$  with 12-bit ADC resolution ( $\sim 0.16 \mu\text{A}$  sensitivity). In experimental validation using a standard  $\text{K}_4[\text{Fe}(\text{CN})_6]$  solution and a three-electrode setup, eSTAT achieved 98.3% accuracy for anodic peak current and 87.8% for cathodic peak current compared to a commercial Zimmer & Peacock potentiostat. The Android interface allows users to set CV parameters, receive live voltammograms, and perform automated peak analysis, all wirelessly—enhancing usability and portability. With production costs under US\$30, eSTAT represents an affordable alternative to expensive instrumentation.

Beduk *et al.* [102] introduce a portable and wireless electrochemical sensing system for on-site detection of Bisphenol A (BPA). The sensor integrates a laser-scribed graphene (LSG) electrode functionalized with a MIP matrix enhanced with gold nanoparticles and PEDOT, creating highly selective recognition sites for BPA. Coupled with a custom-built Bluetooth-enabled potentiostat (“KAUSTat”), the setup enables differential pulse voltammetry (DPV) measurements to detect ultralow concentrations of BPA with a limit of detection of 3.97 nM and a linear calibration range from 0.01 to 10  $\mu\text{M}$ . The device wirelessly transmits data to smartphones, enabling real-time, field-based environmental monitoring without bulky laboratory equipment. Performance was validated across real samples—tap water, bottled water, milk, and baby formula—with successful recovery rates. The combination of high selectivity, sensitivity, and practical portability positions this tool as a cost-effective solution for environmental safety and public health applications.

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Setiyono *et al.* [103] designed UnpadStat, a compact, portable potentiostat tailored for SPCE-based electrochemical sensing. It employs a 3-electrode configuration (working, counter, reference) and uses an STM32 microcontroller handling 16-bit DAC/ADC, analog amplifiers, and a switching resistor network to cover a current range from  $\pm 100$  nA to  $\pm 10$  mA. The DAC can sweep potentials from  $-3$  V to  $+3$  V, though tested CV runs were between  $-1$  V and  $+1$  V at  $80$  mV/s. Performance benchmarking against a commercial unit showed  $>90\%$  accuracy in both RC dummy and ferricyanide SPCE tests, with peak currents around  $\pm 176$   $\mu$ A. Key features include a touchscreen for configuration and on-device plotting, SD-card logging of large datasets, USB PC connectivity, and battery operation offering  $\sim 14$  h runtime. This system offers a low-cost, standalone solution (no PC/smartphone needed), combining portability, high-resolution electrochemical measurements, and data management—ideal for field sensing, educational use, and point-of-care diagnostics.

Järvinen *et al.* [104] introduce the Wireless Customizable Electrochemical Measurement System (WCEMS), which is a compact, open-source potentiostat tailored for cyclic voltammetry on on-chip,  $\text{Ni}(\text{OH})_2$  decorated, vertically aligned  $\text{MoS}_2$  thin-film sensors. The device uses a three-electrode cell (one working electrode) and switches between four current ranges ( $\pm 1.5$  mA to  $\pm 1.5$   $\mu$ A) via a 14-bit ADC, while the DAC supports potentials up to  $\pm 1.5$  V; dopamine testing was conducted between  $-1$  V and  $+1$  V vs.  $\text{Ag}/\text{AgCl}$ . Coupled with BLE connectivity and an Android app, WCEMS enables wireless control, real-time plotting, and data logging—all in a  $28$  g package costing under  $\$100$ . Compared to a commercial benchtop potentiostat, it delivers comparable accuracy for dopamine concentrations from  $1$   $\mu$ M to  $1$  mM, achieving a theoretical LOD of  $0.1$   $\mu$ M. Its open-source design, low cost, and portability make it a promising tool for field-deployable electrochemical sensing, educational use, or rapid assessment of novel electrode materials

Anshori *et al.* [105] introduce ESPotensio, an ultra-affordable, ESP32-driven portable potentiostat that can perform six electrochemical techniques—CV, LSV, SWV, DPV, NPV, and chronoamperometry—across three semi-parallel channels. Each channel uses a standard three-electrode setup and can handle currents up to approximately  $\pm 500$   $\mu$ A. The device sweeps potentials between  $-1$  V and  $+1$  V (although hardware supports  $\pm 3$  V), using an external 12-bit DAC (MCP4725) and reads current via shunt-resistor front-end, converted by the MCU's ADC. Bluetooth (with an Android app) and USB desktop connectivity enable command input, real-time plotting, and CSV data export. Validation against a commercial Emstat Pico, using ferri/ferrocyanide redox measurements, showed over  $90\%$  accuracy across all techniques. Semi-parallel sampling across channels—cycling through ADC pins in under

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10 ms—allows effective multi-sample analysis. At a mere USD 21.4 component cost, ESPotensio stands out as a highly accessible tool for multi-channel, multifunctional electrochemical sensing, ideal for educational labs, fieldwork, and low-resource analytical needs.

Ning *et al.* [106] presents the development of a portable potentiostat based on a three-electrode electrochemical sensor system, designed for integration with smartphones through an OTG interface. The device includes a custom-built board-level circuit with components for excitation generation, signal acquisition, and processing. It utilizes a glassy carbon working electrode, an Ag/AgCl reference electrode, and a platinum counter electrode to detect  $\text{Pb}^{2+}$  ions in  $\text{PbSO}_4$  solution. The system applies bipolar step-shaped voltage excitations ranging from -0.8 V to +0.8 V, and the resulting stripping current ( $\sim 9.95 \mu\text{A}$ ) shows performance comparable to commercial electrochemical workstations. A high-resolution DAC (DAC8562) and precision op-amps (e.g., OP37, INA122P) ensure accurate voltage control and current measurement. The potential control accuracy is within  $\pm 30$  mV. The compact system features smartphone-based control and display, making it highly portable and low-cost, suitable for field applications in environmental monitoring and electrochemical sensing.

Beni *et al.* [107] presents the design and application of a portable electrochemical system for the in-field detection of trace metals, specifically copper, in acetic acid soil extracts. The device consists of a microcontroller-based potentiostat capable of performing anodic stripping voltammetry (ASV), especially underpotential deposition staircase anodic stripping voltammetry (UPD-SCASV), using a gold or glassy carbon working electrode. The system features a wide current range ( $\pm 2.5$  nA to  $\pm 50 \mu\text{A}$ ) and potential control ( $-1.5$  V to  $+1.5$  V), with high precision suitable for detecting trace metal levels. It operates using batteries, supports RS232 PC communication, and includes custom software for sample analysis, calibration, and result generation using standard addition. The portable system was evaluated in both laboratory and field conditions and showed strong agreement with standard laboratory techniques like ICP-OES. Its embedded software allows even non-specialists to operate the device efficiently.

Boni *et al.* [108] presented a highly integrated stand-alone portable potentiostat designed for simultaneous multi-analyte electrochemical analysis using four independent channels. Each channel manages a full three-electrode cell, with real-time control via a microcontroller and

data transmission through Wi-Fi to cloud platforms like ThingSpeak. The system supports Differential Pulse Voltammetry (DPV) and, offering  $\pm 62 \mu\text{A}$  current range with 7 nA resolution, and customizable bias voltages via 16-bit DACs. The potentiostat was used for simultaneous detection of IgG and IgA anti-tTG antibodies (celiac disease biomarkers), and achieved a 76% reduction in analysis time compared to single-channel systems. Advanced signal processing and baseline correction ensure high analytical accuracy, while negligible channel mismatch and crosstalk guarantee reliability. This makes the device ideal for point-of-care (PoC) or home diagnostics, where portability, speed, and accuracy are essential.

Katiyar *et al.* [109] designed a low-cost, portable electrochemical potentiostat circuit for detection of toxic heavy metals, specifically cadmium and lead ions in soil samples. The detection is based on anodic stripping voltammetry (ASV) using both screen printed electrodes (SPE) and glassy carbon electrodes (GCE). The potentiostat is interfaced with a cobalt oxide-modified SPE, enhancing sensitivity. It applies a controlled deposition potential followed by a sweep potential ( $-1.2 \text{ V}$  to  $-0.1 \text{ V}$ ), enabling the oxidation of deposited metal ions, observable as distinct current peaks. The system is Arduino-based, eliminating manual voltage control and enhancing portability. The circuit demonstrates potential for field-deployable environmental monitoring of soil contamination, reducing reliance on expensive and bulky lab equipment.

### 1.6. Objectives and scope of the research

This research focuses on employing MIP strategies for the detection of select phytochemicals such as piperine and thymoquinone. Several experimental parameters were optimized, including pH, buffer composition, and scan rate. The specific objectives of this study are outlined below:

- To design and fabricate MIP-based electrodes capable of selectively detecting phytochemicals such as piperine and thymoquinone in black pepper and black cumin, respectively.
- To optimize electrode fabrication parameters, including the selection of functional monomers, cross-linkers, template-to-monomer ratios, and polymerization conditions, for achieving maximum sensitivity and selectivity.



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- To characterize the developed electrodes using advanced surface and structural characterization techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscopy (FESEM), X-ray Photoelectron Spectroscopy (XPS), and Ultraviolet–Visible (UV–Vis) spectroscopy to confirm successful polymer formation and molecular imprinting.
- To evaluate the electrochemical performance of the developed sensors using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques under optimized pH, buffer composition, and scan rate conditions.
- To apply statistical and machine learning approaches, such as Principal Component Analysis (PCA), Convolutional Neural Networks (CNN), and regression modeling, for analyzing and predicting biomolecule concentrations based on voltammetric signal patterns.
- To design and implement a low-cost, Arduino-based portable potentiostat (LC-PSTAT) integrated with a Python-based user interface to facilitate cyclic and differential pulse voltammetric measurements, providing a compact and affordable alternative to commercial instruments.
- To validate the developed sensors and potentiostat by comparing electrochemical results with reference RP-HPLC data obtained from real spice samples.

Based on these objectives, the thesis is divided into five chapters, summarized as follows:

**Chapter 1** introduces the significance of phytochemicals in medicinal plants and their potential therapeutic applications. It discusses challenges in quality control and standardization of herbal products and proposes electrochemical sensing using MIPs as a cost-effective alternative to traditional analytical methods. The chapter reviews electrochemical techniques, MIP synthesis methods, and literature on MIP-based sensors and portable potentiostats. It highlights the need for selective, sensitive detection of phytochemicals like thymoquinone and piperine. The chapter concludes with the objectives and scope of the thesis, focusing on developing MIP-based electrochemical sensors for plant-derived biomolecules using voltammetric techniques.

**Chapter 2** presents the development of a MIP based graphite electrode using PDMAM for sensitive detection of piperine in black pepper. The sensor, optimized for electrochemical performance, achieved a low detection limit of 0.6 nM. Advanced characterization techniques

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validated the sensor structure. Convolutional Neural Networks (CNNs) and hybrid regression models (KNNR, GBR, RFR) were used to predict piperine content from cyclic voltammetry signals with high accuracy ( $R^2 > 0.999$ ). This AI-integrated electrochemical sensing approach offers a robust, low-cost solution for food quality monitoring, demonstrating strong performance across commercial black pepper brands.

**Chapter 3** presents two innovative electrochemical sensors—TiO<sub>2</sub>-modified graphite paste electrode (GPE/TiO<sub>2</sub>) and thymoquinone-imprinted polymer electrode (TQIP/G)—for detecting thymoquinone (TQ) in black cumin. Both sensors showed high sensitivity, selectivity, and stability, with low detection limits (0.02–0.03  $\mu\text{M}$ ). Principal Component Analysis (PCA) and machine learning, particularly a CNN model, enhanced real-time prediction from voltammetric data with  $R^2 \approx 0.9997$ . The study highlights successful TQ quantification in commercial samples, validating the sensors against RP-HPLC standards. This AI-integrated electrochemical platform offers a cost-effective, portable alternative to traditional analytical techniques for food and pharmaceutical quality monitoring.

**Chapter 4** details the design and development of a low-cost, portable potentiostat (LC-PSTAT) integrated with a molecularly imprinted polymer-based graphite electrode (TQMIP-G) for detecting thymoquinone (TQ) in black cumin. The system uses CV and DPV techniques, achieving a detection limit of 0.03  $\mu\text{M}$ . It is built with Arduino-based hardware and Python software for real-time analysis. The TQMIP-G electrode demonstrated high selectivity, stability, and accuracy ( $\approx 94\%$ ) when validated against RP-HPLC. Its affordability, portability, and user-friendly interface make it suitable for pharmaceutical, food, and agricultural applications, offering a viable alternative to conventional lab-based instrumentation.

**Chapter 5** concludes the thesis, summarizing key findings and highlighting strengths and limitations of the proposed sensor systems. It also suggests potential future directions for advancing this research area.

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## Chapter 2

### Detection of piperine content in black pepper

This chapter introduces a molecularly imprinted graphite electrode for highly selective and sensitive electrochemical detection of piperine in black pepper. Using PDMAM and EGDMA, the sensor showed excellent performance (LOD: 0.0006  $\mu\text{M}$ ) and stability. Real-sample analysis validated its effectiveness. A convolutional neural network (CNN) machine learning model enhanced quantification accuracy, achieving a MAPE of 0.034 and  $R^2$  of 0.9999, demonstrating a powerful fusion of sensing and AI for food quality assessment.

#### List of Sections

- 2.1. Introduction
- 2.2. Experiment with PDMAM@G sensor combined with CNN
- 2.3. Results with PDMAM@G sensor combined with CNN
- 2.4. Discussion
- 2.5. Conclusion
- References

#### **Contents of this chapter are based on mentioned publication:**

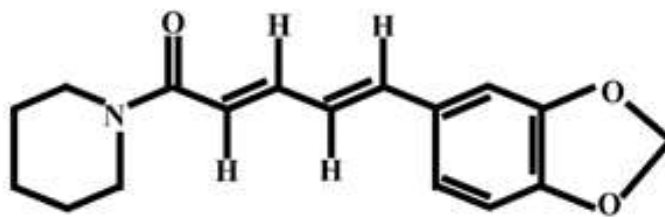
**Sanjoy Banerjee**, Milan Dhara, Hemanta Naskar, Barnali Ghatak, Sk. Babar Ali, Nityananda Das, K. Chezyian, Deepak Kumar Das, Bhimsen Tudu, Arpitam Chatterjee, Bidya Mondal, Dipankar Mandal, Santanu Ghorai, Rajib Bandyopadhyay, Bipan Tudu, "Detection of piperine content in black pepper using a molecular imprinted poly(N,N-dimethylacrylamide) embedded graphite electrode: A machine learning based prediction approach", Microchemical Journal, 207, (2024), 111914.





### 2.1. Introduction

Phytochemicals derived from plants have long played a pivotal role in traditional medicine and drug discovery, offering a diverse array of organic compounds to combat diseases, pests, and environmental stressors [1-2]. Among these, piperine ( $C_{17}H_{19}NO_3$ ), the principal alkaloid in *Piper nigrum* (black pepper), is particularly notable for its pungency and wide-ranging therapeutic potential. Commonly referred to as the “king of spices,” black pepper contains piperine at purities up to 98% [3]. Structurally, piperine is a carboxamide composed of piperic acid and piperidine, existing in four isomeric forms, with only the trans-trans isomer contributing significantly to its pungency [4-5]. The molecular structure of piperine is illustrated in Fig. 2.1.



**Fig. 2.1. Molecular structure of piperine.**

Apart from its role as a spice, it has been traditionally utilized in folk medicine to address a range of health conditions [6,7]. It is used for the treatment and alleviation of pain, muscular pains, chills, rheumatism, influenza, fever, migraine headaches, strep throat, cardiac, digestive disorders, and even coma [8,9]. Moreover, according to pharmacological studies, it possesses anti-inflammatory, analgesic, anti-carcinogenic, and antioxidant activity [10]. Furthermore, PIP can be used as a bio enhancer by enhancing their absorption or slowing down drug metabolism or a combination of two [11–16].

Accurate quantification of piperine in black pepper is critical for quality assurance in both the food and pharmaceutical industries. Various detection methods such as gas chromatography (GC), gas chromatography–mass spectroscopy (GC–MS) [17], near-infrared spectroscopy (NIRS) [18], TLC-UV densitometry [19], high-performance liquid chromatography (HPLC)

## Chapter 2: Detection of piperine content in black pepper

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[20], liquid chromatography–mass spectrometry (LC–MS) [21] and high-performance thin-layer chromatography (HPTLC) [22] techniques have been reported in the last few decades for the quantitative and/or qualitative estimation of PIP from black pepper. However, these analytical methods require costly and complex instruments along with elongated periods for extraction and quantification. In contrast, electrochemical sensing techniques offer advantages such as low cost, fast response, high sensitivity and selectivity, minimal sample preparation, and potential for on-site detection [23]. Jain et al. and Y. Wang et al used square wave voltammetry and cyclic voltammetry techniques to detect PIP using glassy carbon electrode (GCE) [24-25] with a certain linear range. However, achieving high specificity and sensitivity in electrochemical sensors remains a challenge. To address these limitations, molecularly imprinted polymers (MIPs) have emerged as promising materials that enable selective recognition by creating template-specific cavities during polymerization [26].

MIP-based sensors have shown remarkable potential for detecting a wide range of biomolecules and phytochemicals due to their artificial molecular recognition capabilities. Numerous studies have successfully applied MIPs for the detection of compounds such as furaneol, flavones, thymol, and gallic acid, establishing their robustness, reproducibility, and high selectivity [27-36]. In this context, the present study builds upon previous efforts to develop an MIP-based electrochemical sensor specifically tailored for the detection of piperine.

Here, we report the fabrication of a poly(N,N-dimethylacrylamide) (PDMAM) imprinted graphite electrode (PDMAM-MIP@G) using ethylene glycol dimethacrylate (EGDMA) as the cross-linker and piperine as the template molecule. The sensor exhibited a low limit of detection (0.6 nM), wide linear response range, and excellent electrochemical performance in terms of stability, reproducibility, and interference resistance. Real sample applicability was demonstrated using black pepper powders from four commercial brands.

To further enhance detection capabilities, we integrated this sensor with deep learning-based analysis, specifically a convolutional neural network (CNN) framework that extracts features from cyclic voltammetry (CV) signals, showed superior performance, achieving near-perfect correlation with reference HPLC values.

This integrated approach—combining MIP-based electrochemical sensing with AI-driven predictive modeling—presents a novel, rapid, and cost-effective platform for piperine

detection in real-world food samples, offering practical solutions for industrial quality control and large-scale spice processing.

### **2.2. Experiment with PDMAM@G sensor using machine learning approach**

#### **2.2.1. Reagents and chemicals**

Poly(N,N-Dimethylacrylamide) (PDMAM), ethylene glycol dimethacrylate (EGDMA), benzoyl peroxide (BP), and graphite were purchased from Sigma Aldrich, USA. Ethanol and Paraffin oil were obtained from Merck, India. All chemicals were analytical grade and used as received without any further purification. The deionized water received from the Merck Millipore system (Resistance =  $\sim 18 \text{ M}\Omega$ ). All the electrochemical experimental were done at normal room temperature ( $25 \pm 2^\circ\text{C}$ ).

#### **2.2.2. Methods and measurement**

The surface morphology of all synthesized materials was examined using field emission scanning electron microscopy (FESEM, JEOL-JEM-6700F). Fourier transform infrared (FTIR) spectroscopy of the MIP and NIP were obtained by FTIR analysis from IR prestige 21, 200VCE (Shimadzu, Tokyo, Japan), and their spectra were recorded in the range of  $4000 - 400 \text{ cm}^{-1}$ . Three electrodes were utilized in the electrochemical analysis with a Potentiostat/Galvanostat PGSTAT101 (Metrohm Autolab, Netherlands). This setup was equipped with the developed PDMAM-MIP@G as a working electrode, silver/silver chloride ( $\text{Ag}/\text{AgCl}$ ) as a reference electrode and platinum ( $\text{Pt}$ ) wire as a counter electrode. All data plots were constructed using Origin Pro 8.0 (Origin Lab Corporation, USA).

#### **2.2.3. Synthesis of PDMAM-MIP@G and non-imprinted polymer (PDMAM-NIP@G)**

MIPs for the detection of PIP were prepared by a one-step in-situ polymerization approach with a variable molar ratio of template, functional monomer, and cross-linker as given in Table 1. Firstly, 0.90 gm of commercial-grade graphite powder was dispersed in 20 mL of ethanol through ultrasonication for 60 minutes. Concurrently, in the pre-polymerization step, N,N-DMA functional monomer, and piperine as template molecules were also dispersed in 20 mL of ethanol through ultrasonication for 60 minutes until a complex mixture of template molecules and functional monomer through self-assembling was formed via interfacial non-covalent bonding. Subsequently, the as-prepared pre-polymerized mixture was mixed with the dispersed graphite solution for another 60 minutes through ultrasonication along with the

## Chapter 2: Detection of piperine content in black pepper

addition of EGDMA as a cross-linker and 1mg benzoyl peroxide as an initiator. Finally, the entire mixture was heated for 60 minutes under the initial temperature of 60°C and thereafter reduced to 40°C under a pure  $N_2$  atmosphere to facilitate the polymerization. The polymerized product was then taken and kept overnight at room temperature.

For the preparation of the MIP, the piperine was removed from the polymer matrix by washing vigorously with a mixture of ethanol and water (4:1 v/v). A similar procedure was used for the synthesis of MIP of piperine with varying compositions of monomer and template as given in Table 2.1. Non-imprinted polymer composite materials (PDMAM-NIP@G) were prepared under the same procedure without the addition of the template molecules.

**Table 2.1. Materials synthesized and ratio of precursor used.**

| Polymer      | Template (mM) | Monomer (mM) | Crosslinker (mM) | Ratio  |
|--------------|---------------|--------------|------------------|--------|
| PDMAM-MIP@G1 | 0.2           | 0.6          | 2.4              | 1:3:12 |
| PDMAM-MIP@G2 | 0.2           | 0.8          | 2.4              | 1:4:12 |
| PDMAM-MIP@G3 | 0.2           | 1.2          | 2.4              | 1:6:12 |
| PDMAM-NIP@G  | 0             | 0.8          | 2.4              | 0:4:12 |

### 2.2.4. Preparation of the electrode

300mg of the respective synthesized polymers were subjected to proper grinding to get a fine paste with the addition of paraffin oil drop wise. Here, paraffin oil was used as a binder [40]. The paste was further poured into fine capillary glass tubes having an inner diameter of 2.5mm and was stuffed with stainless steel rods. The electrical contact of the electrode was made by connecting a *Pt* wire to the backside of the tube.

### 2.2.5. Preparation of real sample

Four different black pepper samples, labelled as B1, B2, B3, and B4, were purchased from the local market. Twenty grams of each sample were ground into a fine powder using a mixer

## Chapter 2: Detection of piperine content in black pepper

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grinder. Subsequently, 5 mg of each powdered sample was mixed with 20 mL of pure ethanol in separate beakers. The mixtures were ultrasonicated for 30 min to extract PIP from the black pepper dust.

The extracts were then filtered using Whatman filter paper, and the filtrates were collected in four volumetric flasks, each labelled according to the sample. Stock solutions (100 mL each) were further prepared using phosphate-buffered saline (PBS) at pH 6. CV analyses were performed for each sample, and the experiment was repeated 10 times for each sample to ensure accuracy and reproducibility of the results.

### ***2.2.6. Electrochemical characterization and baseline establishment protocol***

To ensure experimental reproducibility and analytical reliability, a systematic electrochemical characterization protocol was adopted. The procedure included electrode preconditioning, baseline stabilization, optimization studies, and transient current analysis for response time determination.

#### ***Step 1: Electrochemical cell configuration***

All electrochemical measurements were performed using a conventional three-electrode system connected to a computer-controlled potentiostat. The system consisted of:

- Working electrode: Molecularly imprinted polymer (MIP)-based modified electrodes (PDMAM@G, GPE/TiO<sub>2</sub>, and TQIP/G)
- Reference electrode: Ag/AgCl (3 M KCl)
- Counter electrode: Platinum wire

All experiments were conducted at room temperature ( $25 \pm 2$  °C) using 20 mL of supporting electrolyte in an electrochemical cell.

#### ***Step 2: Electrode preconditioning and baseline stabilization***

Prior to analyte measurements, each modified electrode was gently rinsed with distilled water and equilibrated in blank supporting electrolyte for 5 minutes to attain interfacial stability.

Cyclic voltammetry (CV) was initially performed in blank buffer solution (without analyte) to establish the electrochemical baseline.

#### ***Step 3: Buffer selection and pH optimization***

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The influence of supporting electrolyte and pH on the electrochemical response was systematically evaluated. Analyte solutions were prepared by mixing analyte stock solution with buffer in a 1:9 ratio (final volume: 20 mL).

The pH ranges investigated were:

- Piperine (PDMAM@G): pH 2.0 – 8.0, Applied CV potential range:  $-1.0$  to  $1.5$  V, Scan rate:  $0.05$  V/s and Step potential:  $0.005$  V (described details in chapter 2, section 2.3.9)
- Thymoquinone (GPE/TiO<sub>2</sub>): pH 4.0 – 7.0, Applied CV potential range:  $-0.7$  to  $1.0$  V, Scan rate:  $0.05$  V/s and Step potential:  $0.005$  V. (described details in Chapter 3, section 3.3.3)
- Thymoquinone (TQIP/G Sensor): pH 2.0 – 7.0, Applied CV potential range:  $-0.6$  to  $1.2$  V, Scan rate:  $0.05$  V/s and Step potential:  $0.005$  V. (described details in chapter 3, section 3.5.4)

For each pH value, CV measurements were performed to evaluate:

- Oxidation peak current ( $I_{pa}$ )
- Oxidation peak potential ( $E_{pa}$ )
- Peak shape and resolution

The optimum pH was selected based on maximum peak current, sharp peak definition, and minimum background interference.

### Step 4: Scan rate studies

To investigate the electrochemical kinetics and mass transport mechanism, cyclic voltammetry was performed at different scan rates.

- Piperine (PDMAM@G): Scan rate in the range of  $0.001$  V/s to  $0.6$  V/s in  $0.1$  M PBS at pH 6. (described details in chapter 2, section 2.3.10)
- Thymoquinone (GPE/TiO<sub>2</sub>): Scan rate in the range of  $0.01$  V/s to  $0.5$  V/s in  $0.1$  M PBS at pH 6. (described details in Chapter 3, section 3.3.4)
- Thymoquinone (TQIP/G Sensor): Scan rate in the range of  $0.005$  V/s to  $0.6$  V/s in  $0.1$  M PBS at pH 6. (described details in chapter 3, section 3.5.6)

The relationship between peak current ( $I_p$ ) and scan rate ( $v$ ) was analyzed to determine whether the redox process was diffusion-controlled or adsorption-controlled. This study also

## Chapter 2: Detection of piperine content in black pepper

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enabled identification of the optimal scan rate for subsequent differential pulse voltammetry (DPV) measurements.

### *Step 5: Optimized measurement conditions for real sample*

(a) Piperine – PDMAM@G Sensor

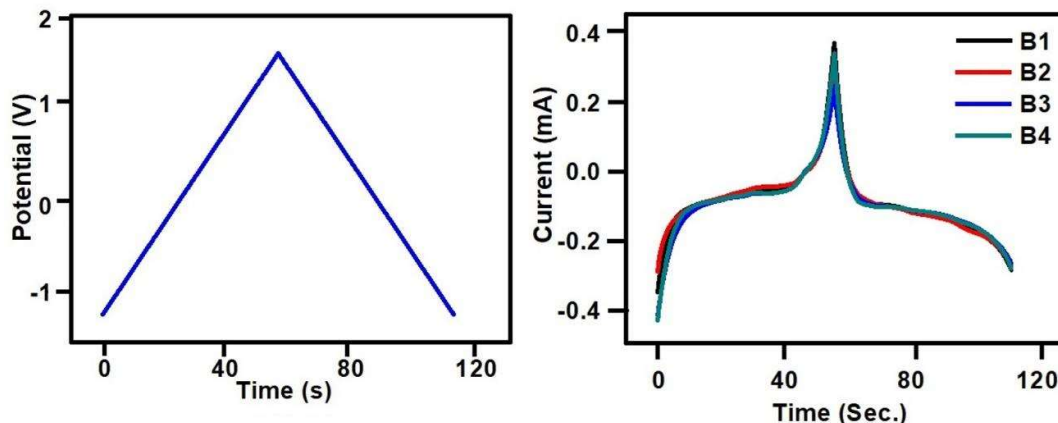
- Cyclic Voltammetry (CV):
  - Potential range:  $-1.0$  to  $+1.5$  V
  - Scan rate:  $0.05 \text{ V s}^{-1}$
  - Step potential:  $0.005$  V
- Differential Pulse Voltammetry (DPV):
  - Potential window:  $0.5$  to  $2.0$  V
  - Scan rate:  $0.05 \text{ V s}^{-1}$
  - Step potential:  $0.005$  V
  - Pulse amplitude:  $0.05$  V
  - Pulse width:  $0.05$  s

(b) Thymoquinone – TQIP/G Sensor (described details in chapter 3)

- Cyclic Voltammetry (CV):
  - Potential range:  $-0.6$  to  $1.2$  V
  - Scan rate:  $0.05 \text{ V s}^{-1}$
  - Step potential:  $0.005$  V
- Differential Pulse Voltammetry (DPV):
  - Potential window:  $0.5$  to  $2.0$  V
  - Scan rate:  $0.05 \text{ V s}^{-1}$
  - Step potential:  $0.005$  V
  - Pulse amplitude:  $0.05$  V
  - Pulse width:  $0.05$  s

### *Step 6: Transient current measurement*

Transient responses were recorded to estimate the response time of the sensors under optimized conditions. The updated transient responses of the piperine in real samples were included in Fig. 2.2.



*Fig. 2.2. Potential-excitation signal and corresponding resulting current of the piperine in real sample.*

### 2.2.7. Data augmentation and dimension reduction

The experimentation with four branded pepper powder was conducted by taking the response of the proposed electrode. The brands have been denoted as *B1*, *B2*, *B3* and *B4* for convenience. Each observation had a feature length of 1401, but there were only 10 observations per sample. The imbalance between the number of observations and the feature length of each observation necessitated data augmentation. A dynamic time warping [41] method was adopted for the augmentation however among different options for augmentation random warping and introduction of noise were used to maintain the characteristic of augmented observations. This resulted in augmented set of dimension  $7200 \times 1401$  while each samples had 1800 observations for experimentation.

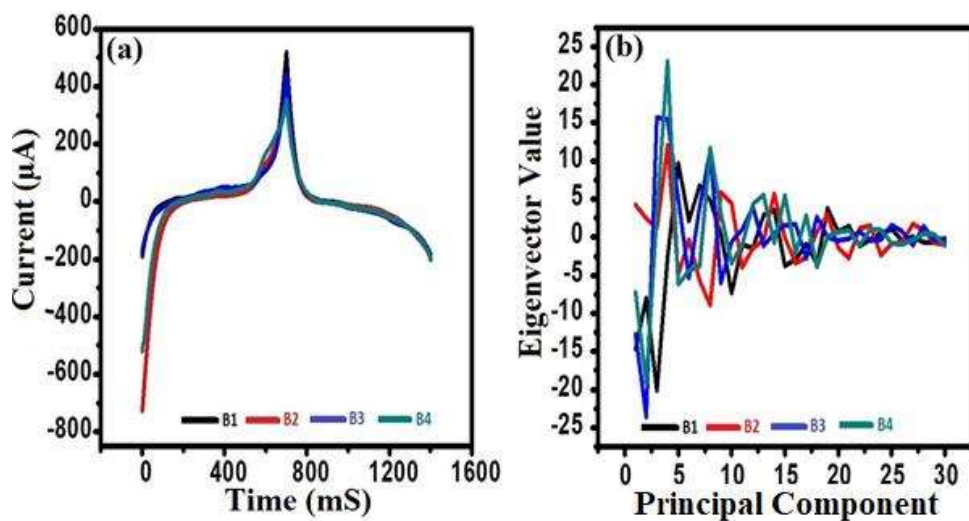
Fig. 2.3.(a) shows the plot of the raw features. It reveals the non-linearity and lower degree of separability present in the recorded observations, particularly between *B2* and *B3*. The plot also revealed that all the 1401 features were not equally important hence a principle component analysis (PCA) [42] based dimensionality reduction was applied. It was found 30 principle components (PCs) can be considered as optimal since those could cover more than 99% variance of the raw feature set.

Fig. 2.3.(b) reveals that apart from dimension reduction PCA also resulted improved separability as can be seen prominent separation between *B2* and *B3* which was absent earlier with the raw features. Consequently, both the PCA-transformed feature set, now with

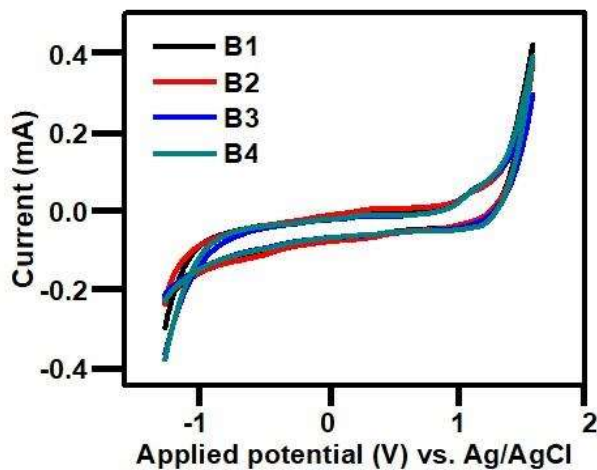


## Chapter 2: Detection of piperine content in black pepper

dimensions of  $7200 \times 30$ , and the original raw signal were used as inputs to the CNN for prediction. Cyclic voltammetric (CV) responses of piperine (PIP) extracted from four different black pepper brands (B1, B2, B3, and B4) recorded using the PDMAM-MIP@G sensor are shown in Fig. 2.4.



*Fig. 2.3. Plot of (a) raw features and (b) PCA transformed features for different brands under experimentation.*



*Fig.2.4. Cyclic voltammetric (CV) responses of piperine (PIP) extracted from four different black pepper brands (B1, B2, B3, and B4) recorded using the PDMAM-MIP@G sensor in 0.1 M PBS (pH 6.0) at a scan rate of  $50 \text{ mV s}^{-1}$  with an Ag/AgCl reference electrode.*

### 2.2.8. *A machine learning based prediction approach*

We explored the potential of machine learning techniques to predict piperine concentration from CV signals using the PADMAN sensor. CNNs were chosen for this task due to their ability to automatically learn hierarchical feature representations from the input data, which is crucial for capturing the complex patterns in the CV signals and PCA features. This capability allows CNNs to effectively identify and extract relevant features for accurate prediction of piperine concentration. For this, we employed four different CNN models. The schematic structures of the models along with the output dimension of each layer are shown in Fig. 2.5.

The structures of the four models are different in respect to input to the model, layer arrangement, number of filters in the convolution layers, kernel size, strides, dropout rate and number of neurons in the dense layers [43]. In the first two models, Model-1 and Model-2, we fed the CV signals as input with shape (none, 1401, 1), whereas PCA features of CV signals have fed as input in the Model-3 and Model-4, respectively. So, model 3 and 4 have input shape (none, 30, 1). Each model consists of a number of convolution layers followed by batch normalization and pooling layers to capture data patterns and extract complex features. Model-1 has three convolution layers with twice the number of filters in the successive layers than its previous layer. This structure increases the number of feature map and hence the dimensionality. The increasing dimensionality is reduced by max pooling operation after each convolution operation aiming to extract a minimal number of features. After the convolution and pooling layers, a flattening layer is used followed by a dropout layer to prevent overfitting. At last, three dense layers are responsible for performing the regression to extract the desired piperine concentration. Model- 2 has a similar structure as that of Model-1, except the number of filters in each convolution layer is kept same. Model-3 has four convolution layers with increasing number of filters in successive layers but has no max-pooling layer. Since the input dimension to this model is very less, we haven't used here any max-pooling operation. The model-4 has four convolution layers with half the number of filters in the successive layers with reducing kernel size. In this model, there are max-pooling layers after each convolution operation with size and stride either 2 or 1.

The model's performance is assessed using three key metrics: mean squared error (MSE) and mean absolute error (MAE) and coefficient of determination or  $R^2$  ( $R$ -squared). The MSE serves as the loss function during the training of the networks, whereas the MAE is employed to evaluate the prediction accuracy of the network.  $R^2$  is a statistical measure of goodness of fit showing how well the regression line predicts the target values. If we denote the expected

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(true) value of the piperine for the  $i^{\text{th}}$  sample as  $y$  and the predicted value by the CNN model as  $\hat{y}$ , then MSE, MAE and  $R^2$  are defined as follows:

$$\text{MSE} = \frac{1}{N} \sum_{i=1}^N (y_i - \hat{y}_i)^2 \quad (2.1)$$

$$\text{MAE} = \frac{1}{N} \sum_{i=1}^N |y_i - \hat{y}_i| \quad (2.2)$$

$$R^2 = 1 - \frac{\sum_{i=1}^N (y_i - \hat{y}_i)^2}{\sum_{i=1}^N (y_i - \bar{y})^2} \quad (2.3)$$

where,  $N$  is the number of samples under test and  $\bar{y}$  is the average value of the targets.

When optimizing the hyper parameters for the four CNN models developed for prediction task, a balanced approach was taken, guided by the performance on a validation dataset, comprising 20% of training data only, to achieve the best MAE. The number of filters was either progressively increased or decreased in deeper layers, starting from the initial layers. The kernel size was kept small, typically  $3 \times 3$  or  $5 \times 5$ , across models to effectively capture fine-grained patterns, though slight adjustments were made based on individual model performance. For pooling layers, a max-pooling with size 1 to 3 and stride 1 to 2 was selected based on performance to reduce dimensionality while retaining essential spatial information. The rectifier linear unit (ReLU) activation function was chosen for its efficiency in handling non-linearity. To avoid overfitting and under fitting problems, batch normalization and dropout regularization layers were used [44]. The dropout rate was selected from a set of  $\{0.1, 0.2, 0.3, 0.4, 0.5\}$ , with the optimal rate determined based on minimizing overfitting while maintaining low MAE of the model. Batch sizes were adjusted typically from a set of  $\{32, 64, 128\}$  based on balancing the convergence speed and computational efficiency. The RMS prop optimizer was chosen for its effectiveness in achieving smoother convergence compared to the Adam optimizer, as observed through validation performance [45]. The models were trained for up to 200 epochs. Additionally, a learning rate scheduler was implemented to adapt the learning rate during training. Early stopping was employed with a patience of 30 epochs, meaning training would halt if the validation loss did not improve for 30 consecutive epochs. This methodical approach to hyper parameter tuning, focusing on

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minimizing the average MAE across the validation dataset, ensured that each model achieved optimal performance on the regression task.

| Model-1                                                            | Model-2                                                           | Model-3                                                           | Model-4                                                             |
|--------------------------------------------------------------------|-------------------------------------------------------------------|-------------------------------------------------------------------|---------------------------------------------------------------------|
| Input<br>CV Signal<br>(None, 1401, 1)                              | Input<br>CV Signal<br>(None, 1401, 1)                             | Input<br>PCA of CV Signal<br>(None, 30, 1)                        | Input<br>PCA of CV Signal<br>(None, 30, 1)                          |
| Conv1D_1 + ReLU + BN<br>(F=32, K=3, S=1, P=1)<br>(None, 1401, 32)  | Conv1D_1 + ReLU + BN<br>(F=32, K=3, S=1, P=1)<br>(None, 1401, 32) | Conv1D_1 + ReLU + BN<br>(F=16, K=3, S=1, P=1)<br>(None, 30, 16)   | Conv1D_1 + ReLU + BN<br>(F=1024, K=5, S=1, P=1)<br>(None, 26, 1024) |
| Max Pooling1D_1<br>(Size=3, S=2)<br>(None, 701, 32)                | Max Pooling1D_1<br>(Size=3, S=2)<br>(None, 701, 32)               | Conv1D_2 + ReLU + BN<br>(F=32, K=3, S=1, P=1)<br>(None, 30, 32)   | Max Pooling1D_1<br>(Size=2, S=2)<br>(None, 13, 1024)                |
| Conv1D_2 + ReLU + BN<br>(F=64, K=3, S=1, P=1)<br>(None, 701, 64)   | Conv1D_2 + ReLU + BN<br>(F=32, K=5, S=1, P=1)<br>(None, 701, 32)  | Conv1D_3 + ReLU + BN<br>(F=64, K=3, S=1, P=1)<br>(None, 30, 64)   | Conv1D_2 + ReLU + BN<br>(F=512, K=3, S=1, P=1)<br>(None, 11, 512)   |
| Max Pooling1D_2<br>(Size=3, S=2)<br>(None, 351, 64)                | Max Pooling1D_2<br>(Size=3, S=2)<br>(None, 351, 32)               | Conv1D_4 + ReLU + BN<br>(F=128, K=3, S=1, P=1)<br>(None, 30, 128) | Max Pooling1D_2<br>(Size=2, S=2)<br>(None, 5, 512)                  |
| Conv1D_3 + ReLU + BN<br>(F=128, K=3, S=1, P=1)<br>(None, 351, 128) | Conv1D_3 + ReLU + BN<br>(F=32, K=5, S=1, P=1)<br>(None, 351, 32)  | Flatten_1<br>(None, 3840)                                         | Conv1D_3 + ReLU + BN<br>(F=256, K=2, S=1, P=1)<br>(None, 4, 256)    |
| Max Pooling1D_3<br>(Size=3, S=2)<br>(None, 176, 128)               | Max Pooling1D_3<br>(Size=3, S=2)<br>(None, 176, 32)               | Dropout_1 (0.1)<br>(None, 3840)                                   | Max Pooling1D_3<br>(Size=1, S=1)<br>(None, 4, 256)                  |
| Flatten_1<br>(None, 22528)                                         | Flatten_1<br>(None, 5632)                                         | Dense_1 + ReLU<br>(None, 256)                                     | Conv1D_4 + ReLU<br>(F=64, K=2, S=1, P=1)<br>(None, 3, 64)           |
| Dropout_1 (0.2)<br>(None, 22528)                                   | Dropout_1 (0.2)<br>(None, 5632)                                   | Dense_2 + ReLU<br>(None, 64)                                      | Max Pooling1D_4<br>(Size=1, S=1)<br>(None, 3, 64)                   |
| Dense_1 + ReLU<br>(None, 512)                                      | Dense_1 + ReLU<br>(None, 256)                                     | Dense_3 + Linear<br>(None, 1)                                     | Flatten_1<br>(None, 192)                                            |
| Dense_2 + ReLU<br>(None, 64)                                       | Dense_2 + ReLU<br>(None, 32)                                      |                                                                   | Dropout_1 (0.1)<br>(None, 192)                                      |
| Dense_3 + Linear<br>(None, 1)                                      | Dense_3<br>(None, 1)                                              |                                                                   | Dense_1 + ReLU<br>(None, 32)                                        |
|                                                                    |                                                                   |                                                                   | Dense_2 + Linear<br>(None, 1)                                       |

*Fig. 2.5. Schematic presentation of the structures of four CNN models used to predict piperine.*

### 2.2.9. Software

Electrochemical measurement and graphical analysis of piperine response were carried out through a computer-controlled Autolab PGSTAT101 electrochemical workstation with NOVA 1.11 (MetrohmAutolab, Netherlands) software. The implementation of CNN was done in python environments using Spyder IDE with tensorflow backend and keras libraries in Windows platform. The Origin Pro 8.0 (Origin Lab Corporation, USA) was used for the CV and DPV plots.

### 2.3. Results with PDMAM@G sensor using machine learning approach

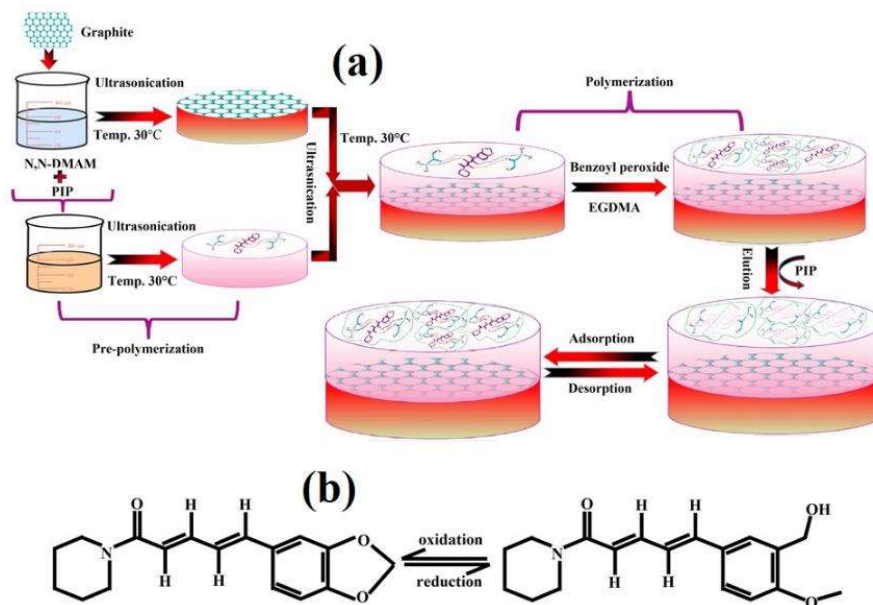
#### 2.3.1. Recognition of PIP using PDMAM-MIP@G/GE

The fabrication of the PDMAM-MIP@G electrode has been presented in Fig. 2.6(a). Due to its weak nature piperine decomposes to a volatile basic piperine, called piperidine ( $C_5H_{11}N$ ) and piperic acid ( $C_{12}H_{10}O_4$ ) on hydrolysis (acid/alkali) [46] as shown in Fig. 2.6(b). Hence, PIP has a  $-OH$  group in solution whereas PDMAM has a *carboxylic* group. During pre-polymerization, non-covalent bonding between PDMAM, and piperine was formed. When polymerized with EGDMA, piperine-trapped EGDMA cross-linked PDMAM material was synthesized in which graphite was used as a conductive and charge-collecting substrate. After elution, when the synthesized PDMAM-MIP@G/GE electrode, was interacted with piperine, the analyte (piperine) was likely to be adsorbed in the pre-defined imprinted cavity form during elution.

PDMAM was selected as the functional polymer due to its strong hydrogen-bonding capability, chemical stability, and excellent film-forming properties, which are highly suitable for molecular imprinting. Piperine contains amide, aromatic, and heterocyclic groups that can interact effectively with PDMAM through hydrogen bonding and dipole–dipole interactions during the pre-polymerization stage, leading to the formation of stable template–monomer complexes. These interactions are essential for generating well-defined and selective imprinted cavities after template removal.

Ethylene glycol dimethacrylate (EGDMA) was chosen as the cross-linker because of its rigid bifunctional structure and high cross-linking efficiency, enabling the formation of a stable three-dimensional polymer network. This rigidity helps preserve the shape and orientation of the piperine-specific recognition sites after elution, thereby improving selectivity and stability.

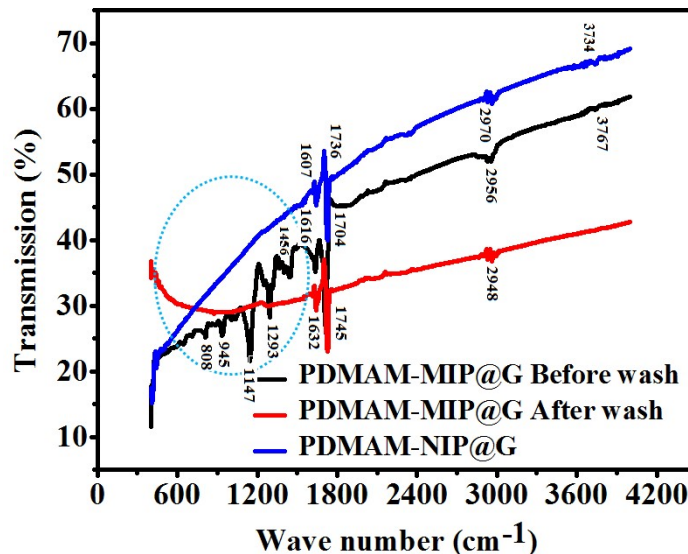
Furthermore, graphite provides high electrical conductivity, large surface area, and efficient charge-collection capability, ensuring effective electron transfer. Thus, the combined use of PDMAM as the functional monomer, EGDMA as the cross-linker, and graphite as the conductive substrate results in a robust electrochemical MIP sensor exhibiting high sensitivity, selectivity, reproducibility, and long-term operational stability.



**Fig. 2.6. (a) Schematic of synthesis of PDMAM-MIP@G sensor and (b) hydrolysis of piperine in acidic/basic solution.**

### 2.3.2. FT-IR spectroscopy of MIP and NIP

FTIR spectroscopy is used to detect the functional groups present in the synthesized polymer and to validate the formation of the polymer, imprinted polymer, and non-imprinted polymer structure as shown in Fig. 2.7. [47]. Small peaks at  $3734\text{--}3767\text{ cm}^{-1}$  recognized to the vibration mode of *O-H* stretching were detected in both MIP and NIP. The vibration mode of *C-H* stretching of the aliphatic compound of MIP was observed in  $2948\text{--}2970\text{ cm}^{-1}$ . The bands at  $1704\text{--}1736\text{ cm}^{-1}$  attributed to the existence of a carbonyl group in the polymer structure. The absorption peaks in the region of  $1590\text{--}1620\text{ cm}^{-1}$  were ascribed to stretching flexural vibration of the *N-H* group of the N,N-DMAM. The FTIR absorption peaks at  $808\text{--}1147\text{ cm}^{-1}$  represented the typical signature of piperine which was absent in NIP and eluted MIP.

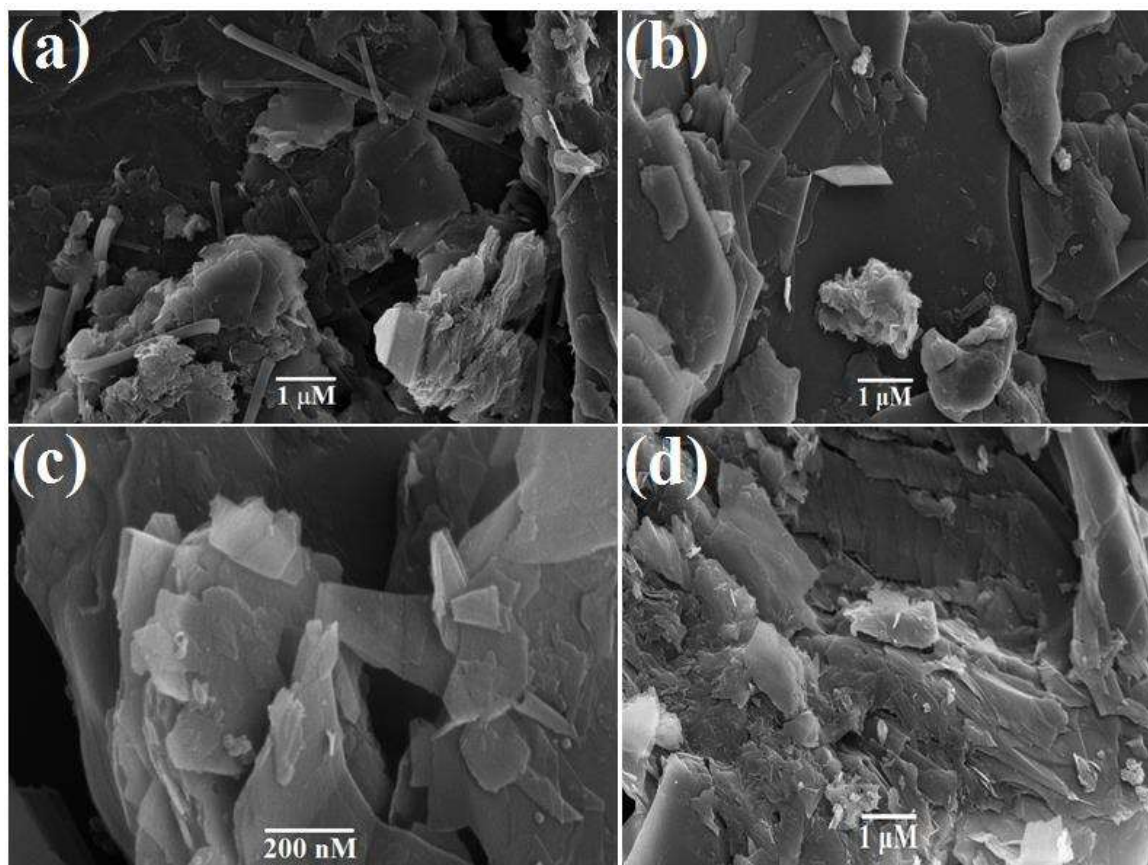


**Fig. 2.7.** FTIR spectrum of PDMAM-MIP@G before elution (black), PDMAM-MIP@G after elution (red), and PDMAM-NIP@G (blue) in the range of 4000-400  $\text{cm}^{-1}$ .

### 2.3.3. Scanning electron microscopy (SEM)

The morphological characterization of the developed sensor materials was studied by using FESEM with an acceleration voltage of 10KV. Fig. 2.8. shows that the polymer layers were uniformly synthesized and immobilized on the surface of the graphite base in PDMAM-MIP@G before washing, after washing with a magnified version, and PDMAM-NIP@G. The micro-size rods representing piperine molecules as shown in Fig. 2.8.(a) were almost absent in Fig. 2.8.(b) which confirms excellent elution of analytes from polymer structure creating cavities for piperine to adsorb during the detection process. On the other hand, as shown in Fig. 2.8.(d) for PDMAM-NIP@G a sheet-like assembly (representing graphite) with a uniform polymer structure on the graphite layer is visible. Fig. 2.8.(c) represents the magnified image of eluted PDMAM-MIP@G with cavities as receptor sites for piperine to adsorb.





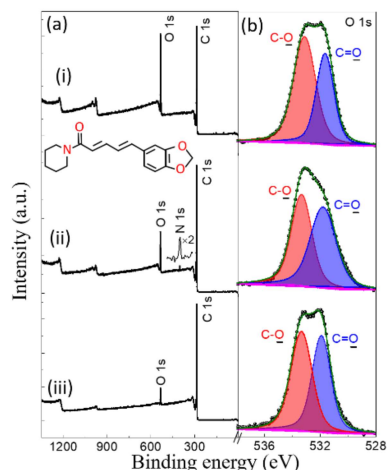
**Fig. 2.8.** FESEM images of (a) PDMAM-MIP@G before elution, (b-c) PDMAM-MIP@G after elution and its magnified section, (d) PDMAM-NIP@G.

#### 2.3.4. X-ray photoelectron spectroscopy (XPS) analysis

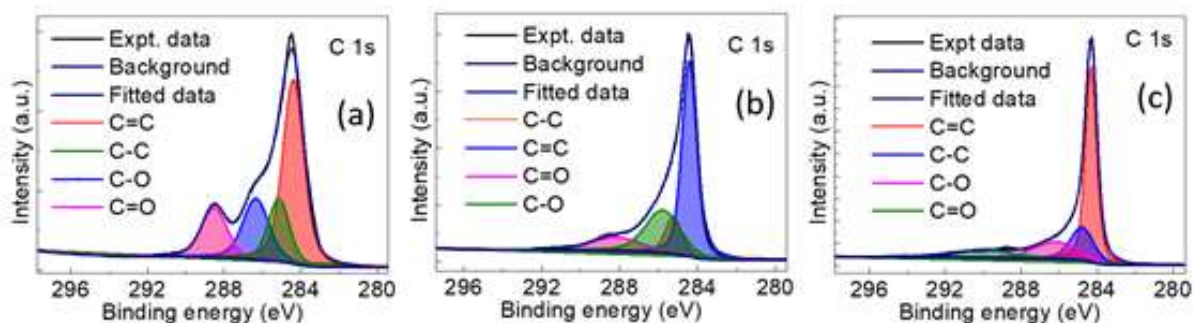
The XPS analysis of NIP, MIP of piperine before wash, and MIP of piperine after wash provides detailed insights into the elemental composition and chemical states of these samples. In the survey XPS scan of NIP, primarily carbon (C) and oxygen (O) atoms are detected, indicating that the polymer matrix consists of these elements (Fig. 2.9.a (i)). In particular, corresponding high resolution spectrum, such as O 1s core level spectrum shows peaks at 531.6 and 533.1 eV which is attributed to C=O and C-O entities (Fig. 2.9.b (i)). Likewise, C 1s spectrum reveals peaks at binding energies of 284.4 eV corresponding to C=C, 285.1 eV for C-C, 286.4 eV for C-O, and 288.5 eV for C=O species (Fig. 2.10.a). In contrast, the survey spectrum of MIP of piperine (PIP) before wash shows the presence of carbon (C), oxygen (O), and nitrogen (N) atoms (Fig. 2.9.a (ii)). Noteworthy, the signature of nitrogen is clear indication that PIP of piperine is present. The corresponding O 1s spectrum



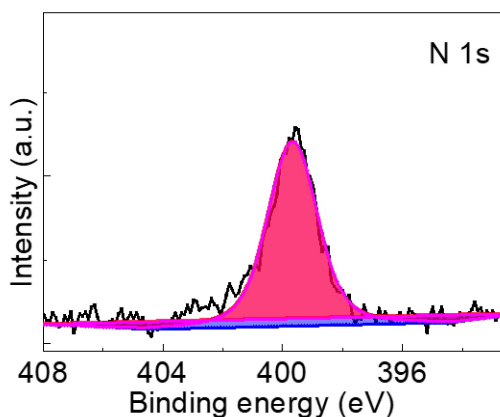
also mirrors that of NIP, with characteristic peaks for C-O and C=O species. The broadening of full width half maximum (FWHM) of O 1s spectral lines is attributed to incorporation of piperine into the NIP (Fig. 2.9.b (ii)). The high-resolution C 1s spectrum exhibits similar peaks to those observed in NIP, indicating the presence of C-C, C=C, C-O, and C=O species, where additional carbon contribution due to PIP is also revealed (Fig. 2.10.b). In addition, the N 1s spectrum in MIP shows a distinct gaussian peak at 399.6 eV, distinctly affirming the incorporation of nitrogen-containing groups, likely from the piperine template molecule (Fig. 2.11). For MIP of piperine, after the piperine template has been washed out, the survey spectrum again shows only carbon (C) and oxygen (O) atoms, similar to NIP (Fig. 2.9.a (iii)). The O 1s spectrum shows peaks at 531.9 eV and 533.4 eV, corresponding to C=O and C-O species, respectively (Fig. 2.9.b (iii)). The high-resolution C 1s spectrum also presents the similar peaks as seen in NIP and MIP of piperine (before wash), corresponding to C-C, C=C, C-O, and C=O species (Fig. 2.10.c). Noteworthy, the absence of the N 1s peak in MIP of piperine after wash suggests that the piperine template has been fruitfully removed, leaving behind a polymer matrix similar in composition to NIP.



**Fig. 2.9. The XPS (a) survey scan and b) high resolution O 1s spectra of (i)NIP, (ii) MIP before wash, and (iii) MIP after wash.**



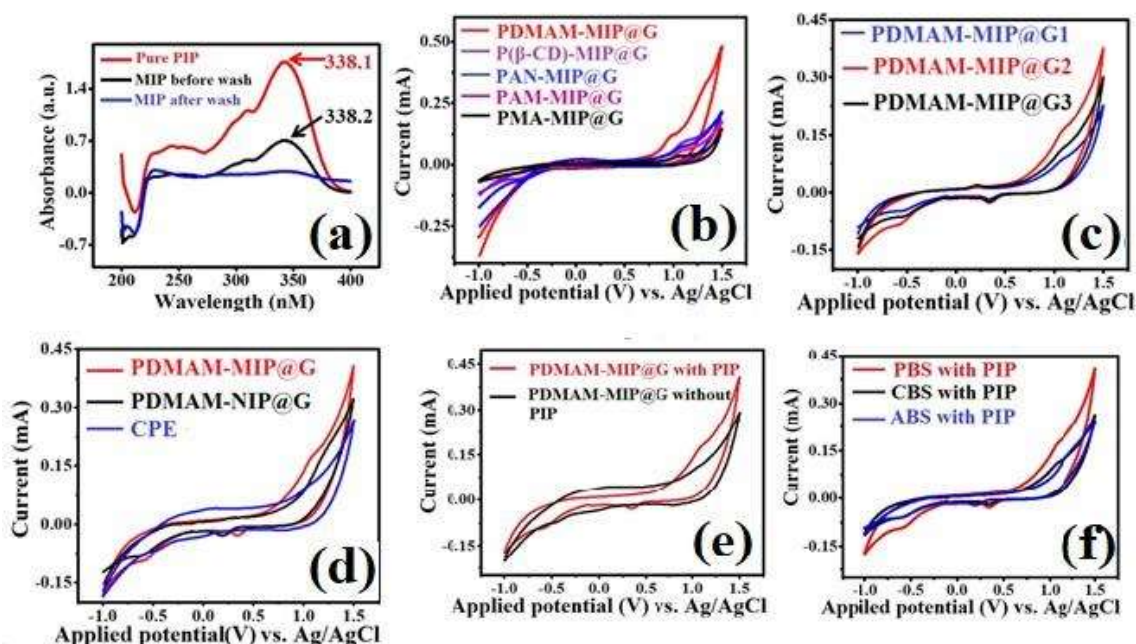
**Fig. 2.10.** High resolution C 1s XPS spectra of (a) NIP, (b) MIP before wash, and (c) MIP after wash.



**Fig. 2.11.** Core level N 1s XPS spectra of MIP before wash.

### 2.3.5. UV-Vis. spectroscopy measurement

UV-Vis spectroscopy technique was applied to explore template-monomer interaction in synthesized polymer materials as shown in Fig. 2.12(a). The absorbance peak of pure piperine molecule was at 338.1 nm which was indicated by red color in Fig. 2.12(a) and that of piperine imprinted (before wash) was at 338.2 nm (Black color in Fig. 2.12(a)) that confirms the successful imprinting of piperine in the PDMAM-MIP@G sensor. Moreover, after elution of piperine with ethanol, the developed material did not produce any peak in the UV spectrum.



**Fig. 2.12.** (a) UV-vis. Spectroscopy of pure PIP, and MIP before and after wash and CV of 100 $\mu$ M of piperine in 0.1M PBS (pH 6.0) with a scan rate of 0.05Vs<sup>-1</sup> (b) at PDMAM-MIP@G, P( $\beta$ -CD)-MIP@G, PAN-MIP@G, PAM-MIP@G, PMA-MIP@G. (c) at PDMAM-MIP@G1 (red), PDMAM-MIP@G2 (black), and PDMAM-MIP@G3 (blue) sensor and (d) at PDMAM-MIP@G, PDMAM-NIP@G, and CPE. CV response of PDMAM-MIP@G sensor (e) in 0.1M PBS (pH 6.0) containing 100 $\mu$ M of piperine (red) and without piperine (black). (f) CV response of PDMAM-MIP@G sensor in 100 $\mu$ M of piperine with different buffer solution.

### 2.3.6. Electrochemical measurements and optimization of experimental conditions

Electrochemical measurements and optimization of Three-electrode electrochemical measurements of developed MIP and NIP electrodes were carried out to measure piperine for electro-catalytic investigation and quantitation, respectively. The optimized scanning potential range for CV and DPV was -1.0-1.5V and 0.5-2.0V, respectively.

### 2.3.7. Optimization of sensing material

The developed PDMAM-MIP@G sensors were compared with PDMAM-NIP@G in 0.1M phosphate buffer saline (PBS-6) containing 100 $\mu$ M concentration of piperine. Fig. 2.12.(c).

shows that PDMAM-MIP@G2 (1:4:12) material showed the highest redox peak current of 0.145mA which is 1.55 and 1.43 times higher than PDMAM-MIP@G1 and PDMAM-MIP@G3, respectively. This is mainly due to the favorable stoichiometric ratio of template, functional monomer, and cross-linker yielding excellent uniform binding sites and thus increasing selectivity of the developed sensor to piperine. Again, the electrochemical response of PDMAM-MIP@G2 was compared with that of PDMAM-NIP@G and bare carbon paste electrode (CPE) as represented in Fig. 2.12.(d). The peak current of the PDMAM-MIP@G2 sensor was 2.42 times greater than PDMAM-NIP@G due to the absence of template molecules in NIP during polymerization resulting in a compact polymer matrix and hence blocking the movement of electrons and analytes. But, CPE showed almost no redox peak current. Hence, the PDMAM-MIP@G2 sensor has served as the primary sensor for all experiments and is subsequently referred to as PDMAM-MIP@G in the following sections. Moreover, different monomers ( *$\beta$ -cyclodextrin, acrylonitrile, acrylamide, and methacrylic acid*) were studied for the synthesis of MIPs. PDMAM-MIP@G sensor yielded 1.04, 1.43, 1.86, 3.29 times better peak current than P( $\beta$ -CD)-MIP@G, PAN-MIP@G, PAM-MIP@G, and PMA-MIP@G, respectively as shown in Fig. 2.12.(b).

### 2.3.8. Effect of buffer

For selecting the proper buffer, CV investigations of PDMAM-MIP@G electrodes with different buffer solutions (*pH* value of 6.0) were conducted to analyse the effect of buffer solution to detect piperine. The developed electrode yielded maximum response in PBS buffer saline with a peak current of 0.18mA at a peak potential of 1.12V as compared to citrate buffer solution (CBS) and acetate buffer solution (ABS) as shown in Fig. 2.12.(f). In the absence of PIP molecules, no peak current was detected as can be seen in Fig. 2.12.(e).

PBS with a fixed ionic strength of 0.1 M was deliberately chosen and maintained throughout all electrochemical experiments to ensure consistency and reproducibility. At this ionic strength, sufficient electrolyte ions are available to effectively screen the surface charge of the electrode, resulting in a compressed electrical double layer. This compression minimizes excessive potential drop across the diffuse layer and promotes efficient electron transfer between the electrode surface and the redox-active analyte molecules.

An increase in ionic strength leads to a decrease in double-layer thickness, which enhances interfacial capacitance and may increase background (non-Faradaic) charging currents. Conversely, lower ionic strength results in a more extended diffuse layer, reduced

capacitance, and weaker charge screening, which can suppress Faradaic current due to slower charge-transfer kinetics. In the present work, maintaining a moderate ionic strength (0.1 M PBS) provided an optimal balance by stabilizing the baseline current while preserving clear and well-defined Faradaic peak. Hence, PBS was preferred as the supporting electrolyte to study for further investigation.

### 2.3.9. Effect of pH

The influence of the *pH* value of the buffered saline on the electro-oxidation peak potential and its corresponding anodic peak current of piperine was investigated in PBS saline with different *pH* values ranging from 2.0 to 8.0 using PDMAM-MIP@G electrode as shown in Fig. 2.13.(a). It was found that with the increased *pH* value of the buffer, anodic peak potentials were negatively shifted, but anodic peak currents were enlarged. On the other hand, when the *pH* value was beyond 6.0, the peak currents were conversely decreased. From this study, it was observed that the adsorption of target molecules (binding efficiency) in acidic and basic conditions was low as compared to the neutral medium. This phenomenon was due to the dissociation of the phenolic moiety in piperine in the oxidation process. At higher *pH* values, *phenol* dissociates to produce the corresponding anion that can be easily oxidized. PB6 buffer saline was chosen for the subsequent analytical experiment. The  $E_{pH}$  vs *pH* plot was linear and the corresponding regression equation was  $E_{pH} = -0.052pH + 1.33$  ( $R^2 = 0.99$ ) with a slope value of  $-0.52 \text{ VpH}^{-1}$  as shown in Fig. 2.13.(b). According to the Nernstain equation, the hypothetical value of this slope is  $0.059 \text{ VpH}^{-1}$  at  $25^\circ\text{C}$  which is very close to the experimental value ( $0.0556 \text{ VpH}^{-1}$ ). This result proved that an equal number of protons and electrons were involved in the PIP redox reaction.

### 2.3.10. Effect of scan rate

The scan rate was also influenced by the nature and kinetics of the electro-oxidation of piperine at the developed electrode. The scan rate effect on peak current and peak potential of piperine in the developed electrode was studied by using the CV procedure over the ranges of  $0.01\text{--}0.6 \text{ Vs}^{-1}$  as shown in Fig. 2.13.(c). piperine shows two irreversible redox peaks (separation of 700 mV) in the voltammogram with a linear increase of redox peak current with scan rates with corresponding linear regression equation of

$I_{OXD(mA)} = 0.63v + 0.0798$  ( $R^2 = 0.996$ ) and  $I_{RED(mA)} = -0.0957v - 0.00084$  ( $R^2 = 0.999$ ) as shown in Fig. 2.13.(d)  $I_p$  vs  $v^{1/2}$  shows linearity which follows the linear regression

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equation of  $I_p(mA) = 0.546v^{1/2} + 0.0041$ , ( $R^2 = 0.953$ ) suggesting that the anodic and cathodic peaks were adsorption-controlled surface electro-oxidation processes.

Based on the above discussion, the surface adsorbs analyte ( $\Gamma_c$ ), charge transfer coefficient, and the number of electrons transferred by the PIP molecules were calculated using Laviron equations [48] as shown in Eq.(2.4)- (2.6).

$$i_p = \frac{nFQv}{4RT} = \frac{n^2F^2Av\Gamma_c}{4RT} \quad (2.4)$$

$$\log(v) = 2.3 \frac{RT}{(1-\alpha)nF} E_p + \text{constant} \quad (2.5)$$

$$\log(k_s) = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log\left(\frac{RT}{nFv}\right) - \alpha(1-\alpha)\frac{nF}{2.3RT}\Delta E_p \quad (2.6)$$

where, all the symbols have their own meaning. The calculated values of  $n$  and  $\Gamma_c$  were obtained 1.68 ( $\approx 2$ ) and  $2.391 \times 10^{-10} \text{molcm}^{-2}$ , respectively. Laviron equation was employed to calculate the charge transfer coefficient ( $\alpha$ ) which was 0.67 and the electron transfer rate constant ( $k_s$ ) which was  $0.075 \text{s}^{-1}$ . The plot of peak potential ( $E_p$ ) vs.  $\log(v)$  was linear with a corresponding linear regression equation as follows:  $E_{pA}(V) = 0.032 \log(v) + 1.33$ , ( $R^2 = 0.98$ ) and  $E_{pC}(V) = -0.11 \log(v) + 0.17$ , ( $R^2 = 0.99$ )

For a totally irreversible process, the theoretical value of the electron transfer coefficient ( $\alpha$ ) was 0.5. From the slope peak potential and the log of scan rate, the electron transfer coefficient ( $\alpha$ ) was calculated as 0.61 which confirms that the electrochemical reaction is an irreversible process. The value of  $n$  was 1.68 ( $\approx 2$ ) that was obtained from the Eq.(5) and hence two electrons were involved in the electrochemical reaction of piperine. For diffusion-controlled process slope close to 0.5 was expected whereas for adsorption-controlled processes slope was close to 1. From the  $\log(I_p)$  vs.  $\log(v)$  as shown in Fig. 2.13.(e), the value of slope was found to be 0.416 which indicates that the redox reaction on the developed sensor also involved diffusion of piperine molecules.

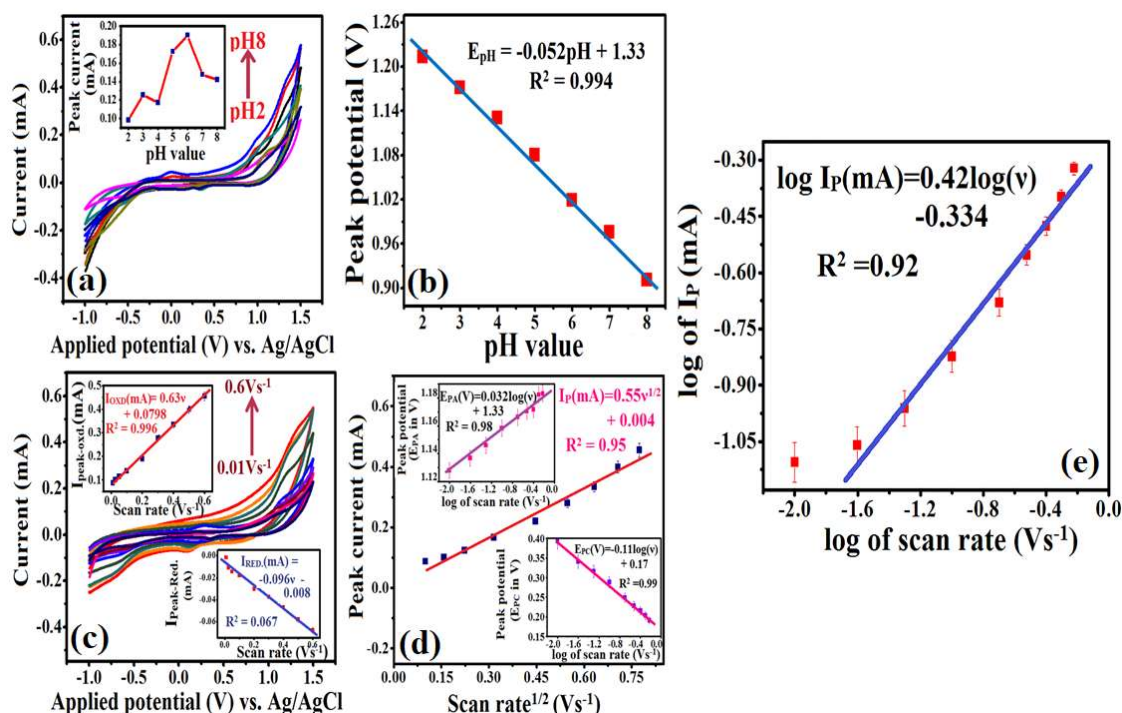
For the reversible process, the Randles-Sevcik equation [49] as shown in Eq.(2.7) was used to estimate the effective surface area of  $0.011 \text{cm}^2$  for the PDMAM-MIP@G sensor.

$$IP = (2.69 \times 10^{-5}) n^{3/2} D^{1/2} v^{1/2} AC \quad (2.7)$$

where,  $I_P$  is the anodic peak current,  $n$  is total number of electrons transferred considered as 1,  $v$  denotes scan rate taken as  $0.05 \text{Vs}^{-1}$ ,  $D$  is the diffusion coefficient with the value of  $6.5 \times 10^{-5} \text{cm}^2 \text{s}^{-1}$ ,  $A$  is the effective surface area and  $C$  is the concentration of piperine taken as  $100 \mu\text{M}$  for this work. The calculated response time was observed as 21.8 sec.

### 2.3.11. Detection of PIP using PDMAM-MIP@G electrode through DPV

DPV procedure was used to estimate the concentration of PIP molecules to obtain better sensitivity by minimizing the background non-Faradaic current due to the creation of double-layer capacitance that was formed during adsorption. Under the optimized conditions, the DPV measurements were carried out to determine piperine using the developed electrode with increasing concentration. In the absence of piperine, no peak was observed. But, when piperine molecules were added in buffer saline, a distinct peak for the oxidation appeared due to charge transfer and adsorption of analyte within the receptor sites of PDMAM-MIP@G. A concentration dependent current response was plotted as shown in Fig. 2.14.(a) that shows an escalation of the peak current of PIP with its concentration ranging from  $0.001$  to  $200 \mu\text{M}$ . A linear dynamic relationship between peak currents and concentration with two linear slopes



**Fig. 2.13.** (a) CV response of PDMAM-MIP@G2 sensor in  $100 \mu\text{M}$  of piperine with different pH values of PBS and (b) linear plot of peak potential vs. pH value. Effect of scan

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*rate on CV (c) at PDMAM-MIP@G sensor (inset: scan rate vs. oxidation peak current and scan rate vs. reduction peak current). (d) Variation of peak current with the square root of scan rate (inset: log of scan rate vs. log of peak potential (below) and log of scan rate vs. peak potential (above) and (e) log of scan rate vs. log of peak current.*

was achieved. The first linear range was from 0.001 to 1  $\mu\text{M}$  with a linear regression equation of with co-relation coefficient value ( $R^2$ ) of 0.98, and the second linear range was from 1 to 200 $\mu\text{M}$  with a linear regression equation of  $R^2$  of 0.99 as shown in Fig. 2.14.(b). Hence, a linear dynamic range with two different slopes (sensitivity of one for 12.92  $\mu\text{A}/\mu\text{M}$  and another for 0.326  $\mu\text{A}/\mu\text{M}$ ) indicated a shift in the sensitivity with piperine concentration which was due to monolayer adsorption followed by multilayer adsorption ascribed to a decrease of the accumulation efficiency. Further, the limit of detection (LOD) and limit of quantification (LOQ) was calculated as 0.6 nM and 1.98nM respectively. Table 2.2 shows a comparative study between the proposed method and all the previously published methods for the detection of piperine molecules and the detection limit of this work is among the lowest ones that have been reported so far.

**Table 2.2. Comparison of the proposed method with other existing techniques.**

| Methodology                                      | Electrode material | Linear range                                                                 | LOD                         | Res.          |
|--------------------------------------------------|--------------------|------------------------------------------------------------------------------|-----------------------------|---------------|
| Square wave voltammetric                         | GCE                | 8 $\mu\text{g}/\text{mL}$ – 48 $\mu\text{g}/\text{mL}$                       | 2.4 $\mu\text{g}/\text{mL}$ | [23]          |
| Semi-circular potential sweep voltammetry method | GC                 | 0.25 mM -5 mM                                                                | 2.2 $\mu\text{g}/\text{mL}$ | [24]          |
| CV method                                        | GCE/GMC@ piperine  | 5 $\mu\text{l}$ – 25 $\mu\text{l}$                                           | -                           | [48]          |
| DPV                                              | SPE                | 0.125 mM – 0.75 mM                                                           | -                           | [49]          |
| DPV                                              | PDMAM-MIP@G        | 1 $\mu\text{M}$ -200 $\mu\text{M}$ and 0.001 $\mu\text{M}$ - 1 $\mu\text{M}$ | 0.0006 $\mu\text{M}$        | Proposed work |



### 2.3.12. Selectivity, reproducibility, and stability test of PDMAM-MIP@G/GE electrode

The selectivity of the developed PDMAM-MIP@G sensor was investigated by electing some normal anions, cations and some common organic molecules in the presence of 50 $\mu$ M piperine. The results reflected that 100-fold concentration of Mg<sup>2+</sup>, Na<sup>+</sup>, Zn<sup>2+</sup>, Ca<sup>2+</sup>, K<sup>+</sup>, and Cu<sup>2+</sup>; and 50-fold of citric acid, sucrose, glucose, ascorbic acid, starch, glycine, and uric acid had no influence on the current response of PIP with deviation not more than  $\pm 7\%$  as consolidated in Table 2.3 which was considered as tolerance level of the developed sensor as shown in Fig. 2.14.(d).

The reproducibility and stability of the developed sensor were evaluated using DPV of 50 $\mu$ M concentration of PIP. Relative standard deviation (RSD) was 1.56% for five same electrodes prepared under similar experimental conditions in different batches as shown in Fig. 2.14.(c) (inset), and also the value was authenticated according to guidance for Industry bioanalytical method validation. Again, to check the long-term stability of the fabricated sensor, the DPV responses were taken after 7 days interval. The DPV peak current was maintained up to 96.15% of initial response after 35 days as shown in Fig. 2.14.(c).

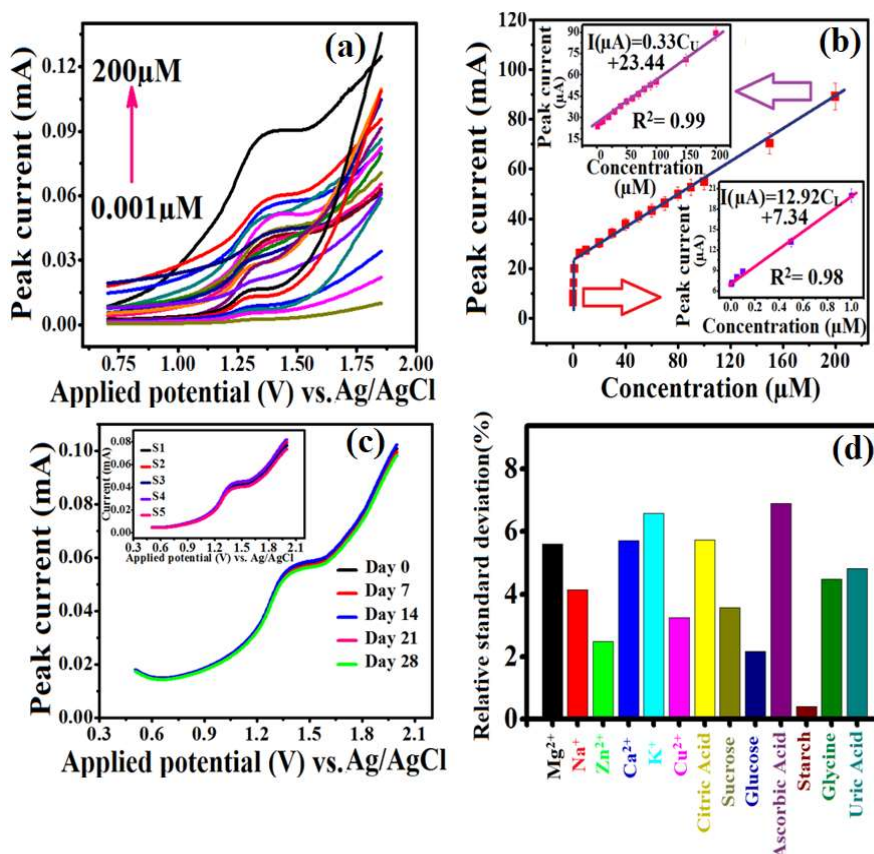
**Table 2.3. Interference analysis of different species for the determination of pip at PDMAM-MIP@G.**

| Interferent      | Interferent Concentration | Peak Current Response ( $\mu$ A) | Relative Deviation% |
|------------------|---------------------------|----------------------------------|---------------------|
| Piperine         | 5 $\mu$ M                 | 41.00                            | -                   |
| Mg <sup>2+</sup> | 1mM                       | 43.30                            | 5.60                |
| Na <sup>+</sup>  | 1mM                       | 42.47                            | 1.91                |
| Zn <sup>2+</sup> | 1mM                       | 41.41                            | 2.49                |
| Ca <sup>2+</sup> | 1mM                       | 43.77                            | 5.69                |
| Cu <sup>2+</sup> | 1mM                       | 39.56                            | 3.25                |
| Citric Acid      | 1mM                       | 41.86                            | 5.73                |
| Ascorbic         | 100 $\mu$ M               | 42.19                            | 6.89                |
| Starch           | 100 $\mu$ M               | 42.73                            | 1.27                |
| Glycine          | 100 $\mu$ M               | 40.82                            | 4.46                |
| Uric Acid        | 100 $\mu$ M               | 38.85                            | 4.82                |

### 2.3.13. Prediction performance of CNN models

#### 2.3.13.1. Experimental procedure

To train and test the CNN models, the dataset was split into training and testing sets using stratified random sampling with a 70:30 ratio. During training, 20% of the training set was further separated as a validation set. The training data was normalized to zero mean and unit standard deviation, and the testing data was normalized using the mean and standard deviation of the training set. The target values of the training set were scaled to the range  $[-1, 1]$  using the minimum and maximum values from the training set. The target values of the testing set were then scaled using the same minimum and maximum values from the training set. The experiment was repeated 50 times, with the dataset randomly permuted before each run. The prediction performance of four CNN models was evaluated using three metrics: mean absolute error (MAE), root mean squared error (RMSE), and  $R^2$  value [50]. These metrics were assessed on both the training and testing sets for each run. Finally, the mean and standard deviation of these metrics over 50 runs are calculated and compared.



*Fig. 2.14. DPV measurements of PIP in 0.1 M PBS (pH of 6.0) using PDMAM-MIP@G-2 (a) Plot of current response against potential (0.5-2.0V) with varying concentration (0.001-200 $\mu$ M). (b) Concentration vs. peak current plot [inset: plot of lower linear range (below) and higher linear range plot (above)]. (c) DPV measurement of 100 $\mu$ M PIP in 0.1 M PBS (pH of 6.0) for stability tests of PDMAM-MIP@G sensor (inset: Repeatability test of PDMAM-MIP@G sensor for five measurements of 50 $\mu$ M of piperine). (d) Study of interference.*

### 2.3.13.2. Experimental results

Table 2.4. showcases the performance of the four CNN models. The results indicate that the performance of the four CNN models in predicting piperine values exhibits only slight variations across different metrics, suggesting that each model has low bias. Notably, Model-1 and Model-2 were trained on the original CV signals, while Model-3 and Model-4 were trained on 30 PCA components of the CV signals. Despite the differences in input data, the minimal differences between training and testing performance across all models indicate that they generalize well without overfitting, maintaining accuracy when exposed to unseen data. Model-2 stands out as the best performer, with the lowest average testing MAE of  $0.268 \pm 0.014$ , testing RMSE of  $0.348 \pm 0.012$ , and the highest testing  $R^2$ -value of  $0.930 \pm 0.005$ . This suggests that Model-2 achieves the best balance between accuracy and generalization among the models trained on the original CV signals. The very low standard deviation of all metrics across 50 runs with random permutations of the data further reinforces this observation, indicating that each model is stable and consistent, performing similarly across different training sets.

Model-1, also trained on CV signals, follows closely behind Model-2, with slightly higher testing MAE and RMSE, and a marginally lower  $R^2$ -value. In contrast, Model-3 and Model-4, trained on PCA components, show slightly higher testing MAE and RMSE values, with Model-3 exhibiting the most noticeable performance degradation when moving from training to testing. However, the consistency in performance suggests that even when using reduced-dimensional input data, the models maintain robust generalization. Model-4 performs slightly better than Model-3 on testing MAE ( $0.281 \pm 0.015$ ) and testing RMSE ( $0.359 \pm 0.015$ ), but still lags behind Model-1 and Model-2.

## Chapter 2: Detection of piperine content in black pepper

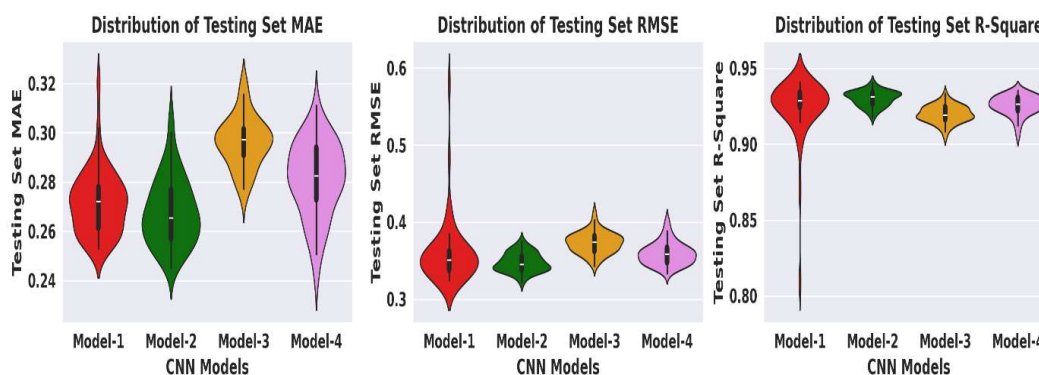
**Table 2.4. Mean values of MAE, RMSE and  $R^2$ - values for training and testing set.**

| CNN     | Training                                       | Testing                                         | Training                          | Testing                                         | Training                                        | Testing                                         |
|---------|------------------------------------------------|-------------------------------------------------|-----------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Model   | MAE $\pm$ std<br>(p-value)                     | MAE $\pm$ std                                   | RMSE $\pm$ std<br>(p-value)       | RMSE $\pm$ std<br>(p-value)                     | $R^2$ -value $\pm$ std<br>(p-value)             | $R^2$ -value $\pm$ std<br>(p-value)             |
| Model-1 | 0.242 $\pm$ 0.015<br>(0.104971)                | 0.272 $\pm$ 0.013<br>(0.220196)                 | 0.309 $\pm$ 0.015<br>(0.206640)   | 0.356 $\pm$ 0.042<br>(0.057491)                 | 0.945 $\pm$ 0.006<br>(0.211471)                 | 0.925 $\pm$ 0.020<br>(0.056459)                 |
| Model-2 | <b>0.237<math>\pm</math>0.014</b>              | <b>0.268<math>\pm</math>0.014</b>               | <b>0.306<math>\pm</math>0.011</b> | <b>0.348<math>\pm</math>0.012</b>               | <b>0.946<math>\pm</math>0.004</b>               | <b>0.930<math>\pm</math>0.005</b>               |
| Model-3 | 0.249 $\pm$ 0.012<br>(1.881 $\times 10^{-5}$ ) | 0.296 $\pm$ 0.010<br>(2.815 $\times 10^{-15}$ ) | 0.307 $\pm$ 0.009<br>(0.476654)   | 0.373 $\pm$ 0.014<br>(2.059 $\times 10^{-12}$ ) | <b>0.946<math>\pm</math>0.003</b><br>(0.465889) | 0.920 $\pm$ 0.006<br>(4.699 $\times 10^{-12}$ ) |
| Model-4 | 0.250 $\pm$ 0.013<br>(2.032 $\times 10^{-5}$ ) | 0.281 $\pm$ 0.015<br>(4.285 $\times 10^{-5}$ )  | 0.309 $\pm$ 0.008<br>(0.132309)   | 0.359 $\pm$ 0.015<br>(5.307 $\times 10^{-5}$ )  | 0.945 $\pm$ 0.003<br>(0.142475)                 | 0.926 $\pm$ 0.007<br>(0.000111)                 |

We conducted a paired t-test to assess whether the differences in the mean performance metrics of Model-2 are statistically significant at a significance level of  $\alpha = 0.05$  compared to the other three models. Since Model-2 demonstrated the best mean performance across MAE, RMSE, and R-squared metrics compared to the other models, we selected it for comparison. The results presented in Table 2.4. indicate that there is no statistically significant difference between Model-2 and Model-1 in terms of both training and testing performance, as measured by MAE, RMSE, and R-squared values. However, significant differences were observed between Model-2 and both Model-3 and Model-4 across all performance measures, except for training RMSE and training R-squared values. These findings highlight the superior performance of Model-2 over the other models, thereby supporting the selection of Model-2 for piperine prediction from CV signals.

The distribution of MAE, RMSE and R-square on testing sets over 50 random runs are shown in Fig. 2.15. for all the four models using violin plot. The violin plots show that Model-1 exhibits a longer tail in its performance metrics (MAE, RMSE, and  $R^2$ ), indicating that while most runs are consistent, there are one or two outlier runs with notably poor performance. Despite this extended range, the paired t-test results reveal no statistically significant difference between Model-1 and Model-2 in both training and testing performance metrics. This suggests that although Model-1 has a few outliers, these do not lead to a meaningful statistical difference in overall performance when compared to Model-2. In contrast, Models-3 and Model-4 demonstrate tighter distributions for MAE, RMSE and  $R^2$ , reflecting more consistent performance across runs, which aligns with the significant differences observed in the statistical tests.

Additionally, the consistently low prediction errors and high  $R^2$ -values across all models suggest that CNNs are highly effective in predicting piperine concentrations from CV signals. The fact that even models trained on reduced-dimensional input data (PCA components) perform comparably to those trained on the original signals highlights the robustness of CNNs in extracting meaningful features from complex datasets like CV signals. This strong predictive performance underscores the potential of CNN-based models in applications related to piperine estimation using the developed sensor.



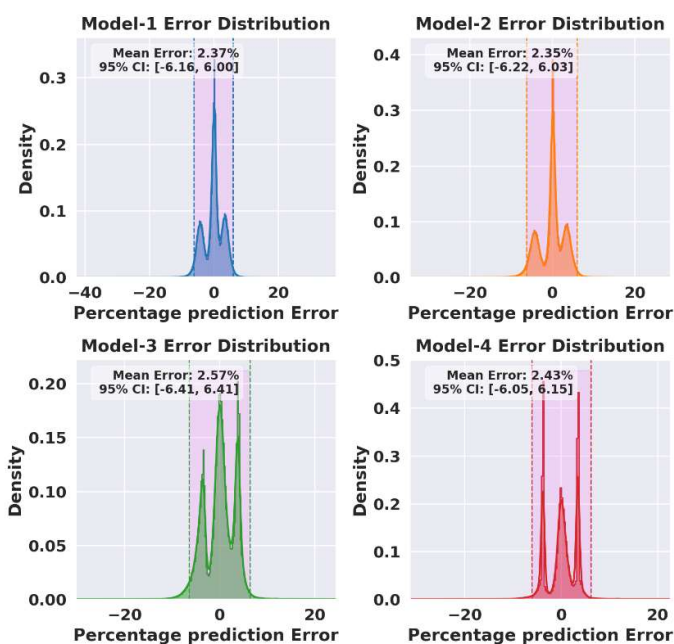
**Fig. 2.15.** *Distribution of MAE, RMSE and R-square on testing samples over 50 random runs for the four models.*

### 2.3.13.3. Analysis of percentage error

We evaluated the percentage error in predicting the piperine content of black pepper samples using four different CNN models. This evaluation was conducted on the testing dataset, randomly selected 30% of the total samples (7200), comprising 2,160 samples per experiment run. Across 50 runs, this resulted in a total of 108,000 testing samples. Fig. 2.16. shows the distribution of percentage error for piperine content predictions across these samples, including the mean absolute percentage error and the 95% confidence interval of the mean.

Model-2 demonstrated a mean absolute percentage error of 2.35%, with a 95% confidence interval of  $[-6.22\%, 6.03\%]$ , indicating a high level of accuracy and reliability in its predictions. The narrow confidence interval suggests that Model-2 consistently provides precise estimates of piperine content. The other three models also exhibited mean absolute percentage errors and 95% confidence intervals very close to that of Model-2, further highlighting the robustness of these CNN models in delivering consistent and accurate predictions. The tight confidence intervals and low percentage errors indicate that these

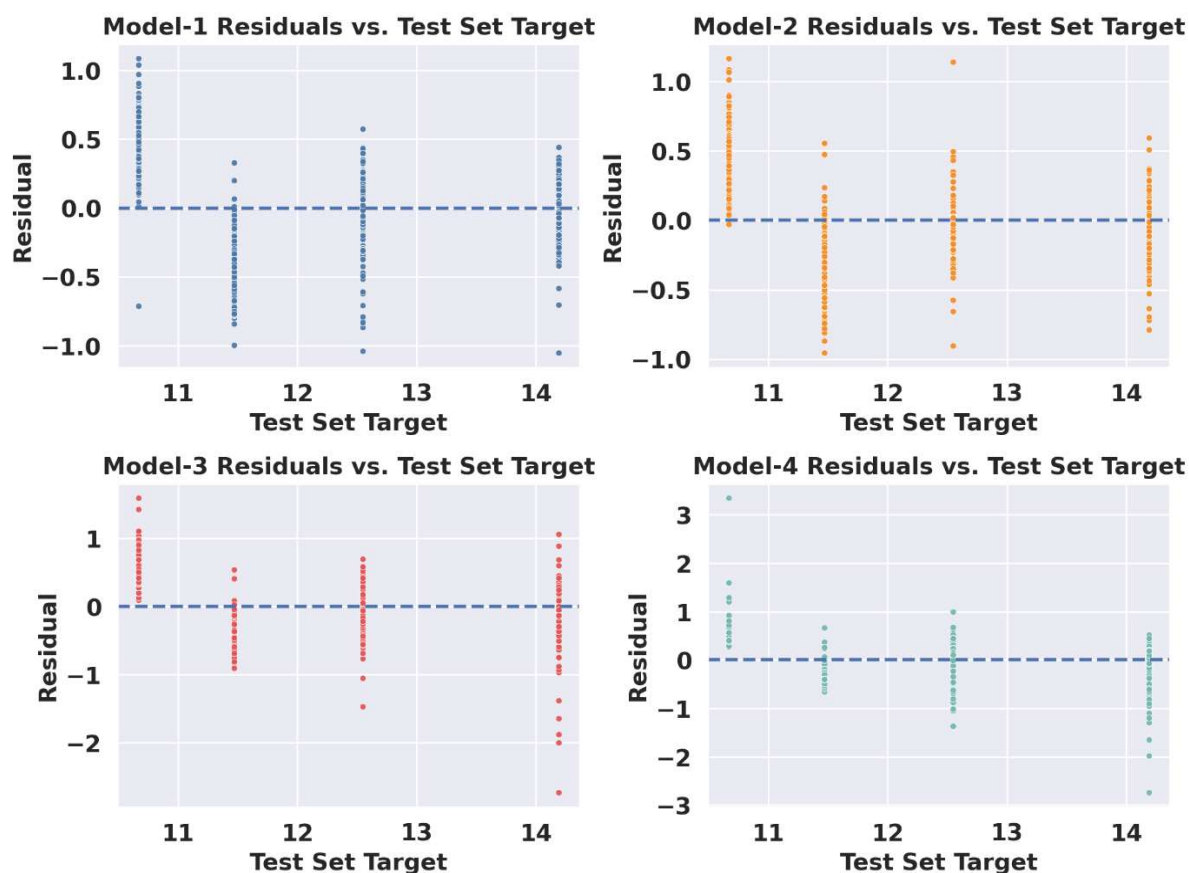
models are highly reliable for precise piperine content determination, which is crucial in applications where accuracy is essential.



*Fig. 2.16. Distribution of percentage error in determining piperine content of testing samples over 50 random runs for the four models.*

#### 2.3.13.4. Residual plot

Fig.2.17. presents the residual plots, illustrating the errors between the actual and predicted piperine concentrations in black pepper for each model. The residuals for Model-1 and Model-2 predominantly fall within the range of -1 to +1.2, indicating a relatively tight distribution around the reference line. In contrast, Model-3 and Model-4 display a higher frequency of outliers, with residuals exceeding  $\pm 1.0$ , suggesting greater prediction errors and potential model instability. These observations highlight the superior predictive performance of Model-2, as it not only maintains a more consistent error distribution but also minimizes the occurrence of significant deviations from the actual piperine content

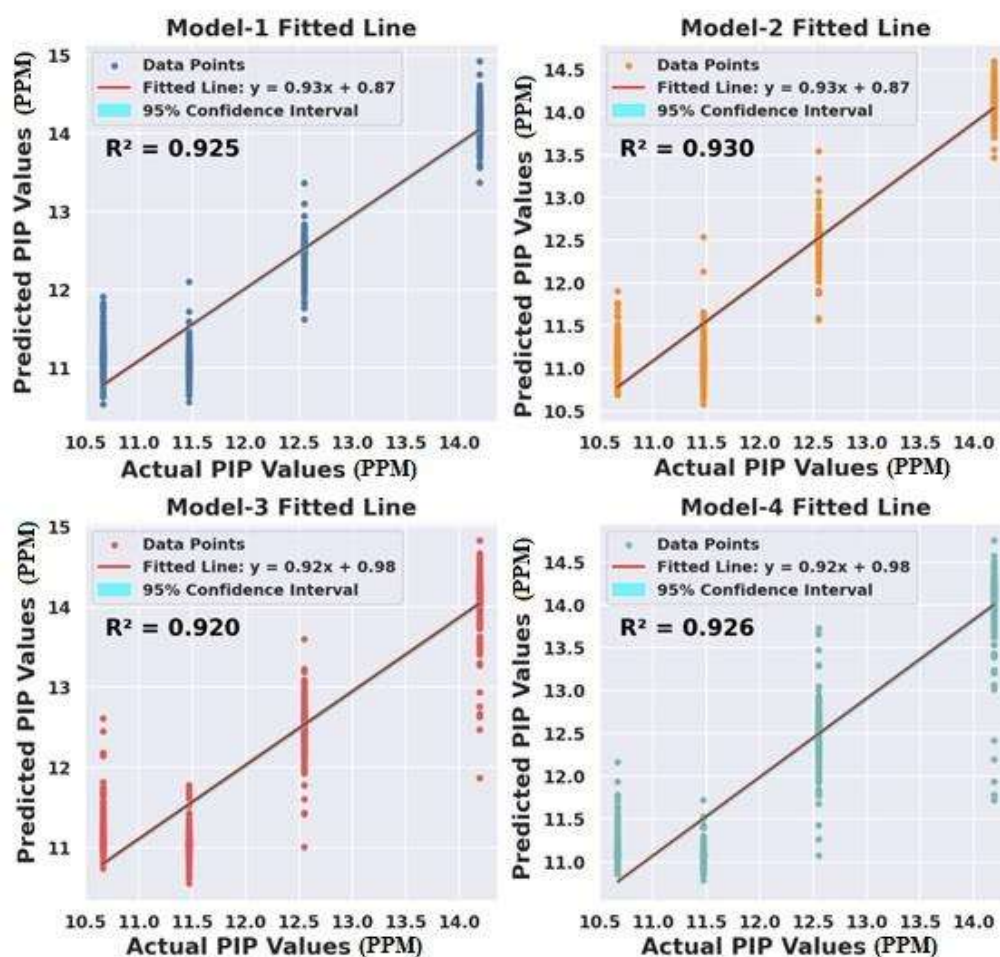


*Fig. 2.17. Residual error in determining piperine content of testing samples.*

### 2.3.13.5. Demonstration of fitted regression line

Fig. 2.18. illustrates the fitted regression lines between the actual PIP values and the predicted PIP values for each model. This figure offers a visual comparison of each model's accuracy, bias, and overall fit to the data from a single random run of the experiment, including the 95% confidence intervals for the predicted lines. The figure reveals that the fitted lines for Model-1 and Model-2 are quite similar, as are those for Model-3 and Model-4. All models demonstrate a comparable fit to the test data, effectively capturing the piperine content across four different brands, which are distinctly clustered into four separate groups. The high  $R^2$  values and the narrow confidence intervals indicate that each model closely approximates the actual data points with minimal uncertainty in their predictions.





*Fig. 2.18. Fitted regression line of the models on the testing samples.*

## 2.4. Conclusion

In this study, a novel molecularly imprinted poly(N,N-dimethylacrylamide)-based graphite electrode (PDMAM-MIP@G) was successfully developed and validated for the sensitive and selective detection of piperine extracted from black pepper. The sensor, synthesized using a polymerization strategy involving graphite, N,N-DMAM, piperine as the template molecule, and EGDMA as the cross-linker, demonstrated excellent electrochemical performance in terms of sensitivity ( $12.92 \mu\text{A}/\mu\text{M}$  in the lower range), a wide linear detection range (0.001–200  $\mu\text{M}$ ), and an impressively low detection limit of 0.6 nM. The optimized stoichiometric monomer-template ratio (1:4) was key to achieving this high performance, along with outstanding reproducibility, long-term stability, and selectivity.



## Chapter 2: Detection of piperine content in black pepper

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To complement the electrochemical sensing, a CNN-driven predictive framework was developed to estimate piperine concentrations from cyclic voltammetry (CV) responses, using RP-HPLC measurements as the reference standard. Among the models tested, the best-performing hybrid CNN-KNNR model achieved a remarkably low mean absolute error (MAE = 0.0339%) and near-perfect prediction accuracy ( $R^2 \approx 0.9999$ ), clearly outperforming traditional methods in terms of speed, cost-efficiency, and practicality. These results underscore the potential of this integrated electrochemical-ML approach to serve as a viable alternative to conventional chromatographic techniques like HPLC, especially in resource-limited or high-throughput environments.

This integrated sensing and prediction strategy offers multiple practical applications, including rapid quality control in food processing, on-site detection of bioactive compounds, and scalable monitoring in commercial settings. Future research should focus on broadening the applicability of the sensor to other food matrices or pharmacological compounds, improving robustness for field conditions, and integrating the system into automated or cloud-connected platforms for real-time, continuous monitoring.

In conclusion, the proposed PDMAM-MIP@G sensor coupled with deep learning analytics provides a powerful, scalable, and cost-effective solution for smart detection and prediction of piperine in black pepper, marking a significant advancement toward next-generation food safety and quality assurance technologies.

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## Chapter 3

### Detection of thymoquinone in black cumin

In this chapter two novel electrochemical methods were developed for detection of thymoquinone (TQ) using a titanium oxide-modified graphite paste electrode (GPE/TiO<sub>2</sub>) and a thymoquinone-imprinted polymer-based graphite electrode (TQIP/G). Both offered excellent sensitivity, selectivity, and stability. Principal Component Analysis (PCA) differentiated black cumin brands, and a convolutional neural network (CNN) analyzed DPV responses with high accuracy. These integrated approaches provide robust, low-cost TQ detection.

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- 3.2. Experiment with TiO<sub>2</sub> modified graphite paste electrode**
- 3.3. Result and discussion with TiO<sub>2</sub> modified graphite paste electrode**
- 3.4. Experiment with molecularly imprinted polymer electrode**
- 3.5. Results with molecularly imprinted polymer electrode**
- 3.6. Machine learning experiment for TQ detection from real samples**
- 3.7. Conclusion**
- References**

#### **Contents of this chapter are based on mentioned publications:**

**Sanjoy Banerjee**, Milan Dhara, Hemanta Naskar, Barnali Ghatak, SK. Babar Ali, Nityananda Das, Deepak Kumar Das, Rajib Bandyopadhyay, Bipan Tudu, “Selective Electrochemical Detection of Thymoquinone in Black Cumin Using Titanium Oxide-Modified Graphite Paste Electrode”, *Nano Life*, 14(2), (2024) 2450001, pp. 1-13.

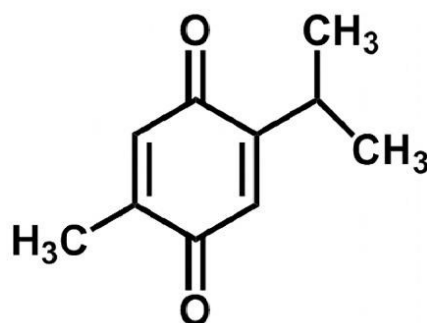
**Sanjoy Banerjee**, Santanu Ghorai, Milan Dhara, Hemanta Naskar, Barnali Ghatak, Sk. Babar Ali, Nityananda Das, Pradip Saha, Bhimsen Tudu, Arpitam Chatterjee, Rajib Bandyopadhyay, Bipan Tudu, “Development of a graphite based molecularly imprinted polymer electrode for the detection of thymoquinone in black cumin: A machine learning-assisted approach”, *Microchemical Journal*, 215, (2025), 114431.



### 3.1. Introduction

*Nigella sativa* L., commonly referred to as black cumin or black seed, is valued for its potent nutritional profile and wide-ranging therapeutic uses. The seeds are notably rich in fixed oils (31–35.5%), essential oils (0.40–0.45%), proteins, carbohydrates, amino acids, minerals, and vitamins, making them suitable for various pharmacological applications. Among the numerous bioactive components found in *N. sativa*, thymoquinone (TQ) stands out as the major therapeutic constituent, representing 18%–24% of the volatile oil and 3.5–8.7 mg/g of the fixed oil content [4].

Thymoquinone (TQ), a major bioactive compound in *N. sativa*, is recognized for its diverse biological effects, such as antioxidative, anti-inflammatory, antihistaminic, antimicrobial, antihypertensive, analgesic, and anticancer properties [5-9]. Recent studies have highlighted its therapeutic potential against a range of illnesses such as diabetes, cancer, bronchial asthma, headache, dysentery, obesity, and gastrointestinal disorders [10]. Remarkably, emerging evidence suggests that TQ may inhibit SARS-CoV-2 proteases, thereby blocking viral replication and entry into host cells by targeting angiotensin-converting enzyme 2 (ACE2) receptors [11]. The molecular structure of TQ is illustrated in Fig. 3.1.



***Fig. 3.1. Chemical structure of Thymoquinone.***

Given its vast therapeutic significance, the qualification and quantification of TQ are of critical importance. Traditional analytical approaches for identifying TQ include techniques like gas chromatography (GC), GC-MS [12], various forms of HPLC, TLC [13-19], and spectroscopic methods such as NIR[20]. While these techniques are known for precision and sensitivity, they often require complex procedures, expensive instruments, and skilled

### Chapter 3: Detection of thymoquinone in black cumin

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operators, limiting their use in rapid or on-site applications. These limitations necessitate the development of novel, simpler, and more accessible methods for TQ detection, especially for rapid or field-based applications.

As an alternative to traditional chromatographic techniques, electrochemical sensors have gained popularity due to their simplicity, low operational cost, high sensitivity, and fast response, making them ideal for trace-level biomolecule detection [21-27]. Techniques like linear sweep voltammetry (LSV), square wave voltammetry (SQV), cyclic voltammetry (CV), and differential pulse voltammetry (DPV) are particularly attractive for detecting trace amounts of bioactive compounds. Carbon paste electrodes (CPEs) are favored for their minimal background signal, broad potential range, easy renewal of surface, and simple customization.

Surface modification of electrodes using nanomaterials and functional coatings has further enhanced electrochemical sensor performance. Titanium dioxide ( $\text{TiO}_2$ ) nanoparticles are widely employed to enhance electrode performance because of their large surface area, wide bandgap (3.2 eV) [28], high electrocatalytic efficiency, chemical stability, and biocompatibility. These features make them suitable for improving detection sensitivity in sensor applications. Earlier research has shown that electrodes modified with  $\text{TiO}_2$  have been effectively utilized for detecting a range of different compounds, including resorcinol, hydroquinone, catechol, gallic acid, acetaminophen, and bisphenol [29-33].

In this work, a simple and cost-effective  $\text{TiO}_2$ -modified carbon paste electrode (GPE/ $\text{TiO}_2$ ) was developed for the sensitive electrochemical detection of TQ in black cumin. Empirical investigations evaluated its sensitivity, repeatability, reproducibility, and stability. The prepared sensor exhibited a wide linear detection range (0.1–200  $\mu\text{M}$ ), an impressively low detection limit (0.02  $\mu\text{M}$ ), and successful application in classifying different black cumin samples (Mousumi, Priya Gold, Sunrise, and DNV) using principal component analysis (PCA) with a separability index (SI) of 67.89. The novelty of this sensor lies in its broad linear range, excellent selectivity, reproducibility, and capability for real sample analysis.

Furthermore, the study introduced a molecularly imprinted polymer (MIP) approach for enhancing the selectivity of TQ detection. MIPs are synthetic polymers fabricated through the imprinting of template molecules, creating highly selective recognition sites complementary in shape, size, and functional groups [34]. MIP-based sensors offer advantages like cost-

effectiveness, ease of fabrication, high selectivity, environmental stability, and reusability. Previous studies have demonstrated the successful application of MIP electrodes for the detection of various compounds such as furaneol, thymol, pantoprazole [35-37] with very low LOD.

In this work thermal polymerization of acrylonitrile, cross-linked with ethylene glycol dimethacrylate (EGDMA), was employed to fabricate the TQ-imprinted MIP electrode. This MIP-modified sensor achieved high specificity for TQ with minimal sample processing and provided rapid, low-cost analysis suitable for decentralized or field testing.

The integration of MIP technology with machine learning-assisted data analysis, offers a robust, sensitive, and selective electrochemical platform for the real-time detection of TQ. This approach addresses the critical limitations of traditional chromatographic techniques and represents a promising advancement for portable, affordable, and practical applications in agricultural, pharmaceutical, and biomedical fields.

### 3.2. Experiment with TiO<sub>2</sub> modified graphite paste electrode

#### 3.2.1. *Reagents and apparatus*

In this experiment, high-quality reagents were employed, all of which were of analytical grade and used as received. Graphite (particle diameter <20  $\mu\text{M}$ ), TQ (Molecular Weight (MW) - 164.20 g/M), and Titanium oxide (TiO<sub>2</sub>) were sourced from Sigma-Aldrich. Ethanol was procured from Merck, India. Millipore water with a resistivity exceeding 18 M $\Omega$  cm was utilized for the preparation of all electrolytic solutions and obtained from the Merck Millipore system. In order to prepare 1 L of phosphate buffer (PBS), 0.63 g of oxalic acid (C<sub>2</sub>H<sub>2</sub>O<sub>4</sub>) was dissolved in 50 mL of millipore water. Then, 4 g of sodium hydroxide (NaOH) pellets were dissolved in 500 mL of Millipore water to form NaOH solution. After that, titration was performed thrice between 10 mL of the oxalic acid and NaOH with phenolphthalein (C<sub>20</sub>H<sub>14</sub>O<sub>4</sub>) as the indicator in order to standardize 0.1 M NaOH. Now, 112 mL of 0.1 M NaOH solution was mixed with 13.6 g of potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) in a volumetric flask. Millipore water was used to fill the 1 L level in the volumetric flask. The pH of the buffer solution was checked repeatedly with litmus paper for confirmation. Phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) or NaOH was used to adjust the pH of the solution. Similarly, acetate buffer solution and citrate buffer solution were prepared by using sodium acetate trihydrate (C<sub>2</sub>H<sub>9</sub>NaO<sub>5</sub>) and sodium citrate dehydrate (C<sub>6</sub>H<sub>9</sub>Na<sub>3</sub>O<sub>9</sub>), respectively [38,39]. A 1.0

## Chapter 3: Detection of thymoquinone in black cumin

mM standard solution of TQ was created by dissolving 16.42 mg of TQ in 100 mL of ethanol. All experiments were conducted under controlled laboratory room temperature conditions.

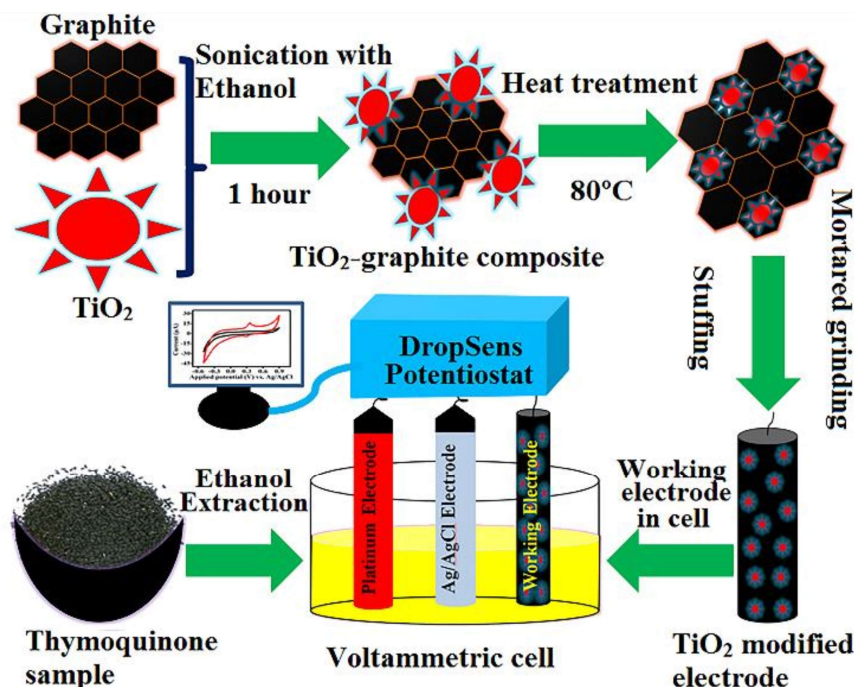
In the conducted research, various electrochemical analysis were carried out employing cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. These measurements utilized a three-electrode system, consisting of a working electrode, a reference electrode (Silver/Silver Chloride (Ag/AgCl) electrode)), and a counter electrode (a platinum electrode). The CVs and DPVs were performed within the potential window of -0.7 to 1.0 V and -0.3 to 0.6 V respectively in a 20 mL solution. The Origin Pro 8.0 (Origin Lab Corporation, USA) was used for the CV and DPV plots. The pH of the supporting electrolyte was measured by using Metrohm pH meter. The PCA model was developed for the data analysis using MATLAB software.

### 3.2.2. *Synthesis of TiO<sub>2</sub> modified graphite material*

Titanium oxide doped graphite material (GPE/TiO<sub>2</sub>) was primarily synthesized by using variable stoichiometric ratio of graphite and TiO<sub>2</sub>, as given in Table 3.1. In the process of synthesis, Titanium oxide nanoparticles were mixed with commercial-grade graphite powder in a beaker containing ethanol solvent [40]. Initially TiO<sub>2</sub> was dispersed in 30 mL of ethanol using an ultrasonicator for 60 minutes. Subsequently, graphite powder were introduced into the dispersed graphite solution, and ultrasonication was continued for 2 hours. The entire mixture was then transferred to a heater. The initial heater temperature was set at 80 °C, and it was gradually reduced to 40 °C until a dry powder was obtained. The resulting TiO<sub>2</sub> doped modified graphite material were collected and dried overnight. The schematic representation of the GPE/TiO<sub>2</sub> synthesis is depicted in Fig. 3.2

**Table 3.1. Materials synthesized and ratio of precursor used.**

| Polymer                 | Graphite (g) | TiO <sub>2</sub> (g) | Ratio |
|-------------------------|--------------|----------------------|-------|
| GPE/TiO <sub>2</sub> @1 | 0.9          | 0.1                  | 9:1   |
| GPE/TiO <sub>2</sub> @2 | 0.8          | 0.2                  | 8:2   |
| GPE/TiO <sub>2</sub> @3 | 0.7          | 0.3                  | 7:3   |
| GPE                     | 1            | 0                    | 1:0   |



*Fig. 3.2. Schematic representation of synthesis of GPE/TiO<sub>2</sub> sensing electrode.*

### 3.2.3. Electrode preparation

To prepare the working electrode, 300 mg of the synthesized TiO<sub>2</sub>-graphite material was combined with paraffin oil to form a smooth paste. This paste was then packed into a glass capillary tube with an inner diameter of 2.5 mm, using a metal rod. Electrical connectivity was achieved by inserting a platinum wire into the rear end of the capillary tube. Prior to each measurement, all electrodes were meticulously smoothed and rinsed with millipore water. Similarly, the graphite paste electrode (GPE) was prepared following the same procedure as the GPE/TiO<sub>2</sub> electrode.

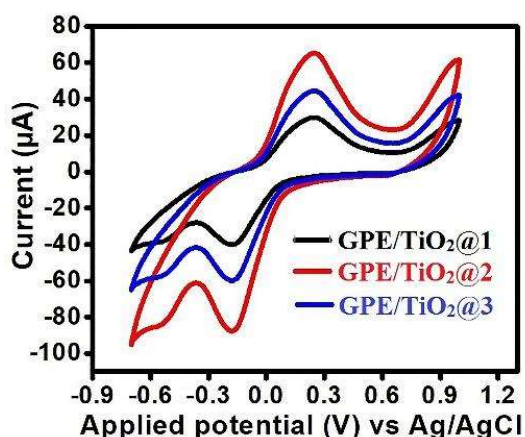
## 3.3. Result and discussion with TiO<sub>2</sub> modified graphite paste electrode

### 3.3.1. Electrochemical properties of the GPE/TiO<sub>2</sub> and GPE electrodes

Cyclic voltammetry (CV) experiments were conducted to investigate the electrocatalytic activities of the TQ molecule at GPE/TiO<sub>2</sub> electrode surface. Three electrodes were developed with varying stoichiometric ratios of graphite and TiO<sub>2</sub>. The measurements were performed in 0.1 M phosphate buffer solution (PBS, pH 6.0) with TQ concentration fixed at 100  $\mu$ M. In Fig. 3.3., it was observed that the GPE/TiO<sub>2</sub>@2 (8:2) material exhibited the

highest redox peak current of 65.36  $\mu\text{A}$ . This value is 2.14 times higher than that of GPE/TiO<sub>2</sub>@1 and 1.47 times higher than that of GPE/TiO<sub>2</sub>@3. This enhancement in electrocatalytic performance can be primarily attributed to the increased concentration of TiO<sub>2</sub> nanoparticles, which resulted in a greater number of charge carriers due to its redox properties. Consequently, this led to a significant improvement in the electrocatalytic activities of the carbon paste electrode, ultimately increasing the sensitivity of the developed sensor for TQ. Hence, the GPE/TiO<sub>2</sub>@2 sensor serves as the primary sensor for all experiments and is subsequently referred to as GPE/TiO<sub>2</sub> in the following literature.

Again, the electrochemical response of GPE/TiO<sub>2</sub> was compared with that of GPE as represented in Fig. 3.4. The GPE/TiO<sub>2</sub> electrode displayed a distinct and sharp peak, indicating a significantly higher peak current compared to the GPE electrode. This voltammetric curve demonstrates exceptional electrocatalytic performance in the redox reaction of TQ.

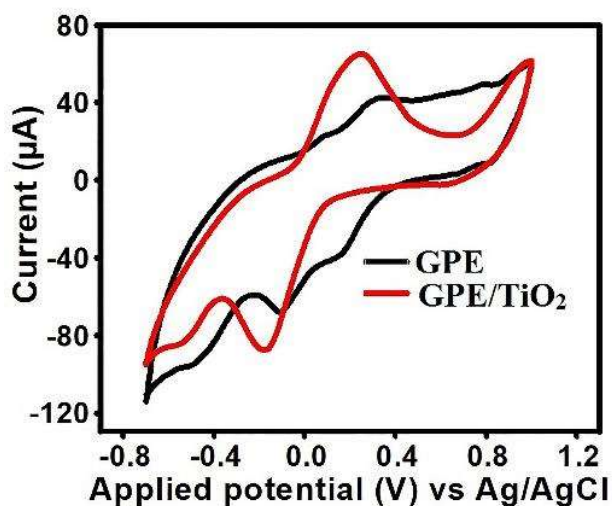


**Fig. 3.3.** CVs of 100  $\mu\text{M}$  TQ at GPE/TiO<sub>2</sub>@1, GPE/TiO<sub>2</sub>@2 and GPE/TiO<sub>2</sub>@3 sensors in 0.1 M PBS (pH 6.0) with scan rate of 0.05  $\text{Vs}^{-1}$ .

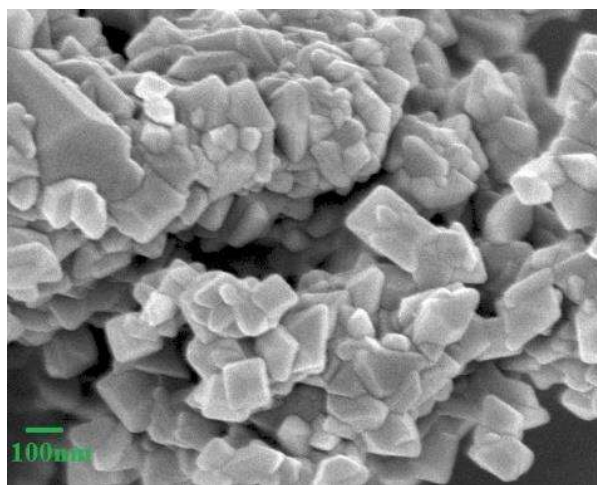
### 3.3.2. Scanning electron microscopy (SEM)

The morphology of GPE/TiO<sub>2</sub> was examined through field emission electron microscopy (FESEM) as depicted in Fig. 3.5. The FESEM image reveals a cubic structure for the TiO<sub>2</sub> powder, with an average particle size of  $51 \pm 8$  nm.





*Fig. 3.4. CVs of GPE and GPE/TiO<sub>2</sub> electrode with 100 μM of TQ.*



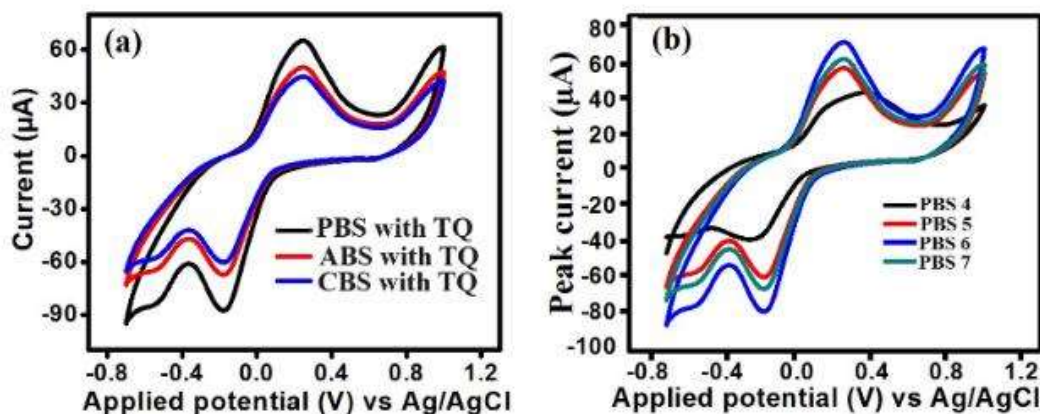
*Fig. 3.5. FESEM images of TiO<sub>2</sub> powder.*

### 3.3.3. Effect of buffer and pH

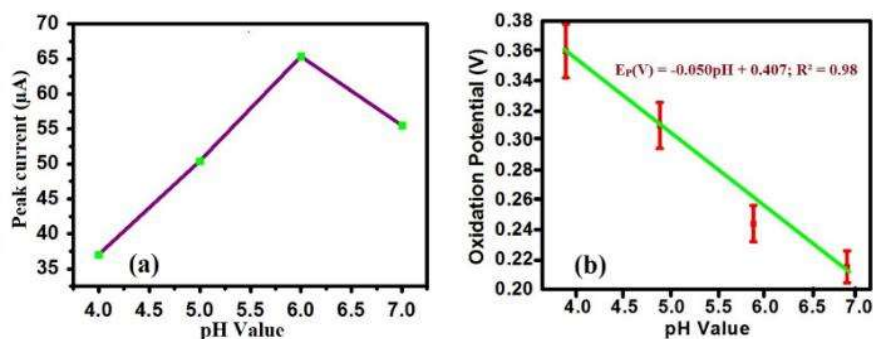
The cyclic voltammetry response of the synthesized TiO<sub>2</sub> doped modified graphite electrode is influenced by the nature of the buffer solution and the pH of the medium. To explore this impact, the GPE/TiO<sub>2</sub> electrode was tested with three different buffer solutions (phosphate, acetate, and citrate), as illustrated in Fig. 3.6.(a). Among these, phosphate buffered solution (PBS) resulted in the sharpest and highest peak current (*I*<sub>p</sub>) compared to acetate buffer solutions (ABS) and citrate buffer solutions (CBS). Consequently, PBS was selected as the supporting buffer solution for studying the electrocatalytic oxidation of the TQ molecule. The

impact of ionic strength of PBS on the CV characteristics are discussed detailed on chapter 2, section 2.3.8.

Fig. 3.6.(b) displays the cyclic voltammetry (CV) response of the GPE/TiO<sub>2</sub> electrode at various pH values, ranging from 4 to 7, as part of the redox process aimed at optimizing the experimental pH. The peak current vs. pH plot was shown in Fig. 3.7.(a). A negative shift in TQ's oxidation peak potential was observed as the pH of the medium decreased (Fig. 3.7. (b)), indicating the dissociation of the phenolic –CO group in the oxidation reaction process. Phenol dissociates into an anion at higher pH, facilitating easier oxidation [41]. The pH versus Oxidation potential was linear with linear regression equation as follows:  $E_p(V) = -0.050pH + 0.407, R^2 = 0.98$ . According to the Nernstain equation, the hypothetical value of this slope is  $0.059 \text{ VpH}^{-1}$  at 25°C which is very close to the experimental value ( $0.050 \text{ VpH}^{-1}$ ) [42]. These findings imply a balanced two proton-electron transfer mechanism is involved in the redox process occurring at the electrode interface. However, as the pH of the PBS further increased from 6 to 7, the weak adsorption of TQ led to a decrease in  $I_p$  (Fig. 6(a)). Consequently, the experimental pH was optimized at 6.

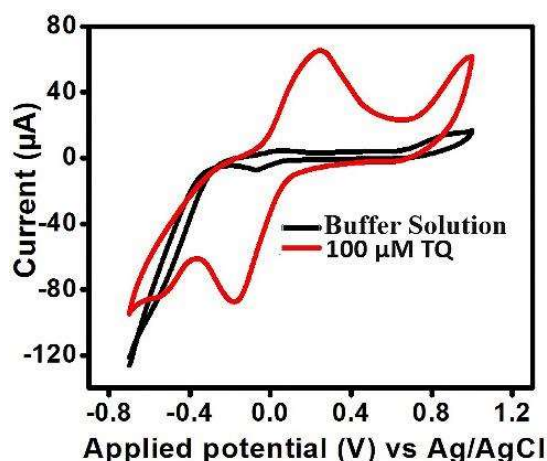


**Fig. 3.6. (a) CV of GPE/TiO<sub>2</sub> sensor in 100 μM TQ in different buffer solution, and (b) CV response of GPE/TiO<sub>2</sub> sensor in 100 μM of TQ with different pH values of PBS.**



**Fig. 3.7. (a) Plot of peak current vs. pH of GPE/TiO<sub>2</sub> sensor in 100 μM of TQ and (b) linear plot of peak potential vs. pH value.**

The CV response of the GPE/TiO<sub>2</sub> electrode both with and without the TQ molecule (100 μM) in PBS buffer solution at pH 6, where no peak current was detected in the absence of TQ molecules as demonstrated in Fig. 3.8.

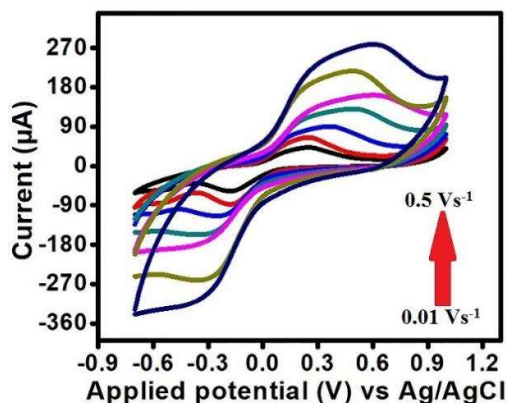


**Fig. 3.8. CV response of GPE/TiO<sub>2</sub> sensor in 100 μM of TQ and without TQ.**

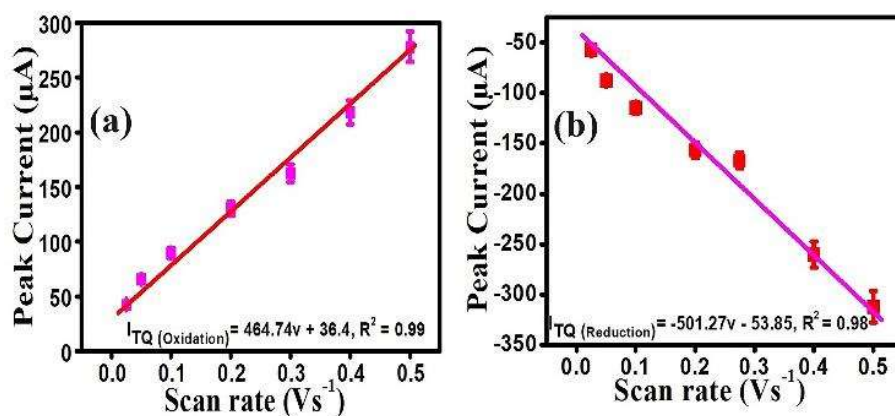
## 3.3.4. Impact of scan rate

The scan rate played a crucial role in determining the electro-oxidation kinetics of thymoquinone on the developed electrode. To explore the influence of scan rate on the peak current and peak potential of TQ at the developed electrode, cyclic voltammetry (CV) was employed over a range of 0.01 Vs<sup>-1</sup> to 0.5 Vs<sup>-1</sup>, as illustrated in Fig. 3.9. The voltammogram displayed two irreversible redox peaks with a separation of 1.019 V. Notably, the redox peak current exhibited a linear increase with varying scan rates, with the corresponding linear

regression equations as follows:  $I_{OXD(\mu A)} = 464.74v + 36.403$  ( $R^2 = 0.99$ ) and  $I_{RED(\mu A)} = -501.27v - 53.85$  ( $R^2 = 0.98$ ).



**Fig. 3.9.** Effect of scan rate on CV in 0.1 M PBS (pH 6.0) using GPE/TiO<sub>2</sub> electrode.



**Fig. 3.10.** (a) Linear plot of oxidation peak current vs. scan rate, and (b) linear plot of reduction peak current vs. scan rate using GPE/TiO<sub>2</sub> electrode in 100  $\mu$ M of TQ.

Fig. 3.10.(a) demonstrated that the scan rate vs oxidation peak current plot and the scan rate vs reduction peak current response represented in Fig. 3.10.(b). Increasing the scan rate resulted in a positive shift in the peak potential. The oxidation process of TQ at the GPE/TiO<sub>2</sub> electrode was determined to involve the transfer of approximately 2 (1.73) electrons, as described in Eqn. (1). This suggests a two-electron transfer system for the electrochemical oxidation of TQ.

The amount of TQ adsorbed on the GPE/TiO<sub>2</sub> electrode surface was calculated using Eq. (1) [43], where parameters such as the number of electrons transferred ( $n$ ), Faraday's constant ( $F$ ), gas constant ( $R$ ), temperature ( $T$ ), cross-sectional area of the electrode ( $A$ ), transfer coefficient ( $\alpha$ ), charge consumed during oxidation ( $Q$ ), scan rate ( $v$ ), and surface concentration of TQ [44]

### Chapter 3: Detection of thymoquinone in black cumin

were considered. The values of  $n$  and  $\Gamma_c$ , determined from eq. (3.1) and (3.2) for the oxidation reaction of TQ, were found to be 1.73 and  $7.88 \times 10^{-10}$  mole  $\text{cm}^{-2}$ , respectively. This aligns well with the observed oxidation peaks of TQ. Additionally, the heterogeneous rate constant ( $k_s$ ) was obtained using Laviron's equation [45,46] validating the analytical data.

$$i_p = \frac{nFQv}{4RT} = \frac{n^2 F^2 A v \Gamma_c}{4RT} \quad (3.1)$$

$$\log(v) = 2.3 \frac{RT}{(1-\alpha)nF} E_p + \text{constant} \quad (3.2)$$

$$\log(k_s) = \alpha \log(1-\alpha) + (1-\alpha) \log \alpha - \log\left(\frac{RT}{nFv}\right) - \alpha(1-\alpha) \frac{nF}{2.3RT} \Delta E_p \quad (3.3)$$

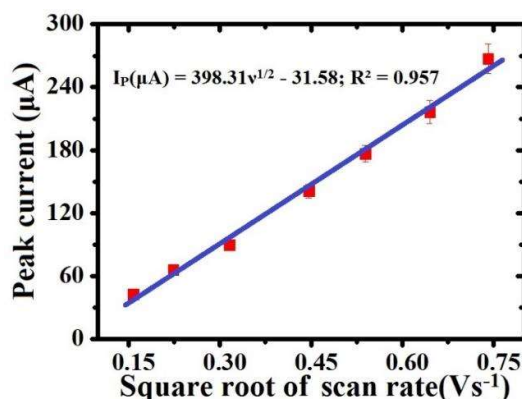
The electron transfer coefficient ( $\alpha$ ) was found to be 0.98 from the slope of the curve (Fig. 3.10(b)), supporting literature-reported values [47]. Using Eq. (3.3), the values of  $k_s$  and  $\alpha$  were calculated as  $5.6 \times 10^{-2} \text{ s}^{-1}$  and 0.99, respectively.

Randles-Sevcik equation (Eqn. 3.4) was used to evaluate the surface area ( $A$ ) using the slope of  $I_p$  versus  $v^{1/2}$  linear plot (Fig. 3.11.). The calculated value obtained was  $0.00232 \text{ cm}^2$ .

$$i_p = 2.69 \times 10^5 A n^{3/2} D^{1/2} C v^{1/2} \quad (3.4)$$

Where,  $D$  is the diffusion coefficient  $= 7.6 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$

The Authors have calculated the number of electrons involved ( $n=1.73$ ), charge transfer coefficient ( $\alpha=0.99$ ) and rate constant ( $k_s=5.6 \times 10^{-2} \text{ s}^{-1}$ ).



**Fig. 3.11. Linear plot of peak current against Square root of scan rate using GPE/TiO<sub>2</sub> electrode in 100  $\mu\text{M}$  of TQ.**

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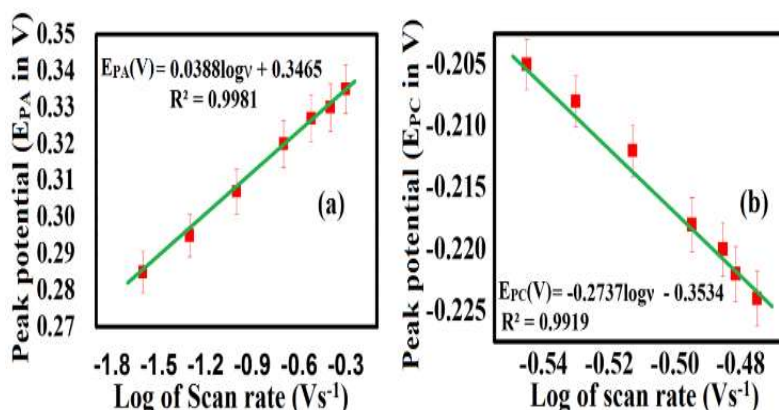
In our study, increasing the scan rate caused the anodic peak potential ( $E_{pa}$ ) to shift in the positive direction, while the cathodic peak potential ( $E_{pc}$ ) moved toward the negative side.

A strong linear correlation was observed between the peak potentials and the logarithm of the scan rate ( $v$ ), as illustrated in Fig. 3.12(a) and (b), with the corresponding linear regression equations as follows:  $E_{PA}(V) = 0.038 \log v + 0.35$ ,  $R^2 = 0.99$  and  $E_{PC}(V) = -0.27 \log v - 0.35$ ,  $R^2 = 0.99$ . By using the slopes of the above equations, the number of electrons transferred ( $n$ ) and the anodic and cathodic charge transfer coefficients ( $\alpha_a$  and  $\alpha_c$ ) were calculated from the following equations (Eqn. 3.5 and Eqn. 3.6).

$$E_{PA} = a + \frac{2.303 RT}{(1 - \alpha_A)nF} \log v \quad (3.5)$$

$$E_{PC} = b - \frac{2.303 RT}{\alpha_C n F} \log v \quad (3.6)$$

The value of  $n$  was calculated to be 1.73 ( $\approx 2$ ) which indicates that the redox reaction at the developed sensor was involved two electrons. The number of protons ( $m$ ) involved was 2.06 ( $\approx 2$ ) obtained using the following equation:  $0.059 \alpha m / n = 0.0612$  ( $\alpha = 0.99$ ,  $n = 2$ ). Hence, the electrochemical behavior of TQ at GPE/TiO<sub>2</sub> electrode was involved equal number of electrons and protons. The calculated response time was observed as 18 sec.



**Fig. 3.12.** (a) Linear plot of anodic peak potential vs log of scan rate (b) Linear plot of cathodic peak potential vs log of scan rate at GPE/TiO<sub>2</sub> electrode.

### 3.3.5. Analytical application of TQ

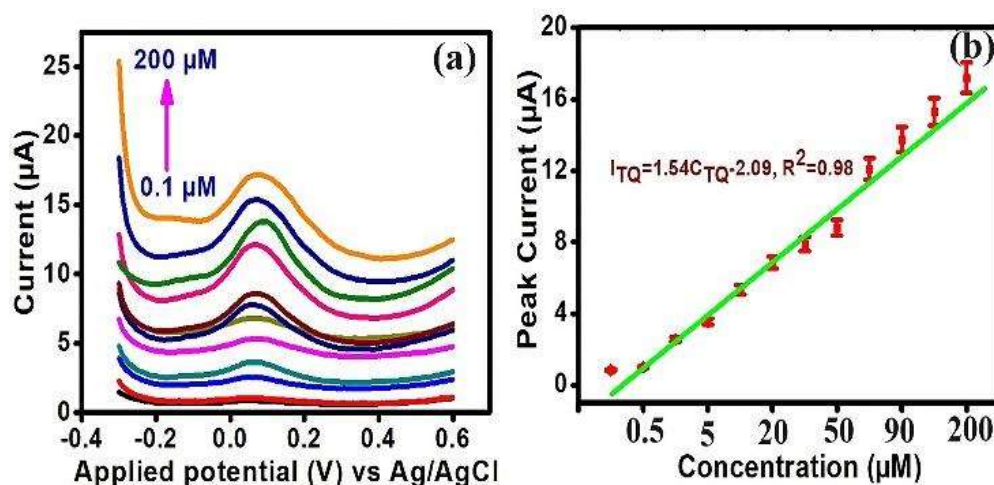
Differential pulse voltammetry (DPV) was selected for further analysis due to its superior resolution and enhanced current sensitivity in comparison to cyclic voltammetry (CV), mainly



attributed to the reduction of non-faradic currents. The concentration of the target molecule, TQ, was systematically increased, resulting in the generation of corresponding DPV curves, as illustrated in Fig. 3.13.(a).

The sensitivity ( $1.54 \mu\text{A}/\mu\text{M}$ ) plot exhibited a linear relationship, indicating a consistent change in oxidation peak currents with the increasing concentration of TQ in Fig. 12(b). Specifically, the GPE/TiO<sub>2</sub> electrode displayed an oxidation peak at a peak potential of 70 mV for the TQ molecule. Notably, the electrode exhibited a broad linear response range, spanning from 0.1  $\mu\text{M}$  to 200  $\mu\text{M}$ , as described by regression equation  $I_{\text{TQ}} = 1.54C_{\text{TQ}} - 2.09$ ;  $R^2 = 0.98$ .

Additionally, the limit of detection (LOD) and limit of quantification (LOQ) for the electrode was 0.02  $\mu\text{M}$  and 0.067  $\mu\text{M}$  respectively, which is lower than the existing methods as shown in Table 3.2. The LOD and LOQ were calculated using formula  $3S_{y/x}/m$  and  $10S_{y/x}/m$  where  $m$  is the slope of the calibration curve and  $S_{y/x}$  is the standard deviation of the blank solution response. From the regression equation the value of  $m$  is 0.98 and the value of  $S_{y/x}$  is 0.0066.



**Fig. 3.13.** DPV measurements of TQ in 0.1 M PBS (pH 6.0) using GPE/TiO<sub>2</sub> sensor (a) Plot of current response against potential with varying concentration, and (b) Concentration vs. peak current plot.

## Chapter 3: Detection of thymoquinone in black cumin

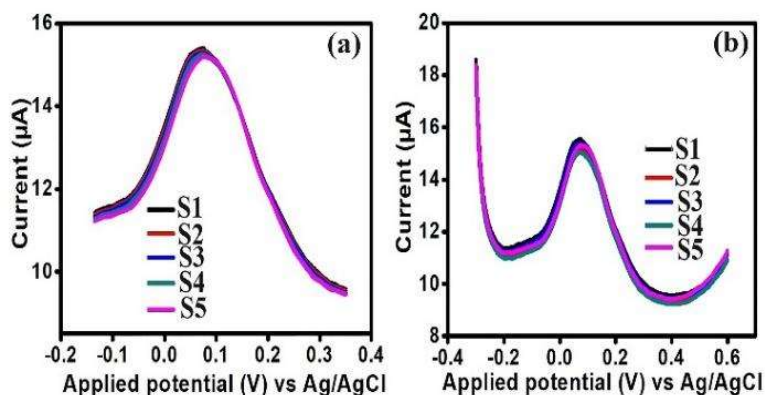
*Table 3.2. Comparison of the proposed method with other existing techniques.*

| Methodology                                                                                                                      | LOD ( $\mu\text{M}$ ) | Range of detection ( $\mu\text{M}$ ) | Ref.             |
|----------------------------------------------------------------------------------------------------------------------------------|-----------------------|--------------------------------------|------------------|
| High-performance thin layer chromatographic method (HPTLC)                                                                       | 0.09                  | 0.004-0.028                          | [48]             |
| Reverse-phased high-pressure liquid chromatography (RP-HPLC) using Silica gel column                                             | -                     | 32.94- 368.30                        | [49]             |
| Differential pulse polarographic method                                                                                          | 3                     | 110–832                              | [50]             |
| <b>Selective electrochemical detection of thymoquinone in black cumin using titanium oxide modified graphite paste electrode</b> | <b>0.02</b>           | <b>0.1- 200</b>                      | <b>This work</b> |

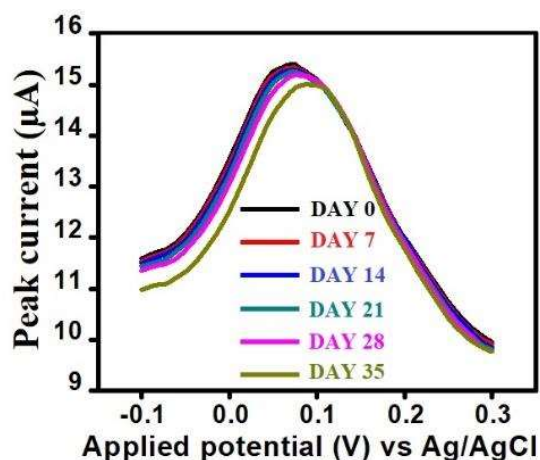
### 3.3.6. Study of repeatability, reproducibility and stability

To assess the precision of our measurements, we conducted evaluations of the developed electrode's repeatability and reproducibility. We have taken 20 mL solution for each experiment. In the case of repeatability analysis, we performed five consecutive measurements using a consistent TQ molecule concentration (100  $\mu\text{M}$ ) at 0.1 M PBS-6 buffer solution (Fig. 3.14. (a)). The relative standard deviation (RSD) for these measurements was calculated and found to be 3.23%. Concurrently, we fabricated and tested five additional GPE/TiO<sub>2</sub> electrodes, following the same synthesis steps, and assessed their response to a 100  $\mu\text{M}$  TQ at 0.1 M PBS-6 buffer solution to determine their reproducibility, as illustrated in Fig. 3.14.(b). To examine the electrode's stability, it was stored at room temperature and its performance was evaluated on a weekly basis. Encouragingly, there was no discernible deviation in peak response after two weeks of preservation (Fig. 3.15).





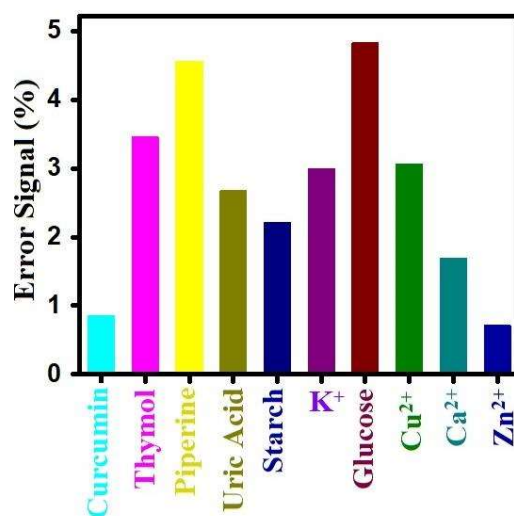
**Fig. 3.14.** DPV measurements of 100  $\mu\text{M}$  TQ using GPE/TiO<sub>2</sub> sensor (a) Repeatability test for five measurements using the same sensor; and (b) Reproducibility test for five different sensors.



**Fig. 3.15.** Peak response of GPE/TiO<sub>2</sub> electrode taken with an interval of 7 days.

### 3.3.7. Interference study

The selectivity of the modified GPE/TiO<sub>2</sub> electrode was investigated by electing some normal anions, cations, and some common organic molecules in the presence of 100  $\mu\text{M}$  TQ. The results reflected that 1 mM concentration of  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^{+}$ , and  $\text{Cu}^{2+}$ ; and 100  $\mu\text{M}$  of curcumin, thymol, piperine, uric acid, starch, and glucose had no influence on the current response of TQ with deviation not more than  $\pm 5\%$  (Table 3.3.) which was considered as tolerance level of the developed sensor (GPE/TiO<sub>2</sub>) as shown in Fig. 3.16.



**Fig. 3.16. Study of interference.**

### 3.3.8. Principle component analysis of TQ

Principal Component Analysis (PCA) serves as a multivariate method for scrutinizing high-dimensional data variables, transforming them into a set of orthogonal variables known as principal components. In this study, PCA was employed to analyze four different samples (B1, B2, B3, and B4 samples) with ten repeated Differential Pulse Voltammetry (DPV) responses. For real sample analysis, four varieties of branded black cumin (certified by the Food Safety Standard Authority of India) were procured from the local market viz. B1, B2, B3, and B4. The real sample has been prepared by taking the black cumin extract (5 mg) in pure ethanol (20 ml) and ultrasonicated for 30 mins. Further, the solution was filtered and collected the filtrated black cumin solution. The procedure has been followed for the four samples.

**Table 3.3. Interference analysis of different species for the determination of TQ at GPE/TiO<sub>2</sub> sensor.**

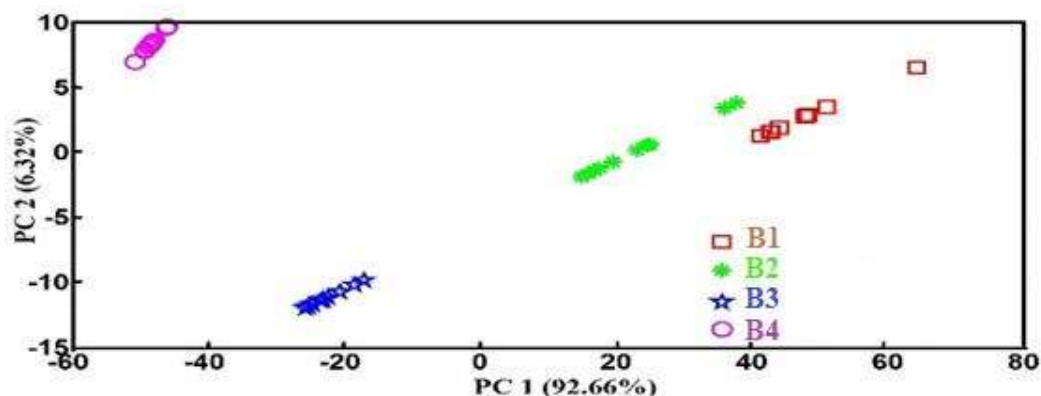
| Interferent  | Interferent Concentration | Peak Current Response (μA) | Error Deviation% |
|--------------|---------------------------|----------------------------|------------------|
| Thymoquinone | 100 μM                    | 15.34                      | -                |
| Curcumin     | 100 μM                    | 14.49                      | 0.84             |
| Thymol       | 100 μM                    | 11.88                      | 3.45             |
| Piperine     | 100 μM                    | 10.77                      | 4.56             |
| Uric Acid    | 100 μM                    | 12.66                      | 2.67             |

|                  |             |       |      |
|------------------|-------------|-------|------|
| Starch           | 100 $\mu$ M | 17.55 | 2.21 |
| K <sup>+</sup>   | 1 mM        | 12.34 | 2.99 |
| Glucose          | 100 $\mu$ M | 10.51 | 4.82 |
| Cu <sup>2+</sup> | 1 mM        | 12.27 | 3.06 |
| Ca <sup>2+</sup> | 1 mM        | 17.03 | 1.69 |
| Zn <sup>2+</sup> | 1 mM        | 16.05 | 0.71 |

Further four stock solutions (100 mL) were prepared in PBS (pH = 6) medium and DPV analyses (n=10) for four samples were carried out. The TQ content in B1, B2, B3 and B4 samples was discriminated using a principle component analysis (PCA) model using MATLAB Version 10.

The resulting score plot in the PCA model (Fig. 3.17.), based on a  $40 \times 358$  data matrix, demonstrated excellent classification of data concerning TQ concentrations in

the four samples. The variance in PC1 accounted for 92.66%, while PC2 contributed 6.32% to the overall variance with separability index (SI) was 67.89.



*Fig. 3.17. PCA plot for four different black cumin samples (B1, B2, B3 and B4).*

### 3.4. Experiment with Molecularly Imprinted Polymer Electrode

#### 3.4.1. Reagents and chemicals

The reagents and chemicals are important for the fabrication and evaluation of the TQIP/G electrode, as their purity and properties significantly influence the sensor's performance and reliability. The details of each chemical, including its source and purity, are provided below to

ensure reproducibility of the experiments. Paraffin oil (99.0% purity) and ethanol (99.8% purity) were sourced from Merck, India. Ethylene glycol dimethylacrylate (EGDMA, MW: 198.22 g/mol), thymoquinone (TQ, MW: 164.20 g/mol), acrylonitrile (AN, MW: 53.06 g/mol), and benzoyl peroxide (BP) were procured from Sigma-Aldrich, USA. For monomer optimization, additional monomers such as acrylic acid and methyl methacrylate were also obtained from Sigma-Aldrich, USA. Interfering agents, including  $\alpha$ -pinene,  $\beta$ -pinene, thymol (TY), carvacrol (CL), dithymoquinone (DTQ), and thymohydroquinone (THQ), were purchased from Sigma-Aldrich, USA. All chemicals used in this study were of the highest purity grade (>99.0%). Solutions were prepared using ultrapure Millipore water with a resistivity of 18 M $\Omega$ .cm.

### 3.4.2. Apparatus

In this investigation, electrochemical analyses were carried out using a three-electrode configuration, which included a working electrode, an Ag/AgCl reference electrode, and a platinum wire as the counter electrode. DPV was utilized for concentration studies, while CV was employed for the optimization of parameters such as scan rate, pH, and buffer selection. These measurements were performed using a PGSTAT 101 electrochemical workstation (Metrohm Autolab, Netherlands). Fourier transform infrared (FTIR) spectroscopy was performed with an IR Prestige 21 spectrometer (Shimadzu, Tokyo, Japan) to identify and analyze the functional groups in the sample. The surface structure of the imprinted electrode was examined using a field emission scanning electron microscope (FESEM) operated at an accelerating voltage of 10 kV.

TQ was quantified using reverse-phase high-performance liquid chromatography (RP-HPLC) analysis. The column temperature was maintained at 30°C, and an ultraviolet-visible (UV-Vis) detector (Absorbance detector 2487 and PDA detector 2996) with wavelength detection at 254 nm was employed.

### 3.4.3. Synthesis of TQ- imprinted graphite-based (TQIP/G) material and NIP material

The synthesis of the thymoquinone-imprinted polymer (TQIP) was initiated by dispersing graphite powder (0.95 g) in ethanol (15 mL) through ultrasonication for 60 minutes to ensure uniform dispersion. Subsequently, the template molecule thymoquinone (TQ, 0.05 g) and the functional monomer acrylonitrile (AN, 0.05 g) were added to the solution and ultrasonicated for an additional 50 minutes. Next, the cross-linking agent ethylene glycol dimethylacrylate

(EGDMA, 400  $\mu$ L) and the polymerization initiator benzoyl peroxide (1 mg) were introduced into the mixture, followed by ultrasonication for 50 minutes. The resulting mixture was heated at 60°C for 1 hour under a nitrogen atmosphere to facilitate polymerization. Once the polymerization was complete, the molecularly imprinted polymer (MIP) adduct was collected and subjected to template extraction. The template removal was performed by washing the polymer with ethanol until the complete removal of TQ was confirmed [51]. FTIR analysis was used to verify the successful removal of the template. For control experiments, a NIP was synthesized using the same procedure but without the addition of the template molecule (TQ). To optimize sensitivity, various monomer-to-template ratios were tested, and the resulting materials were evaluated using CV.

### **3.4.4. Fabrication of TQIP/G and NIP electrode**

To fabricate the electrode, 300 mg of the synthesized TQIP/G material was combined with a few drops of paraffin oil to form a fine paste [52]. This paste was then packed into a glass capillary tube with an inner diameter of 2.5 mm, ensuring compact filling using a metal rod. A platinum wire was attached to the back of the tube to establish the necessary electrical connection. Before each measurement, the electrode surfaces were carefully smoothened and rinsed with ultrapure Millipore water to ensure consistent performance.

The non-imprinted polymer (NIP) electrode was fabricated using the same procedure as the TQIP/G electrode. Buffer solutions required for the electrochemical studies were prepared following the method described by Naskar et al. [36]. The electrodes were preserved under vacuum in a desiccator at room temperature to maintain their stability and functionality.

### **3.4.5. Preparation of real sample**

Four different black cumin samples, labelled as B1, B2, B3, and B4, were purchased from the local market. Twenty grams of each sample were ground into a fine powder using a mixer grinder. Subsequently, 5 mg of each powdered sample was mixed with 20 mL of pure ethanol in separate beakers. The mixtures were ultrasonicated for 30 minutes to extract TQ from the black cumin dust.

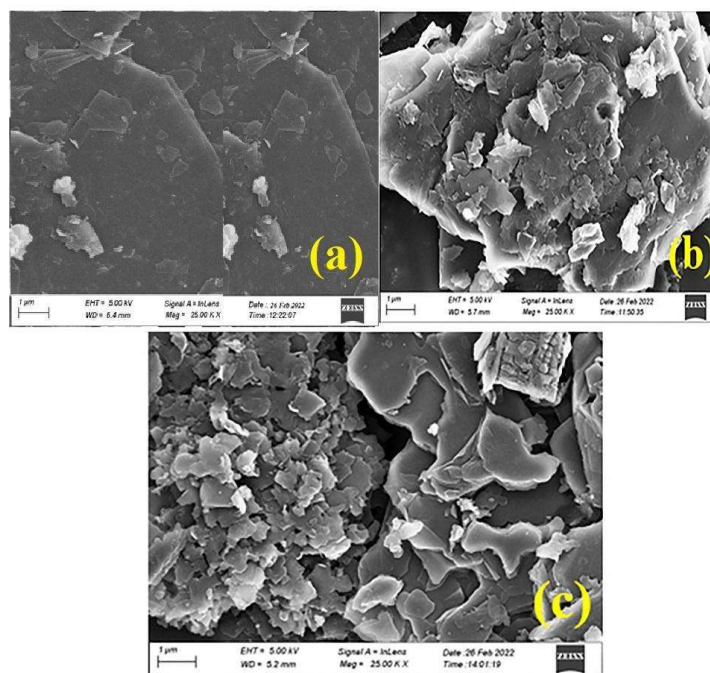
The extracts were then filtered using Whatman filter paper, and the filtrates were collected in four volumetric flasks, each labelled according to the sample. Stock solutions (100 mL each) were further prepared using phosphate-buffered saline (PBS) at pH 6. DPV analyses were

performed for each sample, and the experiment was repeated 10 times for each sample to ensure accuracy and reproducibility of the results.

### 3.5. Results with Molecularly Imprinted Polymer Electrode

#### 3.5.1. Surface morphology of TQIP/G material

The surface morphology of the TQIP/G material and the (NIP) was analyzed using FESEM. Fig. 3.18.(a) illustrates the surface of the NIP material, which lacks cavities, confirming the absence of template-induced features. In contrast, the TQIP/G material before and after template extraction is shown in Figs. 3.18.(b) and 3.18.(c), respectively.

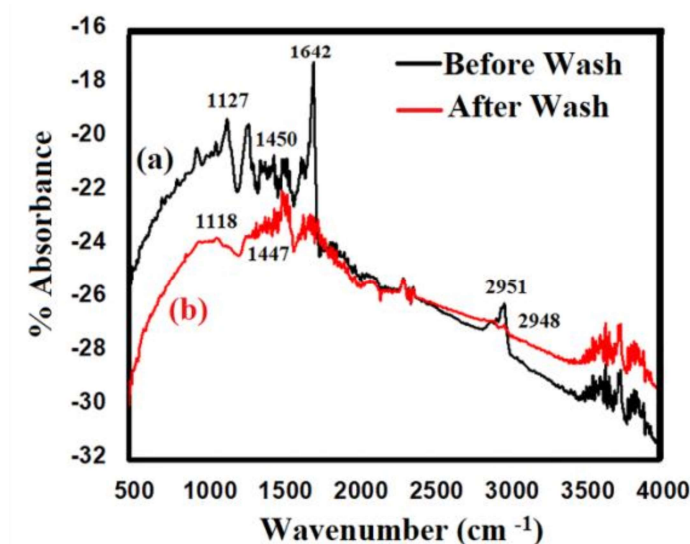


**Fig. 3.18. FESEM image of (a) NIP (b) TQIP/G adduct and (c) TQIP/G material.**

The pre-extraction TQIP/G surface appears relatively smooth and non-porous, characteristic of the polymer matrix containing the template molecule. However, after the removal of TQ using a washing solvent, the surface becomes notably rougher and exhibits visible cavities and porous structures. This transformation highlights the successful imprinting of TQ into the polymer and its subsequent extraction from the matrix. The observed morphological differences between NIP and TQIP/G materials underscore the effectiveness of the molecular imprinting process in creating selective binding sites. These structural features are crucial for the sensor's performance, as the cavities formed during imprinting enhance the material's ability to recognize and bind TQ with high specificity and sensitivity.

### 3.5.2. FTIR analysis of TQIP/G adduct and TQIP/G material

FTIR spectroscopy was conducted to confirm the templating effect and successful polymerization of the TQIP/G material. FTIR spectra of the TQIP/G adduct (before template removal) and the TQIP/G material (after template removal) are presented in Figs. 3.19.(a) and 3.19.(b), respectively. In Fig. 3(a), the TQIP/G adduct exhibits a sharp peak at  $1642\text{ cm}^{-1}$ , indicative of the presence of the template TQ within the polymer matrix. In contrast, Fig. 3.19.(b) shows no peak at this specific absorbance band, verifying the complete removal of TQ from the polymer surface. This observation strongly supports the effectiveness of the molecular imprinting process and the creation of selective binding sites in the polymer matrix.



**Fig. 3.19.** Comparison of FTIR spectra: spectrum of PAN (a) before removal of TQ and (b) after removal of TQ in the scan range of  $4000\text{--}500\text{ cm}^{-1}$ .

Additional absorption bands observed in Fig. 3(a) at  $2800\text{--}3000\text{ cm}^{-1}$  (symmetric stretching of  $\text{CH}_3$ ),  $1420\text{--}1460\text{ cm}^{-1}$  (antisymmetric bending), and  $900\text{--}1150\text{ cm}^{-1}$  (rocking vibrations) confirm the formation of a cross-linked polymer [53]. These characteristic peaks are also present in Fig. 3.19.(b), with negligible shifts, further validating the successful polymerization of monomers into the polymer. The FTIR analysis highlights the templating effect and structural integrity of the TQIP/G material, underscoring its potential for specific and sensitive detection of TQ. The findings confirm that the molecular imprinting process effectively creates well-defined binding sites tailored to the template molecule, which are essential for the sensor's performance.

### **3.5.3. Recognition phenomena of TQ using TQIP/G electrode**

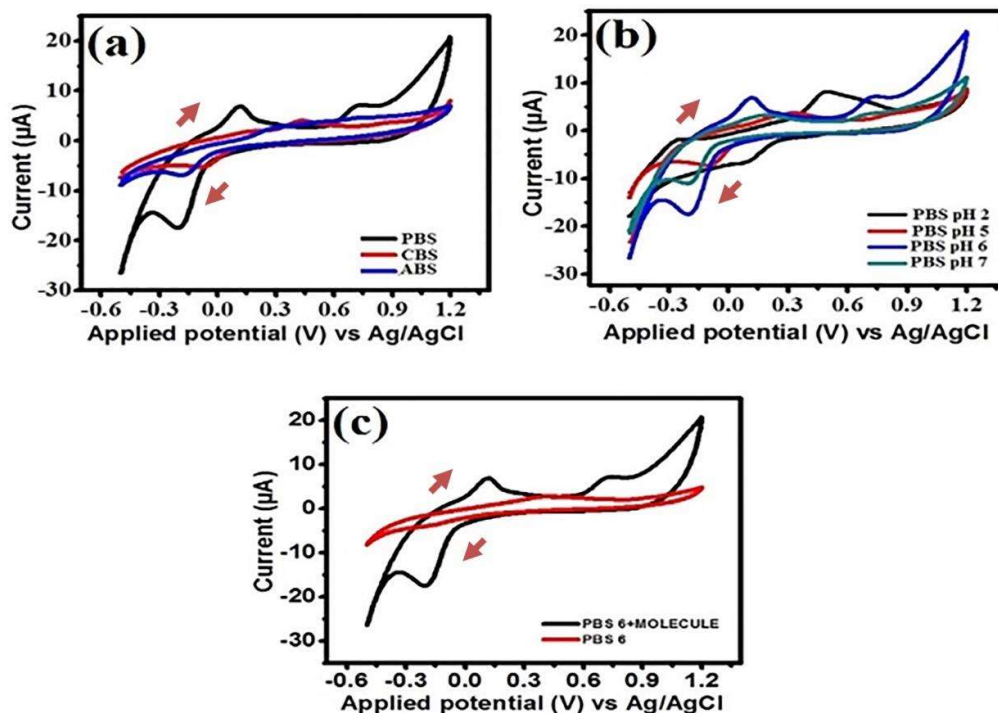
TQ contains a polar phenolic  $-C=O$  group, while ethylene glycol dimethylacrylate (EGDMA) and acrylonitrile (AN) possess polar nitrile and oxygenase groups. This structural complementarity enables dipole-dipole interactions during the pre-polymerization process. Upon polymerization, facilitated by the initiator benzoyl peroxide (BP), a graphite-embedded, TQ-caged, EGDMA cross-linked imprinted polymer is formed. This polymer structure is rigid and insoluble, featuring molecular grooves precisely tailored to accommodate TQ molecules. Following polymerization, the template TQ was extracted from the polymer matrix using an ethanol solution, leaving behind predefined molecular grooves within the TQIP/G material. During electrochemical detection, these molecular grooves selectively adsorb TQ, allowing the TQIP/G electrode to achieve high specificity and sensitivity in its recognition of the target molecule.

This molecular imprinting mechanism ensures the formation of well-defined binding sites that replicate the size, shape, and functional group orientation of the template molecule. This specificity is integral to the electrode's performance, providing a reliable platform for the electrochemical detection of TQ in complex samples.

### **3.5.4. Effect of buffer and pH**

The nature of the buffer solution and the pH of the medium significantly influence the CV response of the TQIP/G electrode. Among the buffer solutions tested—phosphate-buffered saline (PBS), acetate buffer, and citrate buffer—the TQIP/G electrode exhibited the highest and sharpest peak current ( $I_p$ ) when using PBS. This made PBS the optimal supporting buffer solution for studying the electro-catalytic oxidation of TQ throughout the investigation. To determine the optimal pH for the redox process, experiments were conducted at pH values of 2, 5, 6, and 7 (Fig. 3.20.(b)). The CV results for the TQIP/G electrode in 0.1 M PBS containing 200  $\mu$ M TQ at a scan rate of 50 mV/s (Fig. 3.20 (a)) revealed that oxidation peak potentials shifted negatively with decreasing pH. This observation reflects the dissociation of the phenolic  $-C=O$  group in TQ during oxidation. At higher pH levels, the phenolic group ionizes to form an anion, which is easier to oxidize, enhancing the electrocatalytic efficiency.

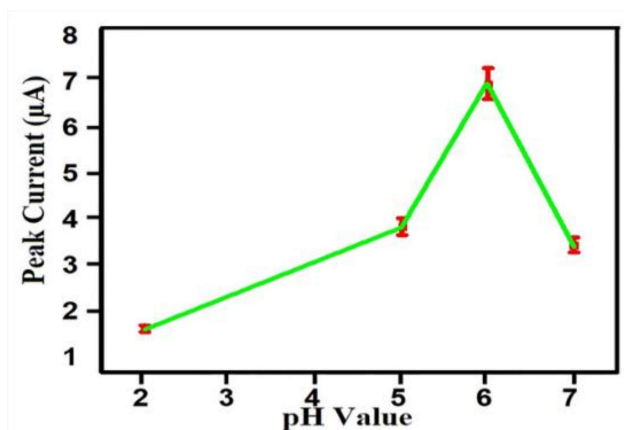




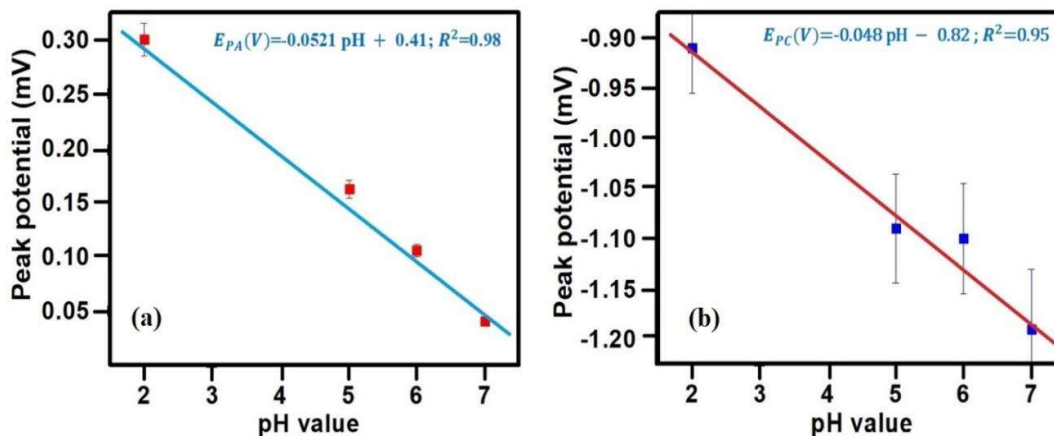
**Fig. 3.20.** (a) CVs of variation of peak current with different buffer solutions (PBS, CBS, ABS), (b) different CVs at different pH of PBS buffer (pH 2-7), (c) CVs of TQ with and without buffer solution.

However, at pH values above 6, such as pH 7, the peak current ( $I_p$ ) decreases due to weaker adsorption of TQ onto the electrode surface (Fig. 3.20.(b)). The plot of peak current versus pH (Fig. 3.21.) confirmed pH 6 as the optimal value for subsequent analyses. Fig. 3.20.(c) illustrates the CV response of the TQIP/G electrode with and without TQ (200 μM) in PBS at pH 6, highlighting the significant peak current enhancement upon TQ adsorption. The oxidative and reductive peak potentials were shifted to more negative values, as pH was increased from 2.0 to 7.0, indicating that protons are involved in the electrochemical reaction. There are linear relationships between the peak potentials and pH as follows:  $E_{PA}(V) = -0.0521 \text{ pH} + 0.41$  ( $R^2 = 0.98$ ) and  $E_{PC}(V) = -0.048 \text{ pH} - 0.82$  ( $R^2 = 0.95$ ). The slopes were found to be equal to -52.1 and -48.00 mV/pH which are close to the theoretical value of -59 mV/pH (Fig. 3.22). This behavior of TQ confirms that the electron transfer is accompanied by equal number of protons in the reaction. The number of protons ( $m$ ) involved is 1.80 ( $\approx 2$ ) obtained using the following equation:  $0.059m/n = 0.0621$  ( $\alpha = 0.99$ ,  $n =$

1.73)(Section: 3.6.). Based on these findings, it was concluded that the electro catalytic oxidation of TQ involves a two-electron, two-proton transfer process [54, 55].



*Fig. 3.21. Plot of peak current versus pH of TQIP/G electrode in 100 mM of TQ.*



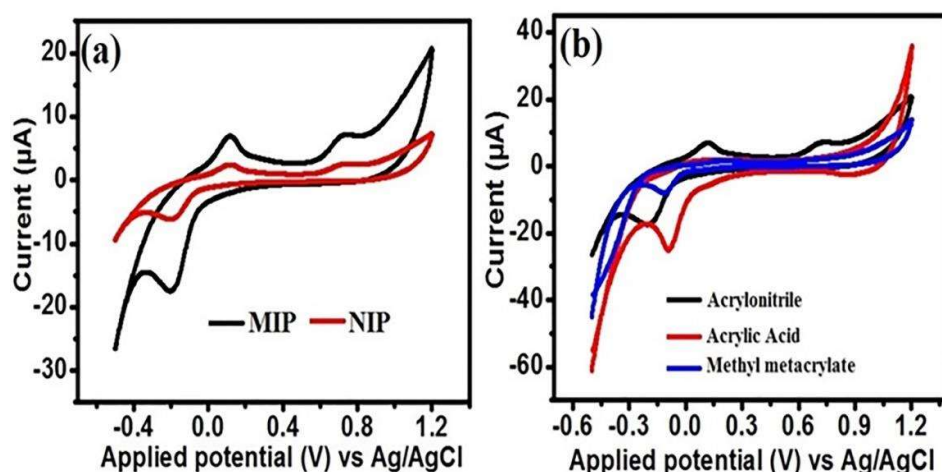
*Fig. 3.22. Linear plot of (a) oxidative and (b) reductive peak potential vs. pH value.*

### 3.5.5. Electrochemical properties of TQIP/G and NIP electrodes

To assess the electrocatalytic performance of the TQIP/G electrode, CV experiments were conducted in parallel with a non-imprinted polymer (NIP) electrode (Fig. 3.23.(a)). The TQIP/G electrode exhibited a well-defined, sharp oxidation peak with significantly higher current compared to the NIP electrode, underscoring its superior electrocatalytic activity. This enhanced response is attributed to the molecular cavities created during the template extraction process, which facilitate selective adsorption of TQ and efficient redox reactions at the electrode surface. In contrast, the NIP electrode, lacking these specific recognition sites,

exhibited a much lower current response, confirming the critical role of molecular imprinting in enhancing the electrode's sensitivity and selectivity.

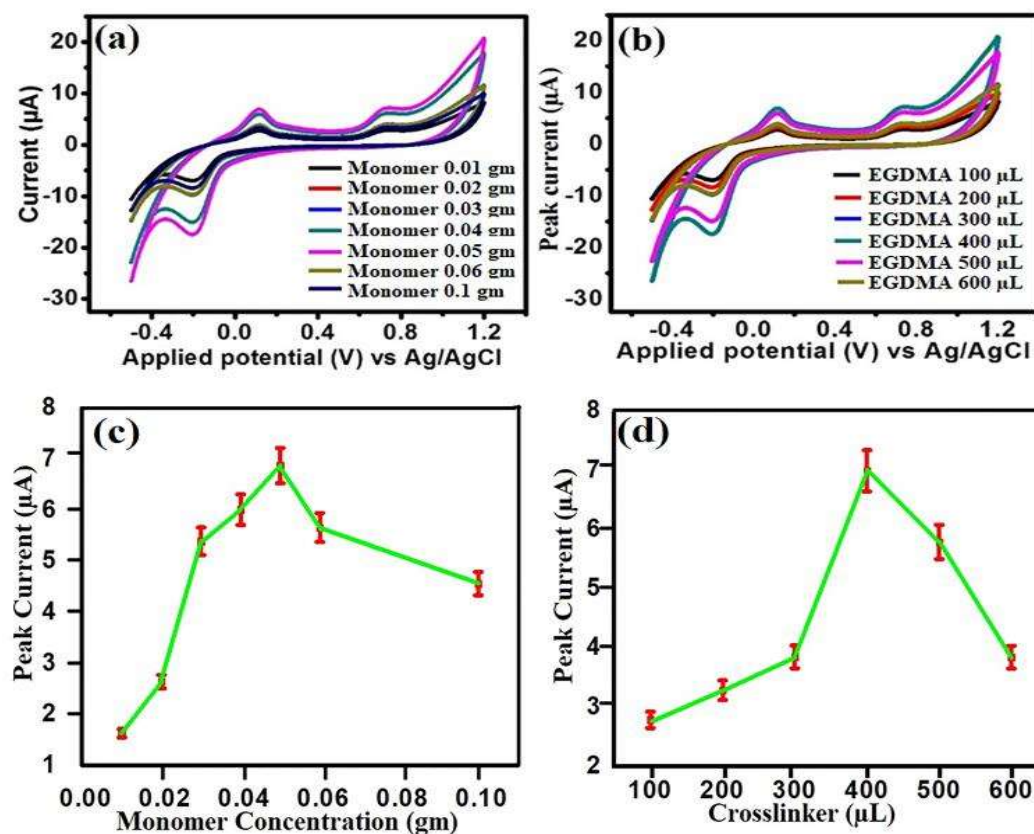
To further investigate the role of monomers in sensor performance, CV responses of molecularly imprinted polymers prepared with different monomers—acrylonitrile, acrylic acid, and methyl methacrylate—were compared (Fig. 3.23.(b)). The TQIP electrode fabricated using acrylonitrile demonstrated the highest peak current, indicating its superior electrochemical activity due to its optimal interaction with TQ during the imprinting process. In contrast, electrodes prepared with acrylic acid and methyl methacrylate exhibited lower responses, likely due to weaker template-monomer interactions or less effective cavity formation.



**Fig. 3.23. (a) Comparative response of TQIP/G and NIP/G electrode at 100  $\mu$ M, (b) CV response of MIP electrode with different monomer at 100  $\mu$ M TQ at scan rate 50 mVs<sup>-1</sup> with Ag /AgCl reference electrode.**

Optimization studies were conducted to determine the ideal compositions of the monomer and cross-linker, as shown in Fig. 3.24. (a) and (b). The results indicate that carefully tuning the monomer-to-cross-linker ratio enhances the electrode's sensitivity and redox efficiency. Line plots of peak current versus monomer concentration (Fig. 3.24.(c)) and versus cross-linker concentration (Fig. 3.24.(d)) provide quantitative insights into these optimizations. These findings collectively establish that the TQIP/G electrode, with its optimized composition and molecularly imprinted structure, offers superior electrochemical properties compared to the NIP electrode. The ability to tailor the electrode's performance by adjusting key fabrication

parameters further highlights its potential for practical applications in the sensitive and selective detection of TQ



**Fig. 3.24. Optimization of the TQIP/G electrode preparation: (a) Effect of monomer concentration (acrylonitrile) on electrode performance (b) Effect of cross-linker concentration (EGDMA) (c) Line plot of peak current versus monomer concentration, and (d) Line plot of peak current versus cross-linker concentration.**

### 3.5.6. Effect of scan rate

The effect of scan rate on the oxidation of TQ at the TQIP/G electrode was investigated using cyclic voltammetry (CV), as shown in Fig. 3.25.(a). The oxidation of TQ was found to be irreversible at the TQIP/G electrode, with the oxidation current ( $I_p$ ) increasing linearly with the scan rate in the range of 0.005–0.6 V/s in 0.1 M PBS at pH 6. This confirms that the oxidation process is diffusion-controlled.

With an increase in scan rate, the oxidation peak potential shifted positively, indicating a kinetic limitation. The number of electrons ( $n$ ) involved in the oxidation process was determined to be approximately 2, confirming that the electrochemical oxidation of TQ

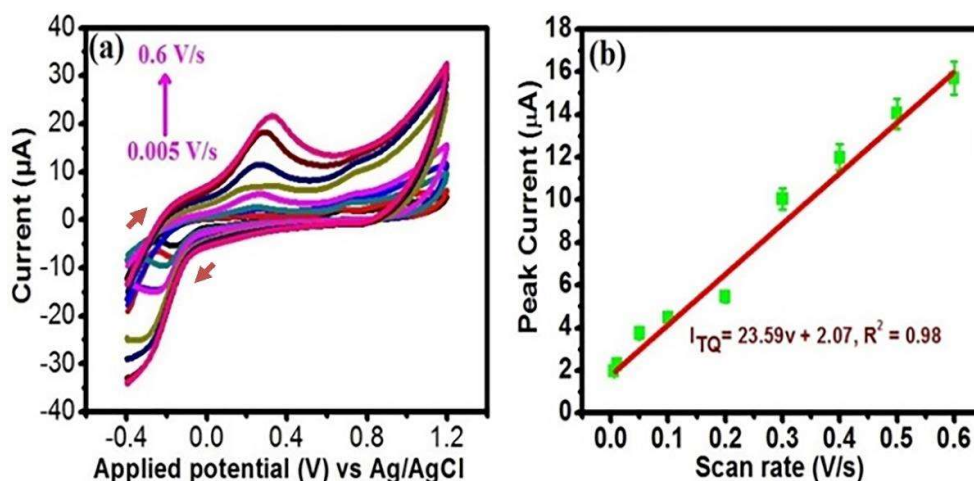
### Chapter 3: Detection of thymoquinone in black cumin

follows a two-electron transfer mechanism. The surface concentration of TQ ( $\Gamma_c$ ) adsorbed on the TQIP/G electrode was calculated using Eq. (3.7) and Eq. (3.8) [56]:

$$n = \frac{I_p \times 4RT}{FQv} \quad (3.7)$$

$$I_p = \frac{n^2 F^2 A v \Gamma_c}{4RT} \quad (3.8)$$

$$\log K_s = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha - \log \frac{RT}{nFv} - \frac{nF\Delta P \alpha(1 - \alpha)}{2.3RT} \quad (3.9)$$



**Fig. 3.25. (a) Cyclic voltammograms showing variation of peak current with scan rate (0.005-0.6 mVs<sup>-1</sup>) and (b) Linear plot of scan rate versus peak current at PBS 6, confirming a diffusion-controlled process.**

where, F is Faraday's constant (96,500 C/mol), R is the gas constant (8.314 J/K·mol), T is the temperature (300 K), A is the electrode area,  $\alpha$  is the electron transfer coefficient, Q is the charge consumed during oxidation, and v is the scan rate [57].

From these equations, n was calculated as 1.73, and the surface concentration ( $\Gamma_c$ ) was determined to be  $7.88 \times 10^{-10}$  mol/cm<sup>2</sup>. These values corroborate the earlier observations of a single oxidation peak for TQ. The heterogeneous rate constant ( $K_s$ ) of the oxidation reaction was calculated using Laviron's equation [58, 59] given in Eq. (3.9), yielding a value of  $5.6 \times 10^{-2}$  s<sup>-1</sup>. The electron transfer coefficient ( $\alpha$ ) was derived from the slope of the curve in Fig. 3.25.(b) and found to be 0.99, which is consistent with values reported in the literature [47]. These results validate the analytical data, confirming the reliability of the TQIP/G electrode for the electrochemical detection of TQ. The calculated response time was observed as 21.68 sec.

### 3.5.7. Analytical application of TQ

We evaluated the analytical performance of the TQIP/G electrode for the detection of TQ using DPV. The importance of this study lies in demonstrating the electrode's sensitivity, selectivity, and practicality for detecting TQ, which is a bioactive compound with significant pharmaceutical and analytical relevance. DPV was employed for enhanced resolution and superior current sensitivity compared to CV, owing to its ability to reduce non-faradaic currents. By incrementally increasing the concentration of the target TQ molecule, a series of DPV curves were recorded, as presented in Fig. 3.26.(a). These curves revealed a linear relationship between the oxidation peak current and the concentration of TQ, confirming the electrode's excellent sensitivity and reliability. This linear response is depicted in Fig. 3.26.(b).

The oxidation peak for the TQ molecule at the TQIP/G electrode was consistently observed at 720 mV, highlighting the electrode's specificity. The electrode demonstrated a broad linear response range from 0.5  $\mu\text{M}$  to 100  $\mu\text{M}$ , with the relationship governed by the regression equation:

$$I_{TQ} = 0.1C_{TQ} + 1.31; R^2 = 0.99 \quad (3.10)$$

The sensitivity of the electrode in this linear range was determined to be 0.1  $\mu\text{A}/\mu\text{M}$ , and the limit of detection (LOD) and limit of quantification (LOQ) was calculated using the formula:

$$\text{LOD} = \frac{3\sigma}{S} \text{ and } \text{LOQ} = \frac{10\sigma}{S}$$

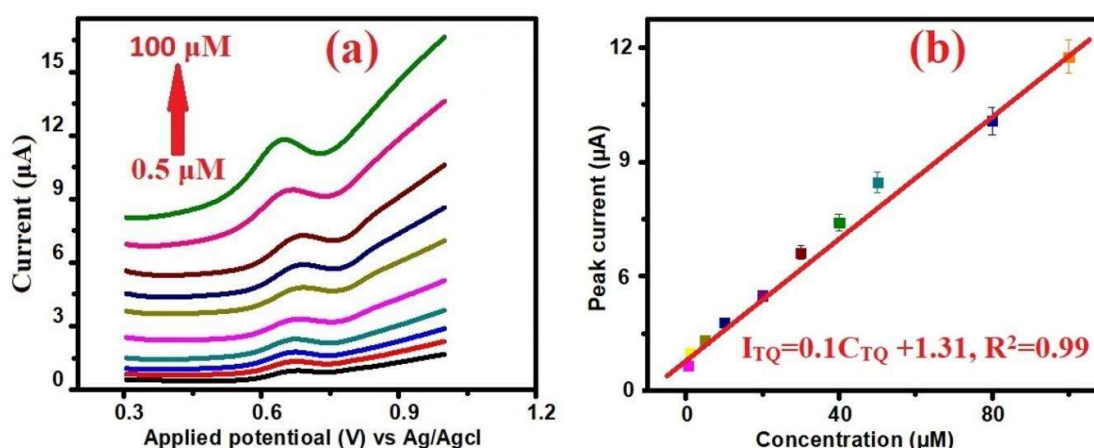
where  $\sigma$  is the standard deviation of the blank measurements and  $S$  is the slope of the calibration curve. Based on this approach, the LOD and LOQ was found to be 0.03  $\mu\text{M}$  and 0.099  $\mu\text{M}$  respectively.

These results underscore the electrode's ability to detect minute concentrations of TQ with high precision compared with other methods present in literature as shown in Table 3.4.



**Table 3.4.** Comparison of proposed MIP performance with the previous reports.

| Methodology                             | LOD (μM)    | Range of detection (μM) | Ref.             |
|-----------------------------------------|-------------|-------------------------|------------------|
| HPTLC                                   | 0.09        | 0.004-0.028             | [67]             |
| RP-HPLC                                 | -           | 32.94-368.30            | [68]             |
| Differential pulse polarographic method | 3           | 110–832                 | [61]             |
| <b>TQIP/G</b>                           | <b>0.03</b> | <b>0.5- 100</b>         | <b>This work</b> |



**Fig. 3.26.** (a) Calibrated DPV measurements for TQ using TQIP/G electrode in 0.1 M phosphate buffer (pH 6), with baseline correction (b) Corresponding calibration plot of TQ concentrations vs. current.

These results not only demonstrate the remarkable sensitivity and selectivity of the TQIP/G electrode for TQ detection but also highlight the broader applicability of MIP-based sensors. Such sensors have been widely used for detecting various analytes in environmental and food samples, showcasing their versatility and practical significance [60]. The robust analytical performance of the TQIP/G electrode - evidenced by its low detection limit, wide linear range, and high sensitivity - suggests that it could be effectively employed for monitoring TQ in pharmaceutical formulations, environmental matrices, or other complex systems. This study further validates the potential of nanocomposite-modified MIP electrodes as reliable tools for advanced electrochemical sensing applications.

### **3.5.8. Stability assessment: repeatability, reproducibility, and RSD analysis**

The stability, repeatability, and reproducibility of the TQIP/G electrode were evaluated to ensure its reliability and precision in practical applications. These factors are critical for validating the long-term usability of electrochemical sensors.

The repeatability of the electrode was assessed by repeatedly measuring the oxidation peak current of TQ at a concentration of 200  $\mu\text{M}$  over five successive trials ( $n=5$ ). The calculated relative standard deviation (RSD) was found to be 3.56%, as shown in Fig. 3.27.(b). This low RSD value reflects the electrode's excellent repeatability, which is vital for consistent and accurate measurements in real-world applications.

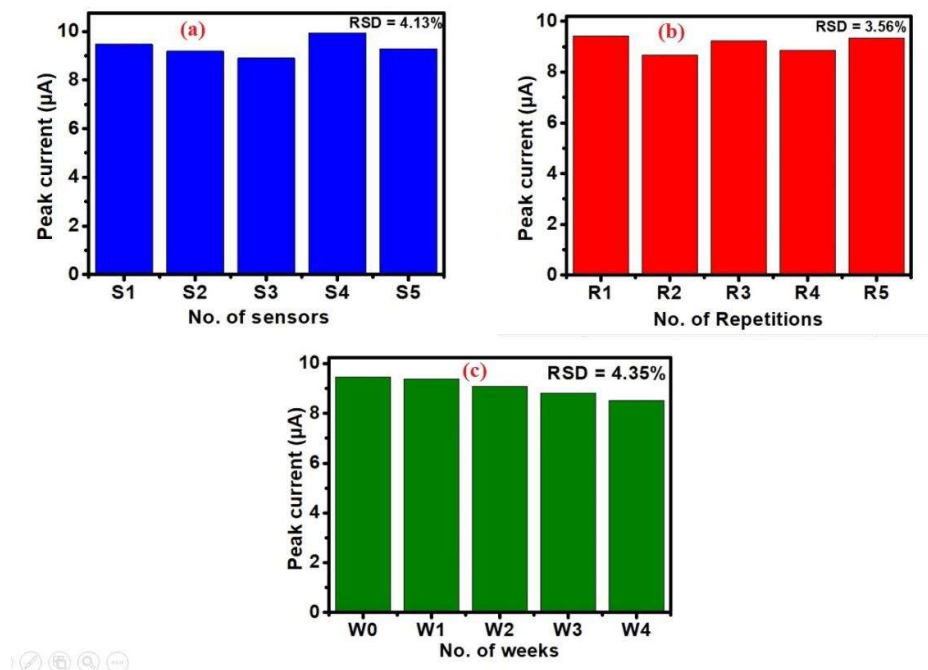
To assess reproducibility, five independent TQIP/G electrodes were fabricated using identical synthesis procedures and tested with a 200  $\mu\text{M}$  TQ solution. The results demonstrated minimal variation among the electrodes' responses, confirming the reproducibility of the fabrication process and ensuring consistent sensor performance with an RSD of 4.13% as shown in Fig. 3.27.(a). This capability is essential for practical deployment, as it indicates that the electrode preparation method is robust and reliable.

The stability of the TQIP/G electrode was investigated by preserving it at room temperature and monitoring its performance over time. Weekly evaluations revealed no significant deviation (an

RSD value of 4.35%) in the oxidation peak current after four weeks of storage (Fig. 3.27.(c)). This demonstrates that the electrode maintains its electrochemical performance over extended periods, making it suitable for long-term applications.

These findings confirm the robustness and reliability of the TQIP/G electrode, making it a strong candidate for practical sensing applications where precision, consistency, and durability are essential.





**Fig. 3.27. Bar plots with RSD values for (a) reproducibility, (b) repeatability and (c) stability measurement.**

### 3.5.9. Selectivity and Structural Analogue Discrimination of the TQIP/G Sensor

Selectivity is a critical parameter in evaluating the performance of a molecularly imprinted polymer (MIP) sensor, as it determines the sensor's ability to distinguish the target analyte in the presence of structurally related and unrelated compounds. In this study, selectivity was assessed by comparing the sensor's response to thymoquinone (TQ) with its response to several common phytochemicals found in black seed oil. Importantly, the analyte panel included compounds that are structural analogues of TQ. Specifically:

- Dithymoquinone (DTQ) – a dimerized derivative of TQ containing similar quinone functionalities.
- Thymohydroquinone (THQ) – a reduced quinone form of TQ.
- Thymol (TY) and Carvacrol (CL) – phenolic monoterpenes with structural resemblance in the aromatic core.
- Additionally,  $\alpha$ -pinene and  $\beta$ -pinene, which are not structurally similar to TQ, were included to represent common volatiles in black cumin oil.

### Chapter 3: Detection of thymoquinone in black cumin

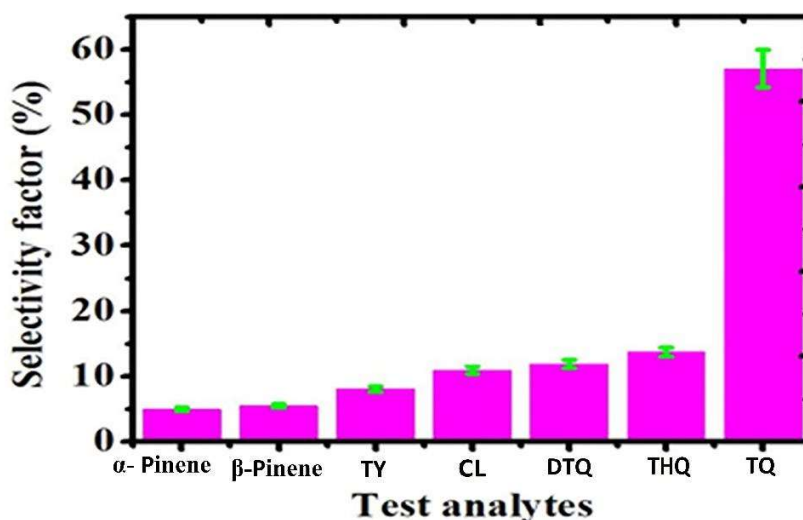
The relative selectivity (%STP) for each analyte was calculated using Eq.(5), which compares the current response of each analyte to the total response of all tested analytes:

$$\%S_{TP} = \frac{R_p}{\sum_{p=0}^T R_{TP}} \quad (5)$$

where, %STP is the relative selectivity, T= number of test volatiles (T=7), and  $R_p$  is the current response of a specific analyte.

The percent selectivity values were observed as follows:

- **$\alpha$ -pinene:** 4.94%
- **$\beta$ -pinene:** 5.44%
- **Thymol (TY):** 8.02%
- **Carvacrol (CL):** 10.95%
- **Dithymoquinone (DTQ):** 11.87%
- **Thymohydroquinone (THQ):** 13.69%
- **Thymoquinone (TQ):** 57.09%



**Fig. 3.28.** Bar-plot representation of selectivity analysis with  $\alpha$ - pinene,  $\beta$ -pinene, Thymol (TY), Carvacrol (CL), dithymoquinone (DTQ), Thymohydroquinone (THQ).

TQ exhibited the highest selectivity at 57.09%, significantly outperforming the other analytes. The sensor showed a significantly higher response to TQ compared to all other compounds, demonstrating a high degree of specificity. The relatively elevated responses to DTQ and THQ affirm the imprinting effect's selectivity toward quinone-based structures. These findings confirm that the MIP sensor effectively distinguishes TQ even in the presence

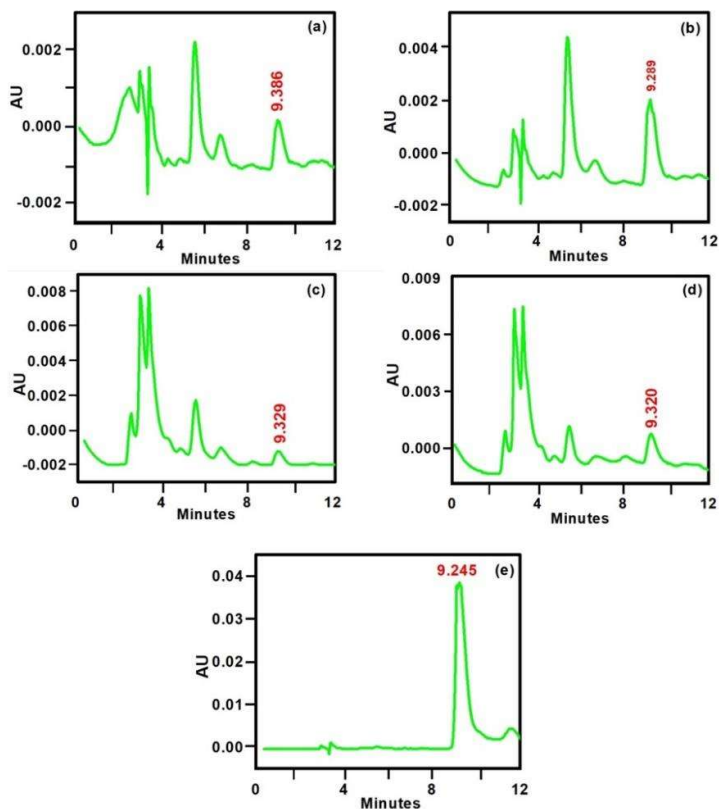
of closely related analogues, reinforcing its utility for precise TQ detection in complex natural matrices. Fig. 3.28. presents a bar plot summarizing the selectivity of the seven analytes, clearly illustrating the superior response for TQ compared to other compounds.

### ***3.5.10. Linearity and recovery studies using RP-HPLC technique***

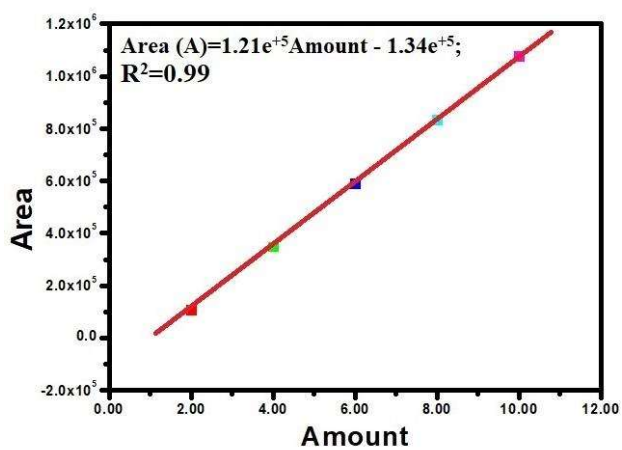
For the linearity study, standard solutions of thymoquinone were prepared at five different concentration levels (ranging from 2 to 10 ppm) in the mobile phase. A volume of 10  $\mu$ L from each prepared solution was injected into the HPLC system under the specified conditions. The chromatograms obtained for various black seed oil samples (B1, B2, B3, and B4) and the TQ standard are illustrated in Fig. 3.29. (a)-(e). The peak areas were plotted against the corresponding TQ concentrations to establish a calibration curve (Fig. 3.30.). The linear concentration range for TQ was found to be 2.19–10.30 ppm, with the regression equation derived from the calibration curve showing a coefficient of determination ( $r^2 = 0.99$ ), indicating excellent linearity.

The accuracy of the method was validated through recovery studies. A working standard of 213.30 ppm was analyzed, yielding a 95.70% recovery, which is well within the acceptable range for analytical methods. Additionally, the relative standard deviation (RSD) for the measurements was calculated as 6.04%, reflecting good precision of the RP-HPLC method.

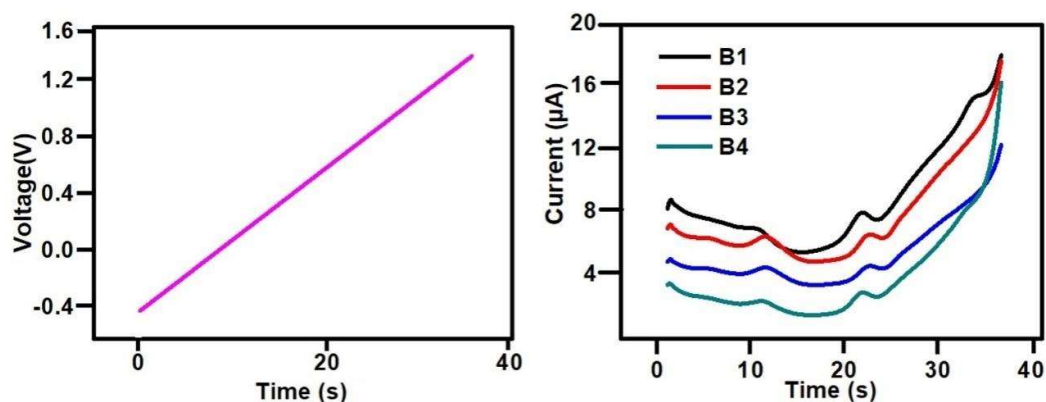
The study demonstrates that the RP-HPLC method is highly sensitive, precise, and accurate for the quantification of thymoquinone across a broad linear range. These results highlight the reliability of the method for analyzing thymoquinone content in black seed oil and related products, making it a valuable tool for quality control and standardization. By combining excellent linearity with robust recovery and precision metrics, the RP-HPLC method proves its efficacy for thymoquinone determination under optimized experimental conditions. The updated transient responses of the thymoquinone in real samples were included in Fig. 3.31.



**Fig. 3.29.** HPLC chromatograms for (a) B1, (b) B2, (c) B3, (d) B4, and (e) Thymoquinone standard.



**Fig. 3.30.** Plot of Peak Area Vs. Concentration of TQ.

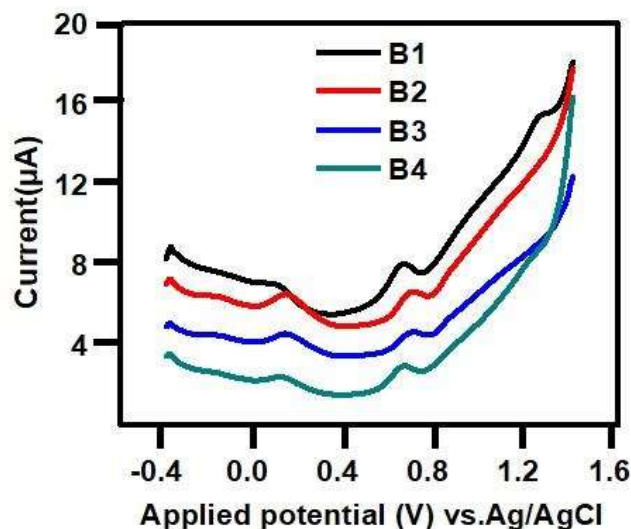


**Fig. 3.31.** Potential-excitation signal and corresponding resulting current of thymoquinone in real samples.

### 3.6. Machine learning experiment for TQ detection from real samples

The real-time applicability of the developed TQIP/G electrode was assessed by quantifying the TQ content in branded black cumin samples. Four certified black cumin varieties, labelled as B1, B2, B3, and B4, were sourced from the local market, adhering to Food Safety Standard Authority of India (FSSAI) regulations. To validate the sensor's performance, the TQ content in these samples was independently quantified using RP-HPLC, which served as the gold standard for comparison.

DPV signals were recorded for each black cumin sample following the preparation protocol outlined in Section 3.4.5. DPV responses of thymoquinone (TQ) extracted from four different black pepper brands (B1, B2, B3, and B4) recorded using the TQIP/G sensor are shown in Fig. 3.32. Each DPV signal comprised 358 data points corresponding to different input voltages, and the measurements were repeated 10 times for each brand, yielding an initial dataset of dimensions  $40 \times 358$ . Due to the imbalance between the number of samples (observations) and the length of features per sample, data augmentation was performed to enhance the dataset. To address this imbalance, dynamic time warping (DTW) [62] was employed for data augmentation. This method applied random warping and controlled noise to preserve the distinct characteristics of the original signals while generating additional synthetic data. This process expanded the dataset by 200 times for each sample type. The original and augmented data were combined to create a comprehensive training dataset of dimensions  $8040 \times 358$ .



**Fig. 3.32.** *Differential Pulse Voltammetry (DPV) responses of thymoquinone (TQ) extracted from four different black cumin brands (B1, B2, B3, and B4) recorded using the TQIP/G sensor in 0.1 M PBS (pH 6.0) at a scan rate of  $50 \text{ mV s}^{-1}$  with an Ag/AgCl reference electrode.*

To automate and improve the reliability of TQ quantification from voltammetric responses, we employed a machine learning approach that directly predicts TQ concentrations from raw DPV signals, bypassing the need for manual signal interpretation or peak analysis. This enables real-time data processing and minimizes the subjective variability typically introduced in conventional analytical workflows.

Among various machine learning algorithms, we selected a one-dimensional convolutional neural network (1D-CNN) [63] due to its superior capability to learn spatial and temporal dependencies in high-dimensional input signals. Unlike traditional models such as support vector machines (SVM), decision trees, or PCA-based regressors—which require handcrafted features like peak height, area, or derivatives—CNNs operate directly on the full signal profile. This eliminates the need for feature engineering, allowing the model to automatically extract relevant signal characteristics.

The augmented dataset was used to train the 1D-CNN model, with RP-HPLC measurements serving as the reference values for supervised learning. This data-driven architecture not only streamlines the analysis pipeline but also enhances robustness, particularly in complex matrices like black cumin oil where multiple overlapping phytochemicals may interfere with electrochemical signals.

The integration of CNN with the MIP-based electrochemical sensor offers a powerful hybrid strategy for precise, scalable, and portable TQ detection. It simplifies the analytical procedure and offers an edge in field-deployable systems where rapid, automated interpretation is essential.

### ***3.6.1. CNN model architecture***

A 1D-CNN [64, 65, 66] model was implemented to predict TQ concentrations from the DPV signals obtained using the TQIP/G electrode. The architecture of the 1D-CNN model, including the details of its layers, is illustrated in Fig. 3.33. The model's structure was optimized by systematically exploring various configurations, such as the arrangement of layers, filter counts, kernel sizes, strides, dropout rates, and the number of neurons in dense layers.

The final model comprises four convolutional layers. The first two convolutional layers utilize 32 filters each, while the third and fourth layers employ 64 and 128 filters, respectively. Pooling layers follow the third and fourth convolutional layers to capture essential signal patterns and extract high-level features. To mitigate overfitting, a dropout layer is included after the convolutional and pooling layers, followed by a flattening layer to prepare the data for dense layers. The model concludes with three fully connected dense layers responsible for the regression task, predicting TQ concentrations.

|                                    |                                                                |                                                                |                                                                |                                                        |                                                                  |                                                        |                            |                          |                               |                              |                                                   |
|------------------------------------|----------------------------------------------------------------|----------------------------------------------------------------|----------------------------------------------------------------|--------------------------------------------------------|------------------------------------------------------------------|--------------------------------------------------------|----------------------------|--------------------------|-------------------------------|------------------------------|---------------------------------------------------|
| Input DPV Signal<br>(None, 358, 1) | Conv1D_1 + ReLU<br>(F=32, K=3, S=1, P=same)<br>(None, 358, 32) | Conv1D_2 + ReLU<br>(F=32, K=3, S=1, P=same)<br>(None, 358, 32) | Conv1D_3 + ReLU<br>(F=64, K=3, S=1, P=same)<br>(None, 358, 64) | Max Pooling_1<br>(Pool Size=3, S=2)<br>(None, 179, 64) | Conv1D_4 + ReLU<br>(F=128, K=3, S=1, P=same)<br>(None, 179, 128) | Max Pooling_2<br>(Pool Size=3, S=2)<br>(None, 90, 128) | Dropout<br>(None, 90, 128) | Flatten<br>(None, 11520) | Dense_1 + ReLU<br>(None, 256) | Dense_2 + ReLU<br>(None, 32) | Dense_3 + Linear<br>(None, 1)<br>Predicted Output |
|------------------------------------|----------------------------------------------------------------|----------------------------------------------------------------|----------------------------------------------------------------|--------------------------------------------------------|------------------------------------------------------------------|--------------------------------------------------------|----------------------------|--------------------------|-------------------------------|------------------------------|---------------------------------------------------|

*Fig. 3.33. CNN Model architecture for TQ prediction from DPV signals*

### 3.6.2. Model training and hyperparameter optimization

The model's performance was evaluated using mean absolute error (MAE), root mean square error (RMSE), and the coefficient of determination ( $R^2$ ). During training, MSE served as the loss function. The model's hyperparameters were selected through an extensive grid search process, optimizing parameters such as kernel size, pooling size, stride, learning rate, batch size, dropout rates, and the number of filters in convolutional layers. Kernel sizes of 3 or 5 were selected to capture detailed signal features; while max-pooling layers with sizes of 2 or 3 and strides of 1 or 2 were employed to reduce dimensionality while retaining critical information. Dropout rates were optimized within the range of 0.1 to 0.5 to balance overfitting control and accuracy retention. Batch sizes of 32, 64, and 128 were tested to optimize computation efficiency and training speed. The Adam optimizer, known for its robust convergence properties, was chosen, and training was conducted for up to 100 epochs with early stopping criteria to prevent overfitting. The optimal hyperparameter set was obtained by selecting the one that yielded the lowest average MAE across 10-fold cross-validation, ensuring optimal generalization.

### 3.6.3. Performance evaluation of CNN model

Once the optimal hyperparameter set is selected, we used it to evaluate the performance of the model using 10-fold CV. Additionally, to assess the model's consistency across diverse data distributions, the experiment was repeated 50 times by dividing the data set into training and testing subsets with a 70:30 ratio, applying stratified random sampling before each run. During model training, 20% of the training set was used as the validation set. For both 10-fold CV and repeated random training and testing, the training data were normalized to the



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range [0, 1], where the testing data were normalized using the minimum and maximum values derived from the training set. The target values in the training set were scaled to the range [-1, 1] based on its minimum and maximum values, with identical scaling factors applied to the testing set. The mean and standard deviation of MAE, RMSE and  $R^2$  were computed for both the training and testing sets across all experimental runs. The results from both 10-fold cross-validation and 50 random runs, are summarized in Table 3.5., providing a detailed evaluation of the model's robustness and generalization ability. The results indicate that there is minimal difference between the outcomes of 10-fold CV and multiple random runs, further supporting the model's reliability. The result shows that there is practically no difference between the results of 10-fold CV and multiple random runs of the experiment.

**Table 3.5. Performance of CNN predictive model on training and testing set.**

| Evaluation Method           | Training MAE( $\pm$ Std) | Testing MAE( $\pm$ Std) | Training RMSE( $\pm$ Std) | Testing RMSE ( $\pm$ Std) | Training R2( $\pm$ Std) | Testing R2( $\pm$ Std) |
|-----------------------------|--------------------------|-------------------------|---------------------------|---------------------------|-------------------------|------------------------|
| <b>10-foldCV</b>            | 0.0513<br>$\pm 0.0354$   | 0.0503<br>$\pm 0.0343$  | 0.0701<br>$\pm 0.0470$    | 0.0700<br>$\pm 0.0469$    | 0.9997<br>$\pm 0.0004$  | 0.9997<br>$\pm 0.0004$ |
| <b>50 random train-test</b> | 0.0579<br>$\pm 0.0298$   | 0.0580<br>$\pm 0.0297$  | 0.0778<br>$\pm 0.0394$    | 0.0779<br>$\pm 0.0394$    | 0.9996<br>$\pm 0.0003$  | 0.9996<br>$\pm 0.0003$ |

The CNN model consistently achieved low MAE and RMSE values across both training and testing sets, with  $R^2$  values approaching 1.0, indicating near-perfect predictive accuracy. Notably, the minimal differences between the average metrics for training and testing sets demonstrate that the model generalizes well to unseen data without overfitting. This robustness is further supported by the low standard deviations observed in all performance metrics across 10-folds or 50 independent runs, each with randomly permuted datasets.

These results underline the model's stability and reliability in predicting TQ concentrations, even under multiple training/testing partitions. The use of stratified sampling and random shuffling ensures a comprehensive evaluation, reinforcing the CNN model's capability to handle the complex DPV signal data effectively. The combination of high accuracy, low variability, and generalization across testing data highlights the suitability of this approach for real-world applications in TQ quantification. This consistent performance makes the CNN

model a dependable tool for analyzing electrochemical sensor data, bridging the gap between experimental observations and accurate TQ concentration predictions.

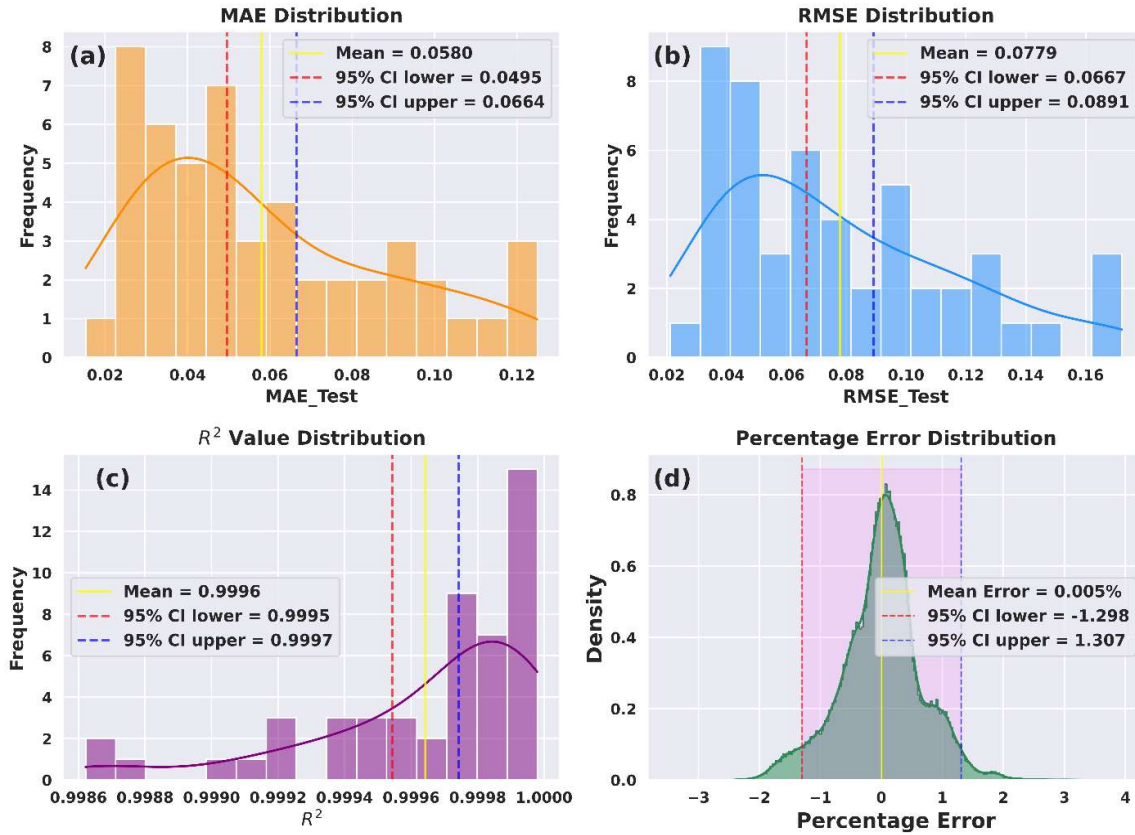
### 3.6.4. Analysis of CNN performance

To assess the performance of the CNN model, we analyze four key statistical metrics: MAE, RMSE,  $R^2$  and percentage error across 50 random runs. Fig.3.34. illustrates the distribution of these metrics across the test dataset.

**MAE distribution:** The MAE quantifies the average magnitude of errors without considering their direction. As seen in Fig. 3.34.(a), the mean MAE value is 0.0580, with a 95% confidence interval (CI) ranging from 0.0495 to 0.0664. The histogram indicates a slightly skewed distribution with a peak at lower error values, suggesting that the model exhibits small but consistent deviations from actual values.

**RMSE distribution:** RMSE measures the standard deviation of residuals and penalizes larger errors more significantly than MAE. Fig. 3.34.(b) depicts an RMSE mean of 0.0779, with a 95% CI of 0.0667 to 0.0891. The histogram shows a concentration of RMSE values in the lower range, indicating that extreme errors are relatively rare, and the model provides stable predictions.

**$R^2$  Value distribution:** The  $R^2$  value, or coefficient of determination, represents how well the model explains the variance in the dataset. Fig. 3.34.(c) shows an exceptionally high mean  $R^2$  of 0.9996, with a 95% CI spanning from 0.9995 to 0.9997. This suggests that the CNN model captures almost all variations in the dataset, reinforcing its robustness and strong predictive power.



**Fig. 3.34.** Statistical analysis of CNN model performance based on 50 random runs of the experiment.

**Percentage error distribution:** The percentage error distribution, presented in Fig. 3.34.(d), reflects the relative deviation of predictions from true values. The mean percentage error is 0.005%, with a 95% CI ranging from -1.298% to 1.307%. This narrow interval reflects the model's high reliability and precision, ensuring that predictions remain accurate even under diverse testing conditions. The symmetrical and sharp distribution of percentage errors further highlights the stability of the CNN model when applied to unseen data.

The statistical analysis of the CNN model demonstrates exceptional predictive accuracy, as reflected in the low MAE and RMSE values, an outstanding  $R^2$  score close to unity, and minimal percentage errors. The 95% confidence intervals further confirm the reliability and consistency of the model across the test dataset. Overall, the CNN effectively generalizes to unseen data while maintaining precise and stable predictions.

### 3.7. Conclusion

This work successfully introduces two novel electrochemical platforms—the TiO<sub>2</sub>-modified graphite paste electrode (GPE/TiO<sub>2</sub>) and the thymoquinone-imprinted polymer graphite electrode (TQIP/G)—for the sensitive and selective detection of thymoquinone (TQ) in black cumin. Both sensors exhibited remarkable analytical performance, achieving low limits of detection (0.02  $\mu$ M and 0.03  $\mu$ M, respectively), wide linear detection ranges, high sensitivity, and robust repeatability and reproducibility. The use of Principal Component Analysis (PCA) further demonstrated their ability to differentiate between black cumin samples from various brands with a significant Separability Index (SI) of 67.89.

Importantly, the integration of a convolutional neural network (CNN) model to interpret differential pulse voltammetry (DPV) signals provided superior predictive accuracy, closely matching RP-HPLC reference results with minimal error margins. These findings position the developed sensors as promising, low-cost, and efficient alternatives to conventional chromatographic methods for TQ analysis.

While the results are promising, further work is needed to assess long-term sensor stability, real-world matrix interference, and sensor performance under diverse environmental conditions. Future research should also focus on automating sensor–AI integration for real-time applications. Nevertheless, the approaches presented here offer a transformative step toward scalable, portable, and accessible quality control systems for food, pharmaceutical, and agricultural industries.

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## Chapter 4

### Design and development of a low-cost potentiostat

This chapter presents a portable, low-cost potentiostat system (LC-PSTAT) integrated with a molecularly imprinted polymer-based graphite sensor (TQMIP-G) for the selective detection of thymoquinone (TQ). The system offers high sensitivity, with a detection limit of 0.03  $\mu\text{M}$  and a broad linear range of 1–200  $\mu\text{M}$ . Electrochemical techniques like CV and DPV confirmed its accuracy and reliability. Ideal for on-site applications, like pharmaceutical quality control, food safety, and agricultural monitoring.

#### List of Sections

- 4.1. Introduction
- 4.2. Design and Development of low-cost potentiostat (LC-PSTAT)
- 4.3. Experiment with LC-PSTAT
- 4.4. Results of LC-PSTAT
- 4.5. Conclusion
- References

#### **Contents of this chapter are based on mentioned publication:**

**Sanjoy Banerjee**, Milan Dhara, Hemanta Naskar, Barnali Ghatak, Nityananda Das, Santanu Ghorai, Bhimsen Tudu, Arpitam Chatterjee, Rajib Bandyopadhyay, Bipan Tudu, “Design and Development of a Low-Cost Potentiostat for Thymoquinone (TQ) Detection in Black Cumin Using a Graphite-Based TQ-Imprinted Polymer Sensor” Measurement Journal, 257, (2026), 118870.



### 4.1. Introduction

Thymoquinone (TQ) is the key bioactive compound in black cumin (*Nigella sativa*) seed oil and is mainly responsible for its medicinal effects [1-2]. It shows strong antioxidant, anti-inflammatory, antibacterial, and immune-boosting properties [3-6]. TQ also reduces stress and supports memory and learning [7-8]. It has been used to manage headaches, cancer, asthma, diabetes, obesity, and digestive disorders, and recent studies suggest its potential against COVID-19 [9-10].

Different analytical methods such as HPLC, HPTLC, RP-HPLC, GC–MS, NIR, and TLC have been used to detect and measure TQ [11-16]. Although accurate, these methods are costly, require complex instruments, and involve lengthy sample preparation. In contrast, electrochemical methods are faster, cheaper, and easier to use, with good sensitivity and selectivity [17-18].

To further improve sensitivity and selectivity, molecularly imprinted polymers (MIPs) are used in sensor development. MIPs offer high selectivity, low cost, and good stability. Many studies have shown the effectiveness of MIP-based electrochemical sensors for detecting TQ [19-24].

Potentiostats play a crucial role in electrochemical sensing by converting sensor outputs into analog signals while maintaining operational stability. Conventional potentiostats are often bulky, expensive, and require sophisticated instrumentation for data acquisition and analysis. However, there is an increasing demand for portable, low-cost devices capable of real-time, on-site analysis. Several commercial potentiostats, including the EmStat3+ (Palm Instruments) [25], PG581 (Uniscan Instruments) [26], 910 PSTAT mini [27], Autolab PGSTAT101 [28], and  $\mu$ Stat-i 400s (Metrohm DropSens) [29], are available but remain costly and often require external devices for data processing via USB or Bluetooth. To overcome these limitations, recent research has focused on developing standalone, low-cost potentiostats that do not rely on proprietary software or external data acquisition systems. Portable electrochemical analyzers are essential in applications such as clinical diagnostics, environmental monitoring, and food safety where they help reduce analysis costs and simplify sample processing. Several studies [30-36] have reported advancements in low-cost potentiostat design.

## Chapter 4: Design and development of a low-cost potentiostat

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The development of cost-effective potentiostats involves the use of affordable components such as microcontrollers and operational amplifiers (OPAMPs), including the ATmega 328 processor and LF347 OPAMPs. While these components help lower costs, they introduce challenges such as reduced dynamic range and limited precision [37]. More advanced OPAMPs with rail-to-rail technology can improve performance but at a higher cost [38]. Thus, a key challenge in potentiostat development is balancing cost, performance, and usability, particularly for applications requiring high sensitivity and flexibility.

The primary objective of this research is to design, develop, and validate a cost-effective, portable potentiostat system coupled with the TQMIP-G sensor for the rapid and selective detection of thymoquinone (TQ) in complex matrices. This work addresses key challenges in electrochemical sensing by integrating molecularly imprinted polymer (MIP) technology with an optimized low-cost electrochemical analyzer, ensuring high sensitivity, specificity, and stability.

A major contribution of this study is the development of a standalone, portable electrochemical detection system that eliminates the need for expensive commercial potentiostats and proprietary data processing software. The integration of the TQMIP-G sensor with a custom-built, low-power potentiostat significantly reduces operational costs while maintaining analytical performance comparable to conventional laboratory-based systems. This innovation holds immense potential for industrial applications, particularly in the pharmaceutical, nutraceutical, and food industries, where rapid and on-site quality assessment of bioactive compounds like TQ is crucial.

Furthermore, this work advances the field of electrochemical sensing by optimizing the MIP synthesis process to enhance sensor selectivity and stability. The proposed system is particularly relevant for industries requiring cost-effective and scalable solutions for real-time quality control and regulatory compliance. By bridging the gap between high-performance electrochemical analysis and industrial feasibility, this study contributes to the development of next-generation, portable sensing technologies for pharmaceutical and food safety applications.



### **4.2. Design and development of low-cost potentiostat (LC-PSTAT)**

#### ***4.2.1. Background of the electrochemical sensing***

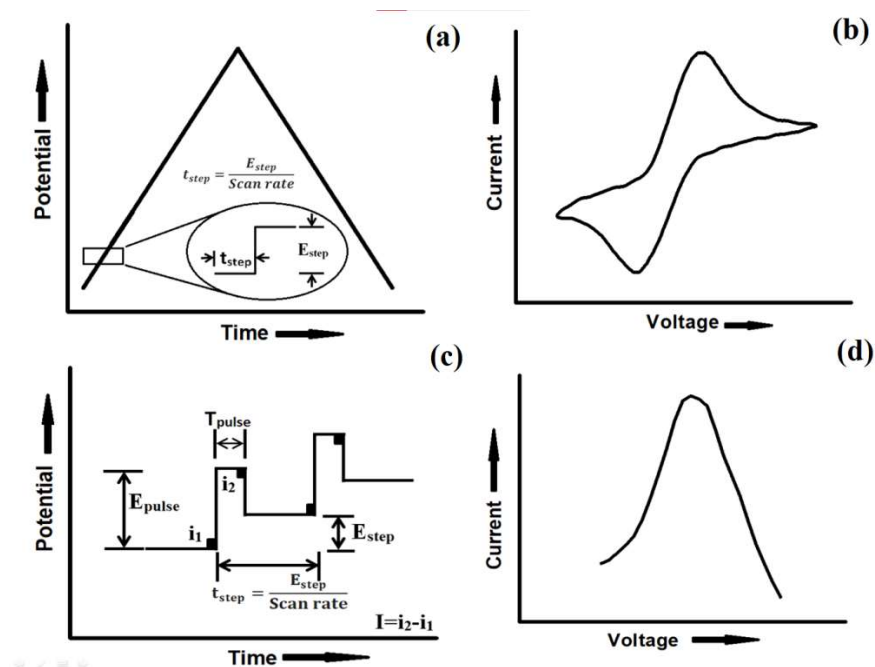
Electrochemical processes involve redox reactions where oxidation (electron loss) and reduction (electron gain) occur simultaneously, converting chemical energy into electrical energy. Electrochemical techniques are classified into potentiometry, which measures the electrochemical cell's potential under static conditions to study ionic activity; coulometry, which involves complete oxidation/reduction of an analyte; and voltammetry, which applies potential and measures current for quantitative and qualitative sensor analysis. Voltammetry, a subclass of amperometry, employs a three-electrode system: counter electrode (CE), working electrode (WE), and reference electrode (RE). A potentiostat maintains the potential between WE and RE using a feedback mechanism while measuring the induced current between WE and CE. A current-to-voltage (I-to-V) converter translates this current into a voltage signal. In this study, a low-cost potentiostat (LC-PSTAT) was developed for voltammetric analysis in applications such as food safety, water quality, and pharmaceutical testing [39].

To characterize the developed sensor, CV and DPV were employed. CV involves linearly ramping potential to a peak value and reversing it, generating a cyclic voltammogram that reveals redox potential and reaction kinetics. DPV, which superimposes small pulses on a linear ramp, enhances sensitivity by eliminating background currents, producing sharper peaks for analyte concentration analysis. The excitation signals and corresponding voltammograms of CV and DPV are illustrated in Fig. 4.1.

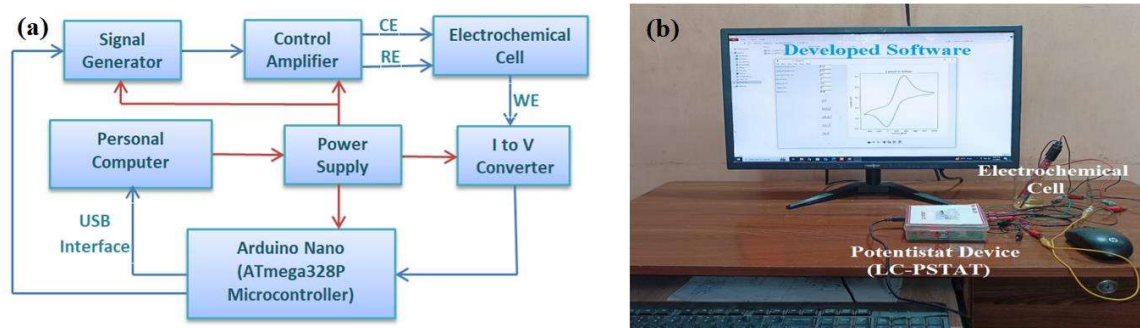
#### ***4.2.2. Design and development of the measurement system***

The LC-PSTAT system was designed for CV and DPV to enable selective and sensitive analysis of thymoquinone in black cumin. As shown in Fig. 4.2(a), the system consists of three major components: LC-PSTAT hardware, an electrochemical cell, and a personal computer with a software for data analysis and visualization.

The LC-PSTAT hardware communicates with the PC via a USB interface, allowing seamless data transfer and real-time monitoring. Fig. 4.2(b) illustrates the experimental setup, where the LC-PSTAT electronic hardware is connected to both the electrochemical cell and the PC for efficient measurement and analysis.



**Fig.4.1.** (a) CV excitation signal potential and (b) it's voltammogram, (c) DPV excitation signal potential and (b) it's voltammogram.



**Fig. 4.2.** (a) Block diagram of the LC-PSTAT system, and (b) Experimental setup of the LC-PSTAT system.

### 4.2.3. *Electronic hardware*

The low-cost potentiostat (LC-PSTAT) is designed using an ATmega328-based microcontroller (Arduino Nano), an LF347 operational amplifier, and a few passive components. The hardware comprises power supply modules, a signal generator, a control amplifier, and a current-to-voltage (I/V) converter.

- **Power Supply and Voltage Regulation:** The system is powered via a USB Type-B connector connected to a PC. Some circuit components require a negative voltage supply, which is provided by the TC7660 integrated circuit. This IC converts a +5V input to a -5V output, ensuring the LF347 op-amp receives a  $\pm 5V$  supply for proper operation.
- **Signal Generation and Voltage Control:** The signal generator produces PWM signals to generate required voltage levels for redox reactions, ranging from -2V to +3V. These PWM signals are processed through a low-pass filter, converting them into three analog signals:
  - $V_D$ : Lower threshold voltage
  - $V_{TR}$ : Triangular wave signal
  - $V_S$ : Shifted voltage (adjusted to range from **0V to 5V**)

The triangular wave's peak value is given by  $(V_H + |V_L|)/2$ , where  $V_H$  and  $V_L$  are the upper and lower threshold voltages, respectively. To achieve the necessary voltage range for the electrochemical cell (e.g., -1V to +1V), a differential amplifier adjusts the output using  $V_{OUT} = 2V_{TR} - V_D$ . The LF347 op-amp is chosen for its high input impedance, high slew rate, low bias current, and cost-effectiveness. The control amplifier maintains the desired potential difference between the reference and working electrodes, injecting current via the counter electrode to stabilize the electrochemical cell voltage.

- **Current Measurement and ADC Conversion:** The current-to-voltage (I/V) converter measures the cell current flowing between the counter and working electrodes. The current passes through a measurement resistor ( $R_m$ ), with the voltage drop across  $R_m$  providing a direct measure of the cell current ( $V_w$ ). Since current

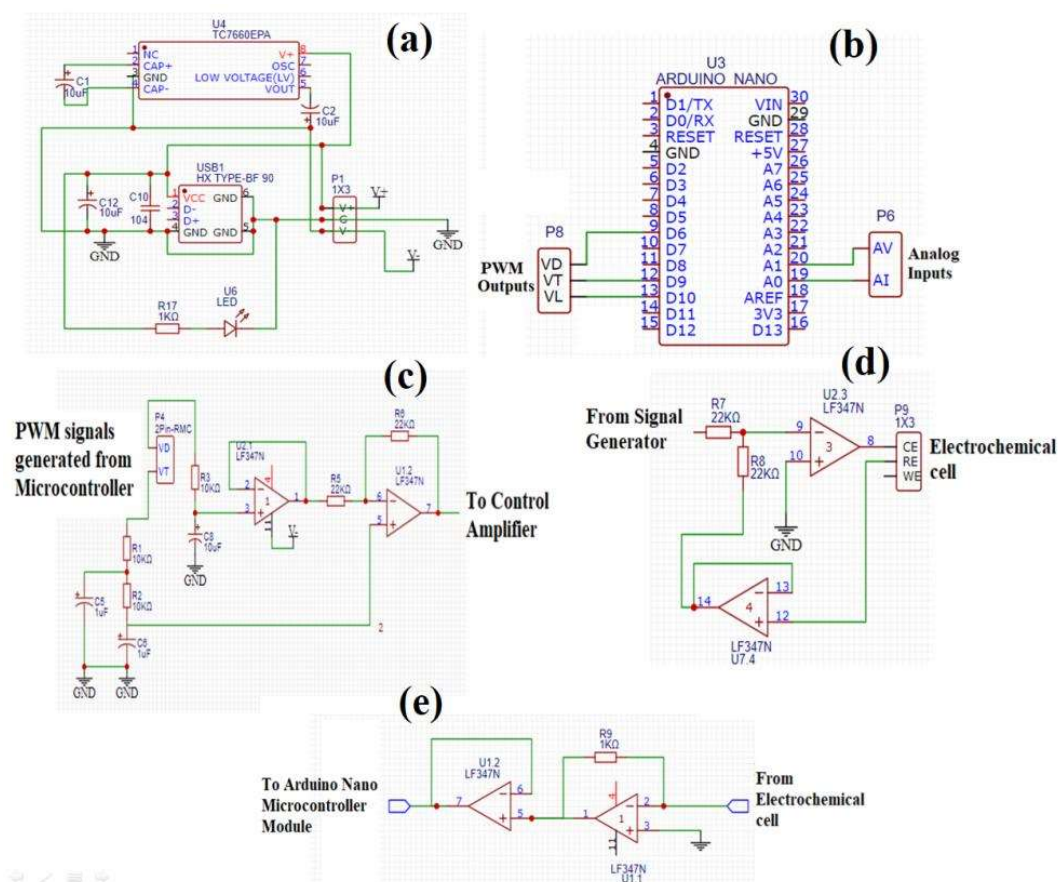
## Chapter 4: Design and development of a low-cost potentiostat

levels may vary significantly across experiments, different  $R_m$  values can be selected manually to accommodate a wide range of current magnitudes. The I/V converter circuit design ensures compatibility with the ATmega328 microcontroller's sensitivity. The resulting voltage is processed through a summing amplifier, adjusting it within a 0V to 5V range before feeding it into the ATmega328's ADC for digital conversion.

A comparative analysis of LC-PSTAT with existing systems is provided in Table 4.1, and the circuit diagram illustrating different hardware modules is shown in Fig. 4.3.

**Table 4.1. Specification Comparison of the proposed LC-PSTAT with existing devices.**

| Specifications       | [25]                          | [26]                          | [28]                          | [29]                          | This work                |
|----------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------|
| Potential Range      | $\pm 3V$                      | $\pm 8V$                      | $\pm 10V$                     | $\pm 4V$                      | $\pm 5V$                 |
| Potential Resolution | 0.1 mV                        | 0.061mV                       | 3 $\mu V$                     | 1 mV                          | 5mV                      |
| Current Ranges       | 1 nA to<br>20 mA              | 1 nA to<br>20 mA              | 10 nA to<br>10 mA             | 1 nA to<br>10 mA              | 50 $\mu A$<br>– 5mA      |
| Current Resolution   | 0.1% of<br>current Range      | 61 fA                         | 0.0003% of<br>current range   | 0.025% of<br>current range    | 0.5% of<br>current range |
| Maximum current      | $\pm 15$ mA                   | $\pm 20$ mA                   | $\pm 100$ mA                  | $\pm 40$ mA                   | $\pm 5$ mA               |
| Working Electrode    | one                           | 2/3                           | one                           | one                           | one                      |
| Type                 | Potentiostat /<br>Galvanostat | Potentiostat /<br>Galvanostat | Potentiostat /<br>Galvanostat | Potentiostat /<br>Galvanostat | Potentiostat             |



**Fig. 4.3. (a) Circuit diagram of different module of the LC-PSTAT: (b) Power supply Microcontroller module (c) Signal generator (d) Control amplifier and (e) Current to voltage converter.**

### 4.2.4. Software

The LC-PSTAT 101 software, developed for the low-cost potentiostat (LC-PSTAT), offers a user-friendly interface and a customizable design to facilitate electrochemical analysis. The software follows an analytical protocol and is developed using Python, while the device's firmware is programmed in the Arduino Integrated Development Environment (IDE). The Arduino-based firmware receives CV and DPV parameters from the user via a PC interface, reads analog voltage and current data from the onboard ADC, and transmits the measured data back to the PC for storage and real-time visualization. The flowchart for CV and DPV measurement is illustrated in Fig. 4.4.

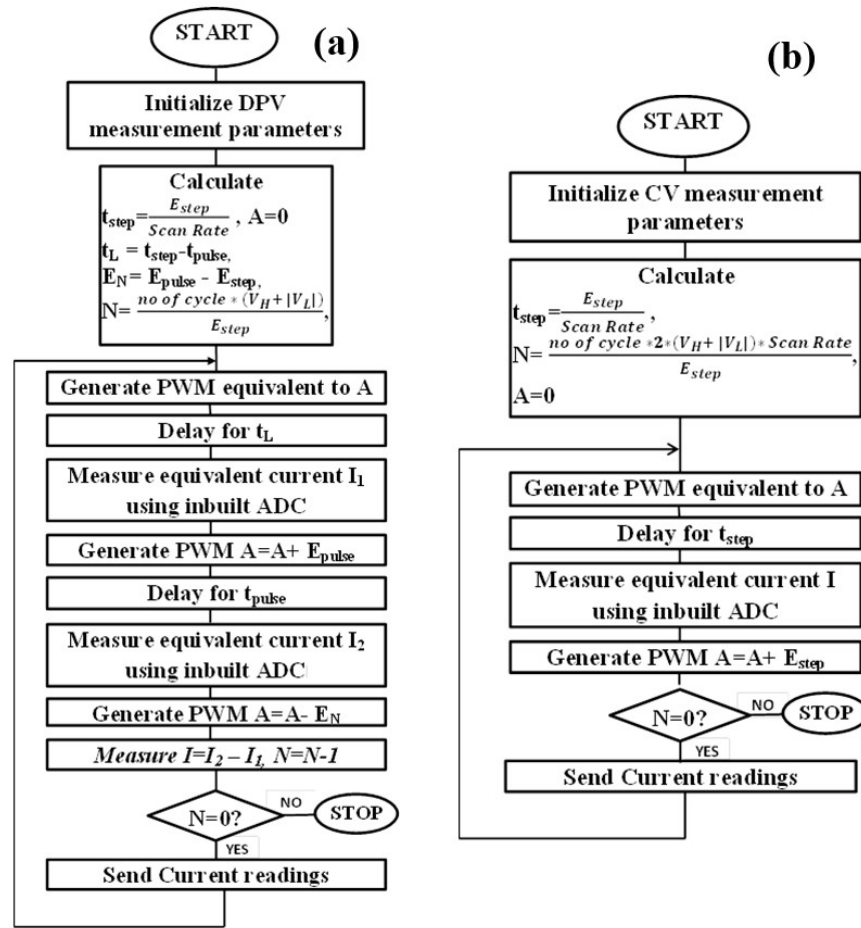


Fig. 4.4. Flow chart for (a) DPV and (b) CV measurement using Arduino IDE.

- **Software Architecture:** The software is structured into several interconnected modules, each serving a specific function:

I. User Interaction Module (Graphical User Interface - GUI)

II. Device Configuration and Execution Module

III. Data Processing, Smoothing, and Visualization Module

- Graphical User Interface (GUI):** The GUI is designed for intuitive user interaction and is implemented using the **Tkinter** library. It provides a clean, customizable layout for setting experimental parameters and monitoring results.

## Chapter 4: Design and development of a low-cost potentiostat

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- **Input Fields:** Users can define experimental parameters such as starting voltage, ending voltage, scan rate, number of cycles, etc.
- **Menu Bar Options:** Includes file operations (Open, Save, Exit) and experimental controls (Start, Plot, Filter).
- **Interactive Buttons:** Enable users to start experiments, visualize plots, and apply filters to the data.

The input parameters differ for CV and DPV measurements and are configured accordingly.

II. **Device Configuration and Execution:** This module handles hardware communication and ensures proper execution of experiments.

- The software configures LC-PSTAT with the selected experimental parameters and transmits them via serial communication (using the serial library).
- The voltage and current data from the potentiostat are acquired in real time and stored in a CSV file, with time stamps added for reference.
- Experimental timing, such as cycle duration, is dynamically computed based on user inputs.

III. **Data Processing and Visualization:** The visualization module is responsible for graphing and analyzing experimental data.

- **Data Plotting:** The current vs. voltage plot is generated using the Matplotlib library within the GUI.
- **Data Handling:** Users can load CSV files using the Pandas library and visualize collected data.
- **Data Smoothing:** A moving average filter is applied to enhance the clarity and readability of the plotted graphs.
- **Interactive Features:** Navigation tools enable users to zoom, pan, and explore data points efficiently.

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Snapshots of the developed software are provided in **Fig. 4.5**, illustrating its interface and functionalities. The following section provides a step-by-step breakdown of the software's algorithm, explaining how CV and DPV data are processed and analyzed.

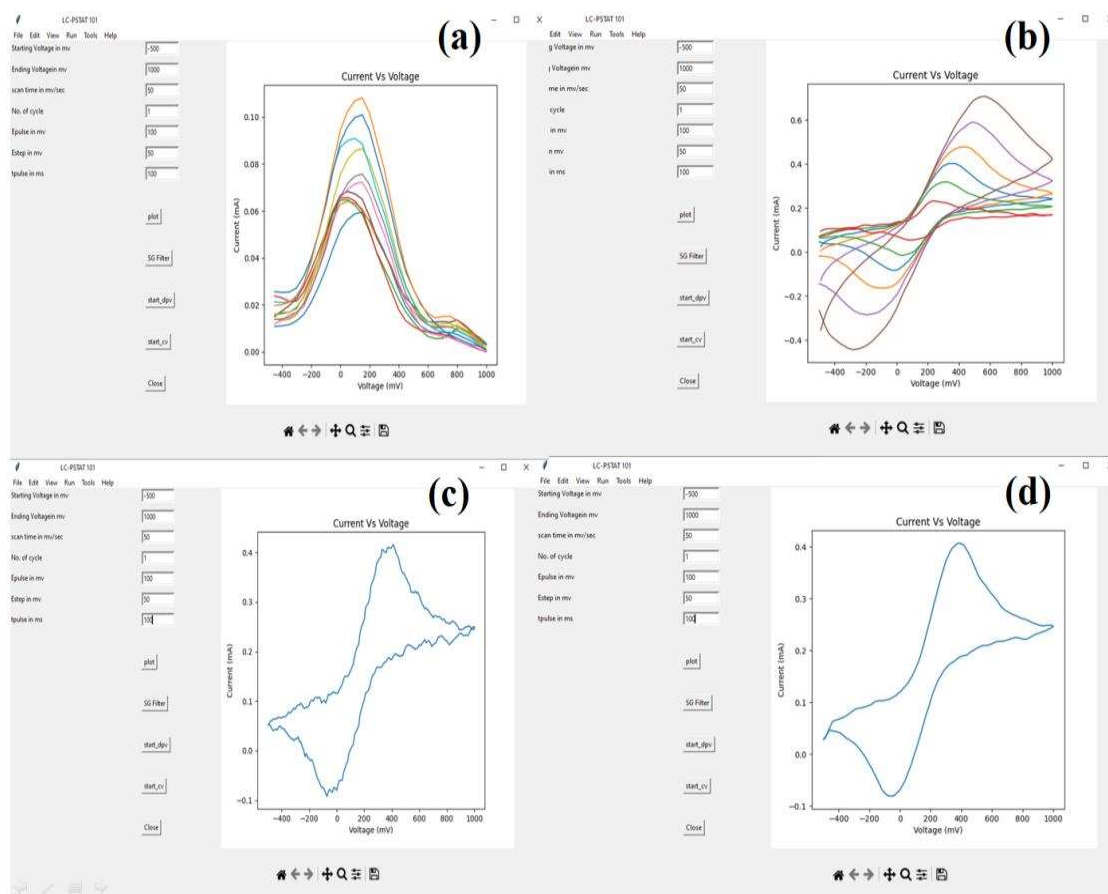
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### *Algorithm 1: Development of the software for LC-PSTAT 101*

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- |                |                                                                                                                                                               |
|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>STEP 1:</b> | <b>Start:</b> Represents the initialization of libraries and the GUI.                                                                                         |
| <b>STEP 2:</b> | <b>User Input:</b> Users enter experimental parameters and select files using the GUI fields and buttons.                                                     |
| <b>STEP 3:</b> | <b>Start Experiment:</b> Retrieve user input values to calculate parameters and establish serial communication with the potentiostat for collecting data.     |
| <b>STEP 4:</b> | <b>Plot Results:</b> Loads the experimental CSV file, extract voltage and current data and plots "Current vs. Voltage" graph using Matplotlib within the GUI. |
| <b>STEP 5:</b> | <b>Smooth Data:</b> Option to apply a smoothing algorithm to the data and re-plot the smoothed graph.                                                         |
| <b>STEP 6:</b> | <b>Clear/Exit:</b> Allows clearing of plots and terminate the application.                                                                                    |
-





**Fig. 4.5.** Some snap shots of the developed software LC-PSTAT 10: (a) DPV voltammogram with variable concentration (b) CV voltammogram with variable scan rate (c) CV voltammogram with noise (d) Removal of noise from CV after applying smoothing algorithm.

### 4.2.5. PCB Design, component layout and instrument packaging

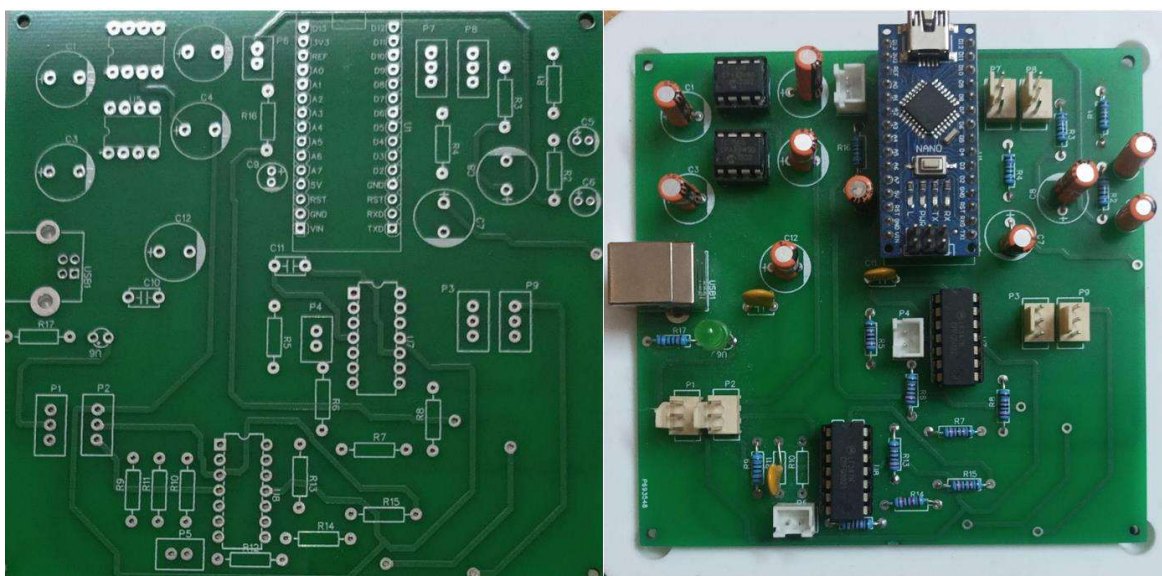
The printed circuit board (PCB) for the LC-PSTAT system was designed using EASY EDA software, while PCB fabrication was carried out by Power PCB Company. The PCB design and component layout are illustrated in Fig. 4.6. A customized casing was designed to house all components securely, ensuring compactness and ease of use. The PCB dimensions are 110 mm × 110 mm, and the enclosure was tailored to fit these specifications, as shown in Fig. 4.7. For device communication and programming, an Arduino Nano is integrated into the PCB. The system features:

- A Type-B Mini USB port for Arduino programming and PC communication.

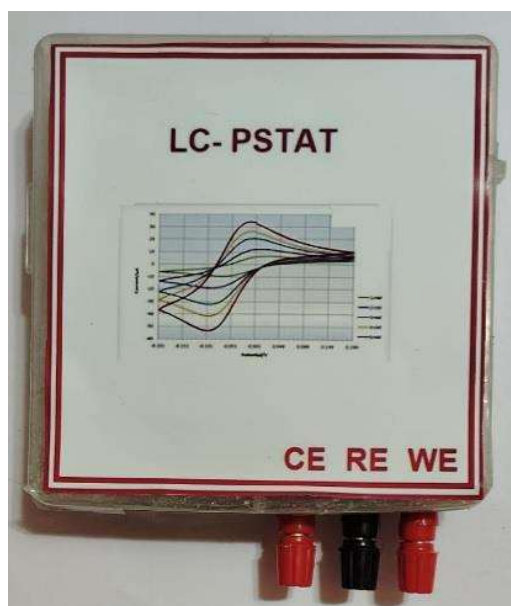
## Chapter 4: Design and development of a low-cost potentiostat

- An additional Type-B USB port for supplementary power requirements.
- Sufficient space in the enclosure design to accommodate both USB cable types conveniently.

The detailed specifications of the LC-PSTAT system are provided in Table 4.2.



**Fig. 4.6. (a) PCB design and (b) Component layout of the hardware of the developed LC-PSTAT system.**



**Fig. 4.7. Top view of the developed potentiostat (LC-PSTAT).**

*Table 4.2. Specifications of the LC-PSTAT system.*

| Parameters                   | Specifications       |
|------------------------------|----------------------|
| Potentiostat                 | Yes                  |
| Galvanostat                  | No                   |
| Cell connections             | 1                    |
| Maximum current              | $\pm 5$ mA           |
| Current Ranges               | 50 $\mu$ A to 5mA    |
| Minimum Current Resolution   | 5 $\mu$ A            |
| Maximum Applied Potential    | $\pm 5$ V            |
| Weight (including packaging) | 225 gm               |
| Dimensions                   | 11cm x 11cm x 3cm    |
| Input Voltage                | 5V through USB       |
| Power Consumption            | 325 mW               |
| Operating Temperature        | 20° C to 45° C       |
| Application                  | Electrochemical Cell |
| Experiment                   | CV & DPV             |
| Maximum Acquisition Rate     | 74074 data points/S  |
| ADC Resolution               | 4.88mV               |

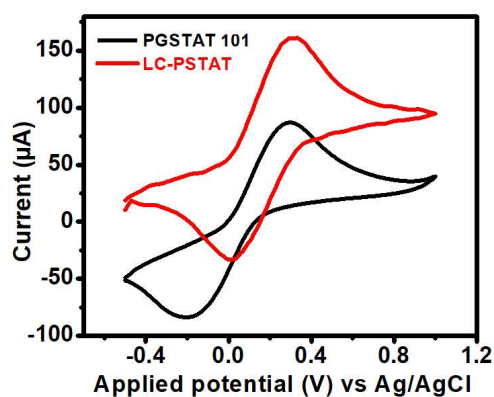
|      |        |
|------|--------|
| Cost | ₹ 5000 |
|------|--------|

### 4.2.6. Sample preparation and testing

The primary objective of developing the low-cost potentiostat (LC-PSTAT) is to perform cyclic voltammetry (CV) and differential pulse voltammetry (DPV) for the selective and sensitive detection of thymoquinone (TQ) in black cumin. The experimental details are further elaborated in Section 4.3 (Experimental) and Section 4.4 (Results and Discussion).

To validate the LC-PSTAT system and determine optimal experimental parameters, initial CV and DPV measurements were conducted in 0.1 M phosphate-buffered saline (PBS), both with and without 100  $\mu$ M TQ, using a glassy carbon electrode (GCE) and a TQMIP-G electrode. For performance evaluation, the measured data from LC-PSTAT were compared with those obtained from a laboratory-grade potentiostat/galvanostat (PGSTAT101, Autolab, Netherlands, Metrohm). The experimental parameters used for CV and DPV experiments are summarized in Table 4.3.

The waveform comparison between LC-PSTAT and PGSTAT101 is presented in Fig. 4.8, clearly demonstrating that the signature voltage of TQ is identical for both instruments. This confirms the reliability of the LC-PSTAT system. Consequently, all experimental parameters listed in Table 4.3 were used for subsequent experiments discussed in the following sections.



**Fig. 4.8.** CVs of TQMIP-G electrode in 0.1 M PBS containing 100  $\mu$ M TQ at 50 mV/s scan rate using developed LC-PSTAT and PGSTAT 101 instrument.

**Table 4.3. Experimental parameter settings for CV and DPV experiments.**

| Experiments | Parameters      | Specifications     |
|-------------|-----------------|--------------------|
| CV          | Initial Voltage | -500mV             |
|             | Final Voltage   | 1000 mV            |
|             | Step Potential  | 10 mV              |
|             | Step Period     | 1000 ms to 50 ms   |
|             | Scan Rate       | 10mV/s to 200 mV/s |
| DPV         | Initial Voltage | -500mV             |
|             | Final Voltage   | 1000 mV            |
|             | Step Potential  | 50 mV              |
|             | Step Period     | 1000 ms            |
|             | Scan Rate       | 50 mV/s            |
|             | Pulse Period    | 50 ms              |
|             | Pulse Potential | 100 mV             |

### 4.3. EXPERIMENTAL

#### 4.3.1. Reagents and chemicals

Acrylonitrile (AN), ethylene glycol di-methacrylate (EGDMA), thymoquinone (TQ), benzoyl peroxide (BP), poly(methyl methacrylate) (PMMA), acrylic acid (AA), and graphite (<20  $\mu\text{m}$ ), along with other interfering agents, were procured from Sigma-Aldrich, USA. Ethanol and paraffin oil were obtained from Merck, India. All chemicals were of analytical grade and were used without further purification. All electrolytic solutions were prepared using Millipore water with a resistance of 18 M $\Omega$ . Four different branded black cumin samples (designated as B1, B2, B3, and B4) were sourced from the local market.

## Chapter 4: Design and development of a low-cost potentiostat

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To prepare 1 L of phosphate buffer (PBS), acetate buffer solution (ABS), and citrate buffer (CBS), the method described by Banerjee et al. was followed [21]. A 1.0 mM standard solution of TQ was prepared by dissolving 16.42 mg of TQ in 100 mL of ethanol. All laboratory experiments were conducted under controlled room temperature conditions.

### 4.3.2. Apparatus

The surface morphology of the synthesized materials was analyzed using field emission scanning electron microscopy (FESEM, JEOL-JEM-6700F), while functional groups were identified through Fourier transform infrared (FTIR) spectroscopy (IR Prestige 21). Various electrochemical techniques were employed in this study, including:

- **Differential pulse voltammetry (DPV)** for concentration studies.
- **Cyclic voltammetry (CV)** for optimizing electrochemical measurement parameters.

All experiments were conducted using a three-electrode system, consisting of:

- **Working electrode:** TQMIP-G
- **Reference electrode:** Ag/AgCl
- **Counter electrode:** Platinum

The electrochemical measurements were performed using the developed low-cost portable potentiostat (LC-PSTAT). For benchmarking accuracy and data validation, identical experiments were also carried out using a Potentiostat/Galvanostat PGSTAT101 (Metrohm Autolab, Netherlands).

### Data visualization and analysis:

- CV and DPV plots were generated using **OriginPro 8.0**.
- Data analysis and Graphical User Interface (GUI) development were conducted using MATLAB and Python.

For thymoquinone (TQ) estimation, reverse-phase high-performance liquid chromatography (RP-HPLC) was performed.

### ***4.3.3. Synthesis of TQ- imprinted (TQMIP-G) material and NIP material***

To synthesize the TQ-imprinted polymer (TQMIP-G), 0.95 g of graphite powder was dispersed in 20 mL of ethanol via ultrasonication for 60 minutes to ensure uniform dispersion. Once dispersed, 0.05 g of TQ and 0.05 g of acrylonitrile (AN) (functional monomer) were added, followed by an additional 50 minutes of ultrasonication. Next, 400  $\mu$ L of EGDMA (cross-linker) and 1 mg of benzoyl peroxide (polymerization initiator) were introduced into the mixture, which was further sonicated for 50 minutes. The polymerization process was completed by heating the mixture at 60 °C for 1 hour. After polymerization, the resulting MIP-adduct was collected and subjected to ethanol extraction to remove any residual TQ template. The successful removal of the template molecule was confirmed through FTIR analysis. For comparison, a NIP was synthesized using the same procedure, except without the addition of TQ.

### ***4.3.4. Fabrication of TQIP/G and NIP electrode***

To fabricate the TQMIP-G electrode, 300 mg of the synthesized TQMIP-G material was mixed with a few drops of paraffin oil to form a uniform paste. This paste was then carefully packed into a glass capillary tube (inner diameter: 2.5 mm) using a metal rod to ensure proper compaction. To establish an electrical connection, a platinum wire was inserted at the back of the tube. Before each measurement, the electrode surface was smoothened and rinsed with ultrapure Millipore water to ensure consistent electrochemical performance. The non-imprinted polymer (NIP) electrode was fabricated using the same procedure as the TQMIP-G electrode, but without the inclusion of TQ during synthesis. Both electrodes were stored at room temperature to maintain their stability and functionality.

### ***4.3.5. Preparation of real sample***

Four distinct brands of black cumin, denoted as B1, B2, B3, and B4, were procured from the local market. Each sample (20 g) was finely ground using a mixer grinder. From each powdered sample, 5 mg was mixed with 20 mL of pure ethanol in separate beakers. The mixtures were then subjected to ultrasonication for 30 minutes to enhance the extraction of thymoquinone (TQ). After sonication, the extracts were filtered using Whatman filter paper, and the filtrates were transferred into separate volumetric flasks, labeled according to their respective samples.

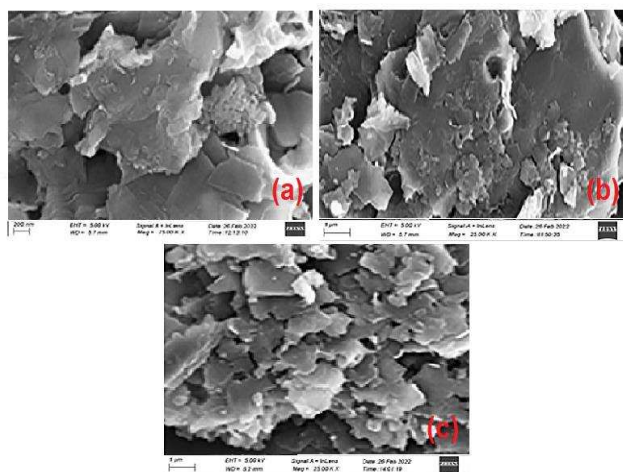
To prepare stock solutions (100 mL each), the filtered extracts were diluted with phosphate-buffered saline (PBS, pH 6). DPV measurements were performed on each sample, with 10 repetitions per sample to ensure the reliability and reproducibility of the results.

### 4.4. Results and discussion

#### 4.4.1. Surface morphology of TQIP/G material

FESEM was used to analyze the surface morphology of both the TQMIP-G and NIP materials. Fig. 4.9(a) presents the surface of the NIP material, which appears smooth and lacks cavities, confirming the absence of template-specific features. In contrast, Fig. 4.9(b) and 4.9(c) display the TQMIP-G material before and after template extraction, respectively.

Before extraction, the TQMIP-G surface is smooth and devoid of visible pores, indicating that the template molecules are embedded within the polymer matrix. However, after extraction, the surface becomes rough with noticeable cavities and pores, signifying the successful removal of the template. These morphological changes confirm the formation of molecularly imprinted binding sites, which enhance the selectivity and sensitivity of the material for detecting thymoquinone (TQ).



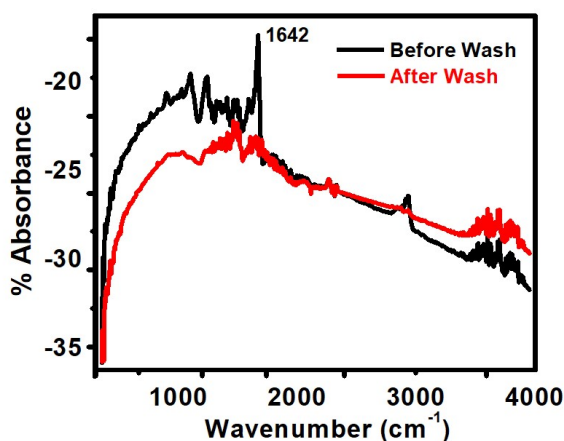
**Fig. 4.9. FESEM image of (a) NIP (b) TQIP/G adduct and (c) TQIP/G material.**



### 4.4.2. FTIR analysis of TQMIP-G adduct and TQMIP-G material

Fourier transform infrared (FTIR) spectroscopy was conducted to confirm the successful polymerization of the TQMIP-G material and the removal of the template from the MIP.

The FTIR spectrum of the TQMIP-G adduct (before extraction), shown in Fig. 4.10 (black), exhibits a sharp peak at  $1642\text{ cm}^{-1}$ , which corresponds to the presence of the TQ template within the polymer matrix [40]. After template extraction, this peak disappears (Fig. 4.10, red), confirming the successful removal of TQ.

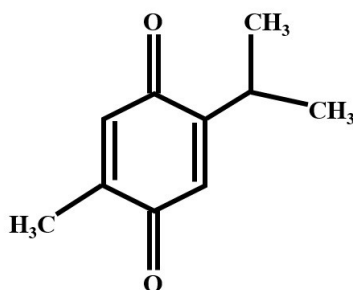


**Fig. 4.10** Comparison of FTIR spectra: spectrum of TQMIP-G before removal of TQ in the scan range of  $4000\text{--}400\text{ cm}^{-1}$  (black), and (b) spectrum after removal of TQ in the scan range of  $4000\text{--}400\text{ cm}^{-1}$ .

Additionally, the FTIR spectrum reveals several characteristic peaks indicative of the polymer matrix's successful formation. Peaks observed in the range of  $2800\text{--}3000\text{ cm}^{-1}$  correspond to the stretching vibrations of methyl ( $\text{CH}_3$ ) groups, reflecting covalent bonding between carbon and hydrogen atoms in the polymer matrix [41]. Similarly, peaks in the  $1420\text{--}1460\text{ cm}^{-1}$  range indicate  $\text{CH}_3$  bending vibrations, associated with the angular deformation of molecular bonds in the cross-linked polymer structure [42]. Peaks in the  $900\text{--}1150\text{ cm}^{-1}$  range represent the rocking vibrations of  $\text{CH}_3$  groups, caused by coordinated movement of hydrogen atoms attached to a carbon atom[43].

### 4.4.3. Detection principle of thymoquinone using TQMIP-G electrode

Thymoquinone (TQ), chemically known as 2-methyl-5-isopropylbenzoquinone, contains two polar carbonyl ( $\text{-C=O}$ ) groups at the 1 and 4 positions of the benzene ring, along with a methyl ( $\text{-CH}_3$ ) group at position 2 and an isopropyl ( $\text{-CH(CH}_3)_2$ ) group at position 5, as illustrated in Fig. 4.11. During pre-polymerization, acrylonitrile (AN), which contains a nitrile ( $\text{-C}\equiv\text{N}$ ) group, and ethylene glycol dimethacrylate (EGDMA), which possesses ester ( $\text{-COO-}$ ) functional groups, interact with TQ through dipole–dipole interactions involving its polar carbonyl ( $\text{-C=O}$ ) groups.



**Fig. 4.11. Chemical structure of thymoquinone (TQ).**

Polymerization is initiated using benzoyl peroxide (BP) as a free radical initiator, leading to the formation of a cross-linked polymer network with TQ molecules embedded within the structure. The cross-linking induced by EGDMA results in a rigid and insoluble polymer matrix. After the extraction of TQ, molecularly imprinted cavities remain in the polymer matrix, which are complementary in size, shape, and functional group orientation to the TQ molecule. These imprinted grooves enable selective recognition and binding of TQ molecules in subsequent applications, enhancing the sensitivity and specificity of the TQMIP-G electrode for TQ detection.

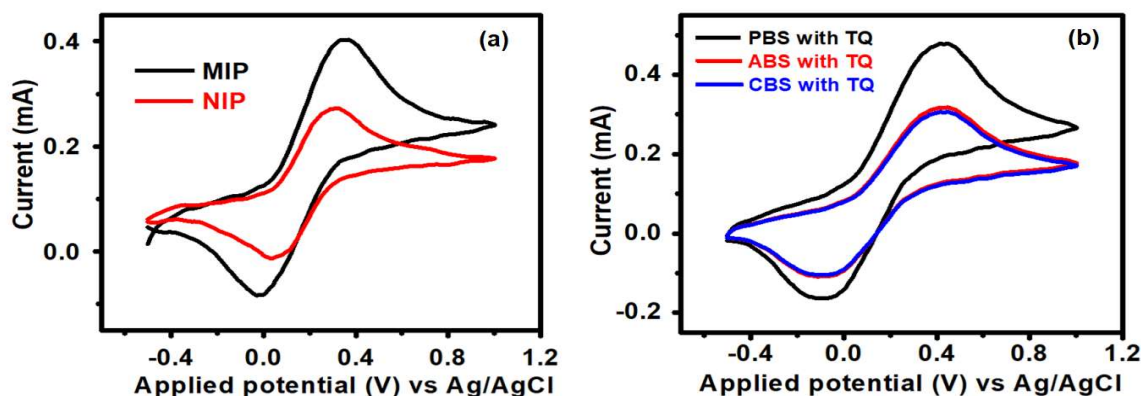
### 4.4.4. Electrochemical properties of TQMIP-G and NIP electrodes

Cyclic voltammetry (CV) experiments were conducted to evaluate the electrocatalytic performance of the TQMIP-G and non-imprinted polymer (NIP) electrodes, as depicted in Fig. 4.12(a). The TQMIP-G electrode exhibited a well-defined and sharp oxidation peak, with a 2.57-fold higher current response compared to the NIP electrode.

This significant enhancement in electrochemical response confirms the successful creation of molecularly imprinted cavities within the TQMIP-G material, which facilitate selective adsorption of TQ molecules. These imprinted cavities improve electron transfer efficiency, enabling more efficient redox reactions at the electrode surface and enhancing the sensor's overall sensitivity.

### 4.4.5. Optimization of buffer and pH

The cyclic voltammetry (CV) response of the TQMIP-G electrode is significantly influenced by both the buffer solution and pH of the medium. To determine the optimal buffer for electrochemical analysis, cyclic voltammograms (CVs) were recorded using three different buffer solutions: phosphate-buffered saline (PBS), acetate buffer solution (ABS), and citrate buffer solution (CBS). Among these, PBS exhibited the highest and sharpest peak current ( $I_p$ ), as shown in Fig. 4.12(b). Based on this observation, PBS was selected for all subsequent electrochemical studies of the TQMIP-G material.



**Fig. 4.12.** (a) CVs of TQMIP-G electrode and NIP electrode, and (b) CVs of TQMIP-G electrode in 0.1 M different buffer solution ((acetate, citrate and phosphate) containing 100  $\mu\text{M}$  TQ at 50  $\text{mVs}^{-1}$  scan rate with Ag/AgCl reference electrode.

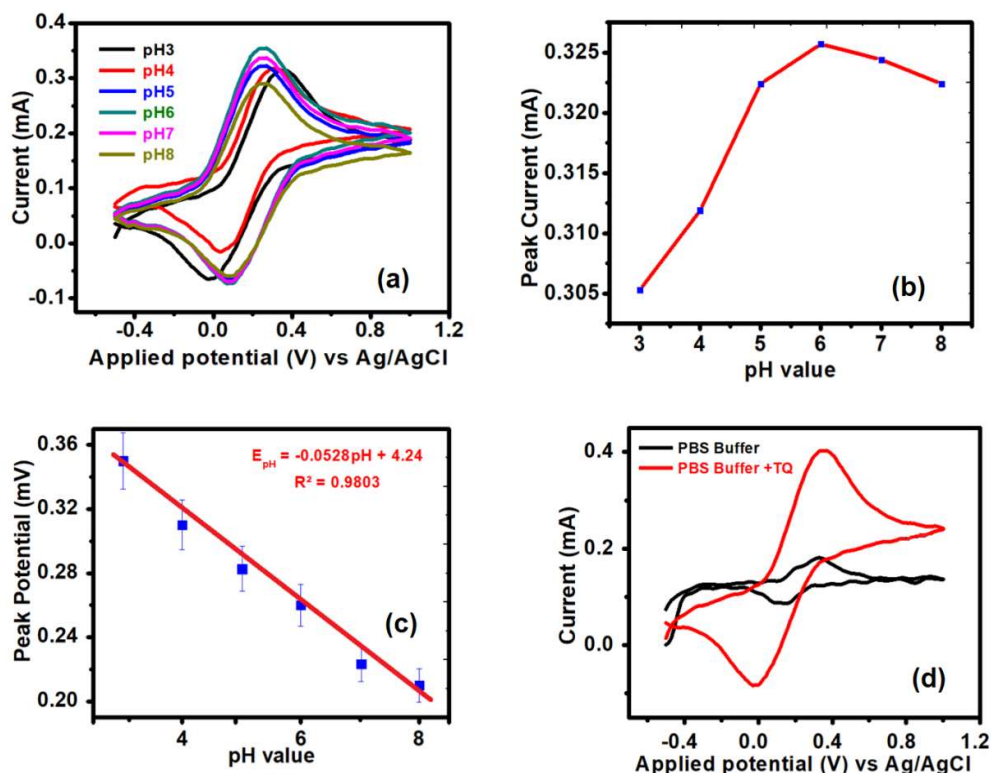
The impact of pH on the redox reaction of TQ was assessed by recording CVs (Fig. 4.13(a)) in 0.1 M PBS containing 100  $\mu\text{M}$  TQ at a scan rate of 50  $\text{mV/s}$ , varying the pH from 3 to 8. The peak current increased with pH from 3 to 6 but decreased at pH 7 and 8, indicating weaker adsorption of TQ at the electrode surface (Fig. 4.13(b)).

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Additionally, the oxidation peak potential shifted negatively with increasing pH (Figure 4.13(c)), suggesting that the phenolic  $-C=O$  group dissociates into an anion at higher pH, facilitating oxidation. This implies that protons actively participate in the oxidation process. A linear relationship was observed between pH and peak potential ( $E_{pH}$ ) expressed by the regression equation:

$$E_{pH} = -0.052pH + 4.24 \quad (R^2 = 0.98). \quad (4.1)$$

The slope value of  $0.052 \text{ V pH}^{-1}$  is close to the theoretical Nernstian value of  $0.059 \text{ V pH}^{-1}$ , confirming that an equal number of electrons and protons participate in the redox reaction [44]. Finally, the CV response (Fig. 4.13(d)) of the TQMIP-G electrode was analyzed in the presence and absence of TQ molecules ( $100 \mu\text{M}$ ) in  $0.1 \text{ M}$  PBS at pH 6. No significant peak current was observed in the absence of TQ, further verifying the electrode's selectivity toward TQ molecules.



**Fig. 4.13.** (a) CVs of TQMIP-G electrode in PBS with different pH solution containing  $100 \mu\text{M}$  TQ at  $50 \text{ mVs}^{-1}$  scan rate (b) Peak current vs different pH value (c) peak potential vs different pH values and (d) CVs of TQMIP-G electrode in  $0.1 \text{ M}$  PBS saline with and without TQ.

### 4.4.6. Optimization of concentration of cross-linker and monomer

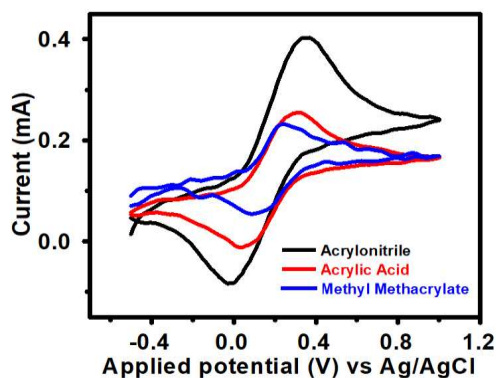
To evaluate the impact of different monomers on sensor performance, TQMIP-G electrodes were fabricated using three distinct monomers: acrylonitrile (AN), acrylic acid (AA), and methyl methacrylate (MMA). Their electrochemical responses were compared using cyclic voltammetry (CV), as shown in Fig. 4.14.

Among the three, the TQMIP-G electrode prepared with acrylonitrile exhibited the highest peak current, indicating superior electrochemical activity. This enhanced response is attributed to stronger interactions between acrylonitrile and TQ during the molecular imprinting process, facilitating better template recognition and charge transfer. In contrast, electrodes fabricated with acrylic acid and methyl methacrylate displayed lower peak currents, likely due to less efficient cavity formation or weaker template-monomer interactions, resulting in reduced electrochemical sensitivity.

To further optimize the polymer formulation, the monomer-to-cross-linker ratio was systematically varied, and its influence on electrode performance was assessed (Fig. 4.15(a), and 4.15(b)). The optimal values were determined to be:

- Monomer concentration: 0.05 g
- Cross-linker volume: 400  $\mu\text{L}$

This ratio was found to significantly enhance sensor sensitivity and redox efficiency, leading to improved electrochemical detection of TQ.



**Fig. 4.14.** CVs of MIP electrode with different monomer in 0.1 M PBS buffer solution containing 100  $\mu\text{M}$  TQ at 50  $\text{mVs}^{-1}$  scan rate with Ag/AgCl reference electrode.

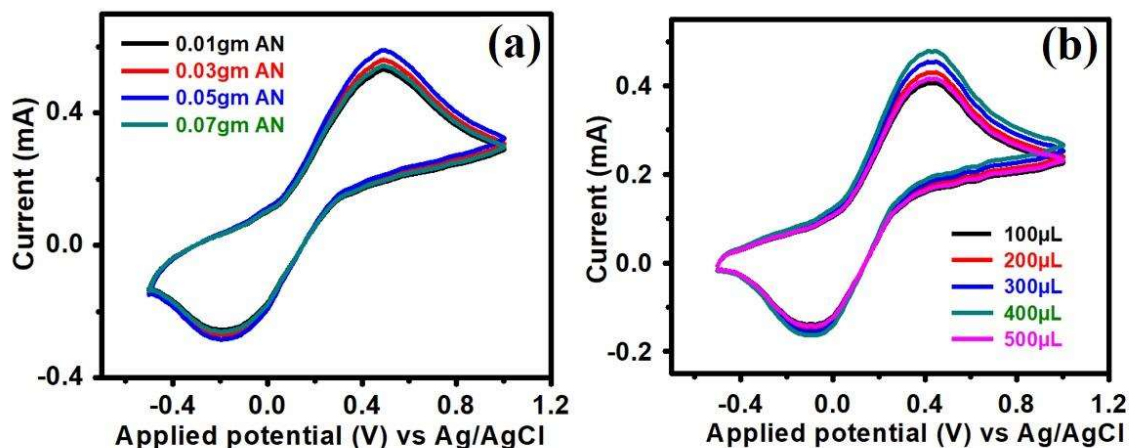


Fig. 4.15. (a) Optimization of monomer (acrylonitrile), and (b) cross-linker (EGDMA) for the preparation of TQIP/G electrode.

#### 4.4.7. Effect of scan rate

The scan rate significantly influences the electro-oxidation characteristics of the TQMIP-G electrode, reflecting its reaction kinetics and surface processes. To investigate this effect, cyclic voltammetry (CV) experiments were conducted at scan rates ranging from 10  $\text{mV s}^{-1}$  to 200  $\text{mV s}^{-1}$  in a 0.1 M PBS buffer (pH 6) containing 100  $\mu\text{M}$  TQ, as illustrated in Fig. 4.16.

Both the oxidation peak current  $I_{\text{OXD}}(\mu\text{A})$  and the reduction peak current  $I_{\text{RED}}(\mu\text{A})$  exhibited a linear increase with increasing scan rate, indicating that the TQ oxidation process at the TQMIP-G electrode is adsorption-controlled, as suggested by Ziyatdinova et al. [45]. The relationships are shown in Fig. 4.17(a) and Fig. 4.17(b), where the oxidation and reduction peak currents are plotted against the scan rate, with corresponding linear regression equations:

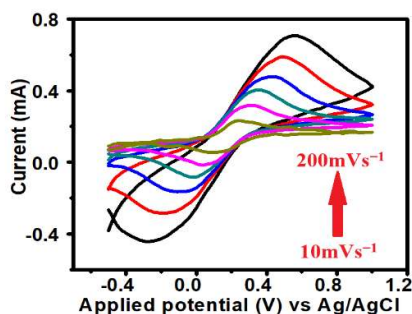
$$I_{\text{OXD}}(\mu\text{A}) = 0.001v + 0.276 \quad (R^2 = 0.98) \quad (4.2)$$

and

$$I_{\text{RED}}(\mu\text{A}) = -0.0012v - 0.027 \quad (R^2 = 0.98) \quad (4.3)$$

Additionally, Figure 4.17(c) demonstrates that the peak current increases linearly with the square root of the scan rate, with the linear regression equation:

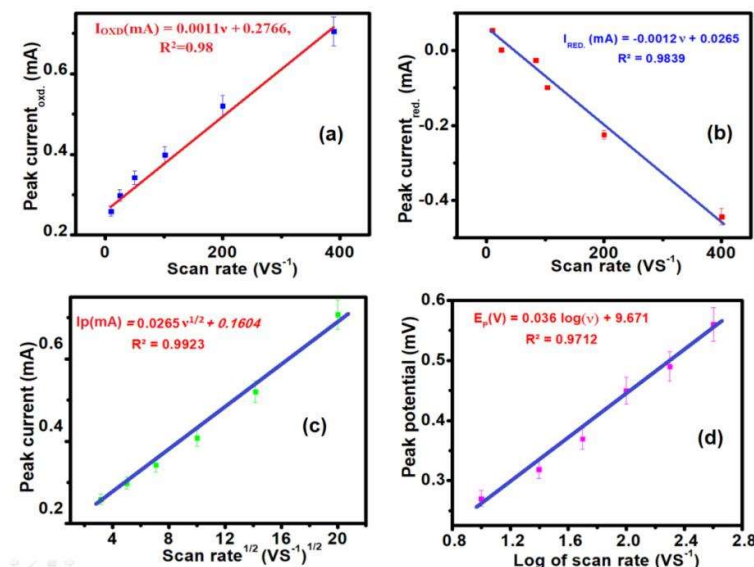
$$I_p(\text{mA}) = 0.026 v^{1/2} + 0.164 (R^2=0.99). \quad (4.4)$$



**Fig. 4.16.** CVs with variation of peak current for different scan rate in 0.1 M PBS solution containing 100  $\mu\text{M}$  Thymoquinone.

This indicates that both the anodic and cathodic peaks are controlled by adsorption on the electrode surface. The linear plot of peak potential versus the logarithm of the scan rate is shown in Fig. 4.17(d), where the oxidation peak potential increases linearly with increasing  $\log(\text{scan rate})$ , with the regression equation:

$$E_{pA}(\text{V}) = 0.036 \log(v) + 9.67 (R^2 = 0.97). \quad (4.5)$$



**Fig. 4.17.** Linear plot of (a) peak current (oxd.) vs scan rate (b) peak current (red.) vs scan rate (c) peak current vs square root of scan rate, and (d) peak potential vs log of scan rate.

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Based on these observations, the number of electrons transferred during the oxidation of TQ molecules ( $n$ ), the charge transfer coefficient ( $\alpha$ ), and the surface adsorbed analyte ( $\Gamma_c$ ) were calculated using the following three equations:

$$I_p = \frac{nFQv}{4RT} = \frac{n^2F^2Av\Gamma_c}{4RT} \quad (4.6)$$

$$\log(\vartheta) = 2.3 \frac{RT}{(1-\alpha)nF} E_p + Constant \quad (4.7)$$

$$\log K_s = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha - \log\left(\frac{RT}{nF\vartheta}\right) - \alpha(1 - \alpha) \frac{nF}{2.3RT} \Delta E_p. \quad (4.8)$$

Where the parameters are, peak current ( $I_p$ ), number of electrons transferred ( $n$ ), charge ( $Q$ ), scan rate ( $\vartheta$ ), electrode ( $A$ ), universal gas constant ( $R$ ), Temperature ( $T$ ), and Faraday's constant ( $F$ ). The value of  $n$  and  $\Gamma_c$  were calculated using equation (4.6), where  $\Gamma_c$  was found to be  $7.88 \times 10^{-10}$  mole  $\text{cm}^{-2}$ , and  $n$  was calculated as 1.73 ( $\approx 2$ ). The value of  $n \approx 2$  indicates that two electrons are involved in the redox reaction. The charge transfer coefficient ( $\alpha$ ) was calculated using equation (4.7) and was found to be 0.97. The standard rate constant ( $K_s$ ) was calculated using Laviron's equation (4.8) and was calculated to  $5.6 \times 10^{-2} \text{ s}^{-1}$  [46]. The number of protons ( $m$ ) involved in the redox reaction can be calculated using the equation:

$$0.059 \frac{\alpha m}{n} = 0.0612 \quad (4.9)$$

This results  $m = 1.86 (\approx 2)$ , indicating that the redox reaction involves an equal number of protons and electrons.

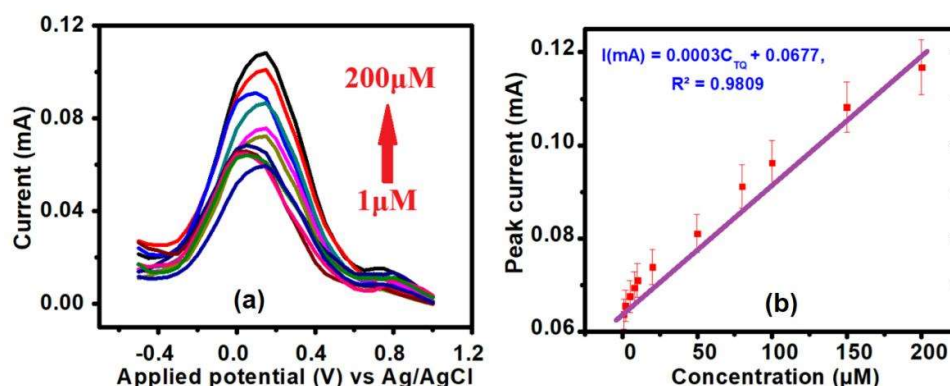
### 4.4.8. Determination of TQ

Differential pulse voltammetry (DPV) technique was used to evaluate the analytical performance of TQMIP-G electrode for detecting TQ. DPV exhibits a better current sensitivity and higher resolution over CV as it minimizes non-faradic current. For determination of TQ, DPV were taken for different concentration ranging from 1  $\mu\text{M}$  to 100  $\mu\text{M}$ , as shown in Fig. 4.18(a). The oxidation peak potential was found at 250 mV, which is the signature voltage of TQMIP-G electrode. Fig. 4.18(b) shows a linear response for the range of 1  $\mu\text{M}$  to 200  $\mu\text{M}$ , with regression equation  $I_{TQ} = 0.0003C_{TQ} + 0.0677$ ; ( $R^2 = 0.98$ ). The limit of detection (LOD) was calculated as 0.20  $\mu\text{M}$  using formula  $3.3 S_{y/x}/m$ ; where  $S_{y/x}$  (0.0066) and  $m$  (0.98) are the standard deviation of the blank solution response



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and slope of the calibration curve respectively. The Table 4.4 shows the comparative study between proposed PQMIP-G electrode with previously published methods, and found that the detected LOD of TQMIP-G electrode using portable low-cost potentiostat is better than other related works.



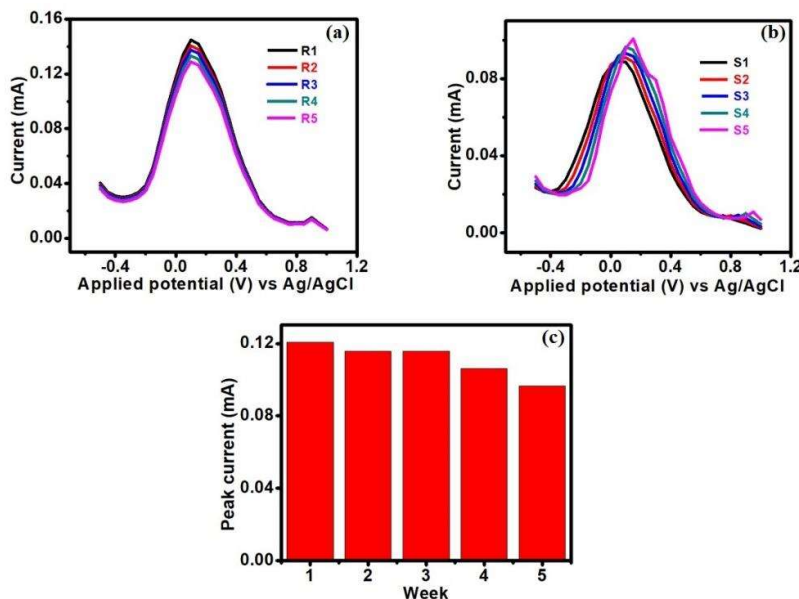
**Fig. 4.18.** (a) DPV measurement of TQMIP-G electrode in 0.1 M PBS saline containing different concentration (from bottom are 1, 2, 5, 8, 10, 20, 50, 80, 100, 150, 200  $\mu$  M) of TQ at  $50\text{mVs}^{-1}$  scan rate with Ag/AgCl reference electrode (b) corresponding peak current vs concentration linear plot.

**Table 4.4.** Comparison of proposed MIP performance with other reported literature.

| Methodology                                                                          | LOD ( $\mu\text{M}$ ) | Range of detection ( $\mu\text{M}$ ) | Ref.             |
|--------------------------------------------------------------------------------------|-----------------------|--------------------------------------|------------------|
| High-performance thin layer chromatographic method (HPTLC)                           | 0.09                  | 0.004-0.028                          | [12]             |
| Reverse-phased high-pressure liquid chromatography (RP-HPLC) using Silica gel column | -                     | 32.94- 368.30                        | [13]             |
| Differential pulse polarographic method                                              | 3                     | 110–832                              | [47]             |
| <b>TQMIP-G electrode measured with low cost –potentiostat (LC-PSTAT)</b>             | <b>0.03</b>           | <b>0.2- 100</b>                      | <b>This work</b> |

### 4.4.9. Stability, repeatability and reproducibility

In practical applications, the performance of the developed TQMIP-G sensor was evaluated in terms of stability, repeatability, and reproducibility to assess its reliability and usability.



**Fig. 4.19.** (a) *Repetability test of five DPV measurements using same TQMIP-G electrode,* (b) *Reproducibility test of five DPV measurements using five different TQMIP-G electrodes,* and (c) *Peak current of TQMIP-G electrode taken with an interval of 7 days containing 100  $\mu$ M TQ.*

Repeatability of the TQMIP-G sensor electrode was tested by performing repeated measurements ( $n=5$ ) of the oxidation peak current. The results, shown in Fig. 4.19.(a), yielded a relative standard deviation (RSD) of 3.23%, demonstrating excellent repeatability and consistent performance of the sensor electrode under the same conditions. To assess reproducibility, five independent TQMIP-G electrodes were fabricated and tested. The results, presented in Fig. 4.19 (b), indicated minimal variation in the sensor responses, confirming that the sensor fabrication process is reproducible. For evaluating long-term stability, the sensor performance was monitored over a period of 4 weeks, with measurements taken at weekly intervals. During this time, the electrodes were stored at room temperature. The bar plot in Fig. 4.19(c) shows that there was negligible change in the performance of the electrode, indicating its stability over an extended period of time. These findings suggest that the developed TQMIP-G electrode maintains its performance over long-term storage and usage, making it suitable for practical, long-term applications.

### 4.4.10. Study of selectivity

The selectivity of the developed TQMIP-G electrode was investigated to evaluate its ability to specifically measure thymoquinone (TQ) while rejecting structurally similar compounds. Several phytochemicals found in black seed oil, such as  $\alpha$ -pinene, thymol (TY), carvacrol (CL), and thymohydroquinone (THQ), are structurally similar to TQ. Therefore, their influence on the sensor's selectivity was studied.

In the selectivity study, various interfering agents, including anions, cations, and phytochemicals, were introduced at selected concentrations (100  $\mu$ M of TQ, 100  $\mu$ M of thymol, carvacrol, and thymohydroquinone, and 1 mM of  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{K}^+$ ) during the TQ determination. The results, presented in Table 4.5, show that the presence of these interfering agents had minimal effect on the current response for TQ determination, with deviations less than 7%, as depicted in Fig. 4.20. These findings confirm that the TQMIP-G electrode demonstrates excellent selectivity, allowing for the accurate determination of TQ even in the presence of structurally similar compounds and common interfering ions.

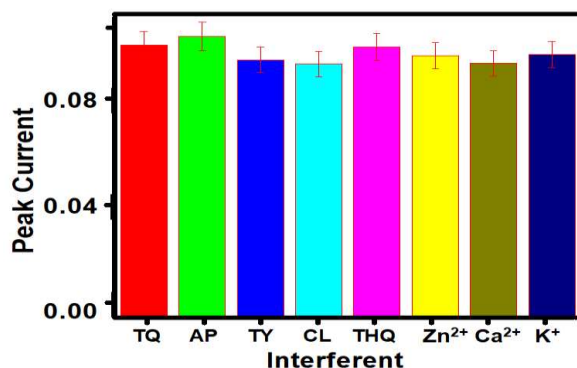


Fig. 4.20. CV peak currents for different interferent with 100 $\mu$ M TQ in 0.1M PBS buffer at 50mVs<sup>-1</sup> scan rate.

**Table 4.5. Real sample analysis of TQ with TQMIP-G electrode.**

| Interferent             | Interferent Concentration | Peak Current Response (mA) | Error Deviation % |
|-------------------------|---------------------------|----------------------------|-------------------|
| Thymoquinone (TQ)       | 100 $\mu$ M               | 0.1032                     | 0                 |
| $\alpha$ -pinene        | 100 $\mu$ M               | 0.1065                     | 3.19767           |
| Thymol (TY)             | 100 $\mu$ M               | 0.0976                     | 5.426357          |
| Carvacrol (CL)          | 100 $\mu$ M               | 0.0961                     | 6.879845          |
| Thymohydroquinone (THQ) | 100 $\mu$ M               | 0.1025                     | 0.678295          |
| Zn <sup>2+</sup>        | 1mM                       | 0.0991                     | 3.972868          |
| Ca <sup>2+</sup>        | 1mM                       | 0.0963                     | 6.686047          |
| K <sup>+</sup>          | 1mM                       | 0.0997                     | 3.391473          |

### 4.4.11. Determination of TQ through real sample analysis

The real-time application of the TQMIP-G electrode was evaluated by quantifying the thymoquinone (TQ) content in various branded black cumin samples. Four varieties of branded black cumin, certified by the Food Safety and Standards Authority of India (FSSAI), were procured from the local market, labeled as B1, B2, B3, and B4. To validate the TQ quantification, the RP-HPLC (Reverse Phase High-Performance Liquid Chromatography) method was used as the gold standard for comparison [13]. The TQ contents in these black cumin samples were predicted using a Partial Least Squares Regression (PLSR) model, implemented in MATLAB. The model was calibrated with the Differential Pulse Voltammetry (DPV) response from the developed TQMIP-G electrode, using the RP-HPLC data as the reference.

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During cross-validation, a training dataset was created by excluding data points from a specific black cumin sample, and the model was tested using this excluded dataset. This process was repeated for each of the four samples, and prediction accuracies (%) were calculated accordingly. Table 6 illustrates the actual TQ concentration, the mean predicted TQ concentration, and the prediction accuracy for each sample. A comparison of the actual and predicted TQ concentrations is presented in Fig. 4.21. The bar plot indicates a strong correlation between the actual and predicted TQ concentrations, with an average prediction accuracy of approximately 94.39% for all four samples. These results confirm the satisfactory performance of the TQMIP-G electrode for the qualitative and quantitative estimation of TQ in B1, B2, B3, and B4 black cumin samples. The developed TQMIP-G electrode demonstrated promising sensing characteristics, including a low limit of detection (LOD), a wide detection range, and excellent prediction accuracy when compared to the RP-HPLC data. The successful application of this electrode for real sample analysis highlights its significant societal value and industrial applicability.

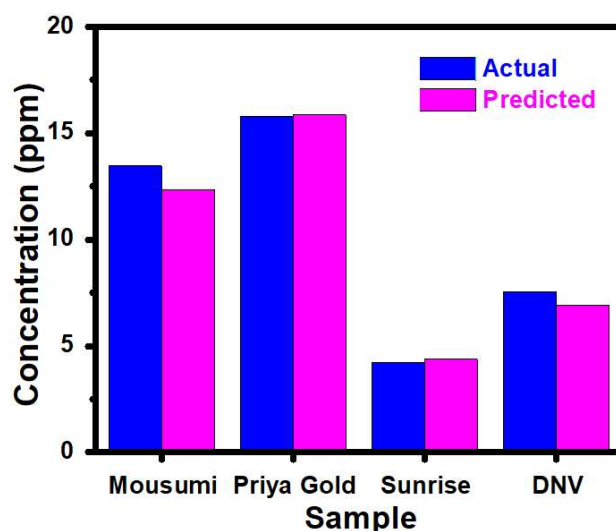


Fig. 4.21. Determination of TQ from Real Samples using PLS Model

**Table 4.6. Determination of TQ from Real Samples using PLS Model.**

| Sample ID  | Actual<br>TQ (ppm) | Predicted<br>TQ (ppm) | Predicted<br>Accuracy (%) |
|------------|--------------------|-----------------------|---------------------------|
| Mousumi    | 13.46              | 12.34                 | 90.90                     |
| Priya Gold | 15.81              | 15.85                 | 99.72                     |
| Sunrise    | 4.20               | 4.38                  | 95.82                     |
| DNV        | 7.55               | 6.93                  | 91.11                     |

### 4.5. Conclusion

This study successfully addresses the need for a low-cost, portable potentiostat system (LC-PSTAT) designed for the selective and sensitive detection of thymoquinone (TQ) in black cumin, utilizing a molecularly imprinted polymer-based TQMIP-G sensor. The primary challenge this system resolves is the need for an affordable, reliable, and portable solution to quantify TQ, a valuable bioactive compound, in a variety of real-world settings. Traditional methods for TQ detection, such as RP-HPLC, are typically expensive, require skilled operators, and are not easily deployable in field environments. The LC-PSTAT system offers a much-needed alternative with its microcontroller-based hardware and user-friendly software, which are both cost-effective and capable of providing real-time, on-site analysis.

The TQMIP-G sensor was meticulously characterized using advanced techniques such as FESEM and FTIR, confirming its high sensitivity and specificity for TQ detection. The electrochemical properties of the sensor were further validated through cyclic voltammetry (CV) and differential pulse voltammetry (DPV), which demonstrated the sensor's ability to detect TQ at low limits of detection (LOD), exhibit high reproducibility, and maintain excellent stability over extended use. These performance metrics highlight the sensor's potential for real-world applications, where consistent and reliable results are crucial.

A particularly notable achievement of this study is the system's ability to predict TQ concentrations in real sample analyses, achieving a prediction accuracy of 94% when

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compared to the gold-standard RP-HPLC method. This demonstrates the LC-PSTAT system's high accuracy and precision in quantifying TQ, providing a strong case for its application in industries that require rapid, on-site analysis.

The features related to application and design of this system are summarized below:

**Low-current optimization:** The potentiostat was optimized for  $\mu\text{A}$ –mA level currents relevant to MIP-based sensors by appropriate selection of the current-sensing resistor and scaling circuitry, enabling reliable detection of small Faradaic currents from the TQMIP-G electrode.

**Tailored potential window:** The operational potential range ( $-1\text{ V}$  to  $+1\text{ V}$ ) and resolution were specifically designed to match the redox behavior of thymoquinone, ensuring accurate CV and DPV measurements while avoiding unnecessary circuit complexity.

**Standalone operation:** A custom Python-based GUI was developed to enable real-time data acquisition, visualization, and storage without reliance on proprietary software, facilitating calibration and data analysis.

**Sensor compatibility:** Hardware–software co-design ensured stable baseline currents, minimal signal distortion, and reproducible responses when interfaced with MIP-modified electrodes.

**Portability:** The compact, low-power design allows on-site and real-sample analysis, supporting practical deployment in food and pharmaceutical applications.

**Cost-effectiveness:** The LC-PSTAT system is far less expensive than conventional methods, making it accessible for resource-limited settings.

However, there are also some limitations and potential areas for improvement:

- The system currently focuses on the detection of TQ in black cumin, but it could be used to detect a broader range of bioactive compounds in various matrices.
- The sensor sensitivity could be improved to detect lower concentrations of TQ, expanding its utility in more sensitive applications.

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- While the system performs well in its current form, incorporating wireless communication capabilities such as Bluetooth or Wi-Fi could enhance usability by enabling remote monitoring and data collection. This feature would be particularly useful in industrial settings where real-time data sharing and analysis are critical.

Looking to the future, the LC-PSTAT system holds great promise for a wide range of applications:

1. Agricultural sector: The system can be used for rapid detection of TQ levels in black cumin and other crops, aiding in quality control and traceability.
2. Pharmaceutical industry: Given TQ's medicinal properties, the system could be used for the development of quality-assured herbal medicines or for quality assurance during manufacturing.
3. Food industry: The sensor could be deployed to ensure food safety and quality by detecting the concentration of TQ in food products like black cumin oil, with minimal delay.

In conclusion, the LC-PSTAT system represents a significant advancement in bioanalytical detection. Its combination of affordability, portability, and high performance offers tremendous potential for application across a range of industries.

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## **Chapter 5**

### **Conclusion and future scopes**

This chapter introduces MIP-based electrochemical sensors for detecting thymoquinone and piperine in spices. The sensors are low-cost, portable, and highly sensitive, offering real-time analysis. Combined with AI models, they provide an efficient alternative to conventional methods for quality control in food, pharmaceutical, and agricultural applications. The summary of findings along with recommendation and future scopes have been presented.

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- 5.2. Summary of findings**
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### 5.1. Introduction

In recent years, there has been a renewed scientific focus on medicinal and culinary plants, particularly spices, due to their potent bioactive properties and minimal adverse effects. Indian spices, in particular, are recognized not only for their flavor and aroma but also for their therapeutic potential. Researchers worldwide are now investigating these botanicals as valuable sources for drug development and functional food ingredients. To ensure the reliability and standardization of such spice-based products, the identification and quantification of specific biomolecules—referred to as biomarkers—has become a critical aspect.

The therapeutic efficacy and safety of these natural products largely depend on the concentration of key phytochemicals present in the raw materials. Conventionally, laboratories rely on sophisticated instruments such as HPLC and GC-MS to assess biomarker content. Although highly accurate, these methods are expensive, time-consuming, and often not suitable for on-site or real-time analysis.

To overcome these limitations, the current research presents the development of a cost-effective, portable, and sensitive measurement system tailored to quantify essential biomolecules in selected Indian spices. This thesis emphasizes the fabrication of functionalized electrodes utilizing the MIP technique—a method that allows for the creation of synthetic recognition sites within polymer matrices, mimicking the shape and functional groups of target molecules.

Two customized MIP-based electrochemical sensors were designed and extensively characterized for the detection of piperine and thymoquinone, two prominent phytochemicals found in Indian spices such as black cumin and black pepper respectively. These MIP sensors offer several key advantages:

- a) They serve as a cost-efficient substitute for traditional laboratory instruments, as the components used in their synthesis are inexpensive and readily accessible
- b) Their fabrication process is straightforward, and the sensors exhibit excellent sensitivity, specificity, and repeatability, making them highly suitable for real-time monitoring in both lab and field settings.

By integrating these sensors into a comprehensive measurement system, this work aims to enable non-destructive, rapid, and reliable quantification of bioactive compounds directly from spice samples. The outcomes of this study not only contribute to the standardization of herbal and spice-based formulations but also open up avenues for broader applications in food safety, pharmaceuticals, and quality control industries.

### 5.2. Summary of findings

A concise review of existing literature reveals that several studies have explored the electrochemical detection of piperine and thymoquinone individually. Table 5.1 presents the findings from the current thesis, showing a notable enhancement in detection limits across all sensor types [1-5]. The application of MIP technology in the development of these sensors played a crucial role in achieving improved sensitivity, as evidenced by the significantly lower limits of detection (LOD) relative to conventional electrochemical techniques. In contrast to earlier works, where electrode fabrication often involved complex procedures or the use of expensive materials, this research employed simple, low-cost, and readily available precursors. This approach not only reduced fabrication costs but also contributed to the sensors' environmental compatibility, stability, and biocompatibility. Furthermore, while many prior studies lacked a detailed evaluation of electrode selectivity, the current work emphasizes and validates the high selectivity of the developed MIP-based sensors. Overall, the two proposed MIP electrodes exhibit excellent potential as sustainable and effective tools for detecting piperine and thymoquinone in real-world applications. The research underscores the advantages of combining low-cost materials with MIP technology to develop reliable, selective, and eco-friendly electrochemical sensors. The key contributions and outcomes of this study are summarized below.

a) To detect piperine in black pepper samples, a selective electrochemical sensor using a molecularly imprinted polymer (PDMAM-MIP@G) was developed. The molecularly imprinted polymer (MIP)-based graphite electrode was fabricated using poly(N,N-dimethylacrylamide) (PDMAM) as the functional monomer and ethylene glycol dimethacrylate (EGDMA) as the cross-linker. The optimized electrode (1:4:12 ratio of template, monomer, cross-linker) showed excellent sensitivity with a detection limit of 0.6 nM and a wide linear range (0.001–200  $\mu$ M) [1, 2]. Characterization techniques (FTIR, SEM, XPS, UV-Vis) confirmed successful imprinting, and electrochemical analysis revealed a two-

electron irreversible, adsorption-controlled mechanism. The sensor demonstrated high selectivity, stability, and reproducibility.

To enhance predictive accuracy, CV data from 1000 real samples were analyzed using CNN and hybrid CNN-regression models. The best-performing model (CNN+KNNR) achieved an MAE of 0.03389, RMSE of 0.04548, and  $R^2$  of 0.99991. Real-sample validation confirmed the system's effectiveness for piperine quantification. This integrated sensing and AI approach offers a practical tool for quality control in the spice and food industries. Experimental details and results are presented in Chapter 3 of this thesis.

b) Two novel electrochemical sensors was developed for detecting thymoquinone (TQ) in black cumin: a titanium oxide-modified graphite paste electrode (GPE/TiO<sub>2</sub>) and a thymoquinone-imprinted polymer electrode (TQIP/G). Both sensors showed excellent sensitivity, low detection limits (0.02  $\mu$ M and 0.03  $\mu$ M respectively), and broad linear ranges [3, 4]. They demonstrated high selectivity, reproducibility, and stability. Principal Component Analysis (PCA) effectively distinguished cumin brands based on TQ content. For enhanced prediction accuracy, a 1D convolutional neural network (CNN) model was trained using DPV signals, achieving near-perfect agreement with reference RP-HPLC data ( $R^2 \approx 0.9996$ ). The CNN model enabled direct, real-time prediction of TQ levels without manual signal interpretation. These results position the developed sensors, particularly when combined with AI, as promising low-cost alternatives for portable, accurate TQ detection, suitable for food, pharmaceutical, and agricultural quality control. Experimental details and results are presented in Chapter 4 of this thesis.

c) A cost-effective, portable electrochemical system (LC-PSTAT) was developed combined with a molecularly imprinted polymer-based graphite electrode (TQMIP-G) for the selective detection of thymoquinone (TQ), in black cumin (*Nigella sativa*) [5]. The TQMIP-G sensor was fabricated using acrylonitrile as the monomer and EGDMA as the cross-linker, forming a polymer matrix with selective binding sites for TQ.

The LC-PSTAT device, designed using Arduino Nano and LF347 OPAMPs, supports both cyclic voltammetry (CV) and differential pulse voltammetry (DPV). It showed a detection limit of 0.03  $\mu$ M and a linear detection range of 1–200  $\mu$ M. The sensor maintained high repeatability (RSD 3.23%), reproducibility, and stability. Real-sample analysis of four branded black cumin samples using DPV and Partial Least Squares Regression (PLSR)

showed a prediction accuracy of ~94% when compared with RP-HPLC, confirming practical applicability. The developed LC-PSTAT system, with user-friendly Python-based software, costs ₹5000 and is suitable for on-site TQ detection in food, pharmaceutical, and agricultural industries.

**Table 5.1. Major findings of the thesis.**

| Sl. No. | Target compound | Electrode            | Method | Linearity (μM) | LOD (μM) | LOQ (μM) | Sensitivity (μA/μM) | Response Time (S) | Ref.  |
|---------|-----------------|----------------------|--------|----------------|----------|----------|---------------------|-------------------|-------|
| 1       | Piperine        | PDMAM-MIP@G          | DPV    | 0.001–200      | 0.006    | 0.019    | 12.92 & 0.326       | 21.8              | [1,2] |
| 2       | Thymoquinone    | GPE/TiO <sub>2</sub> | DPV    | 0.1-200        | 0.02     | 0.067    | 1.54                | 18                | [3]   |
| 3       | Thymoquinone    | TQIP/G               | DPV    | 0.05-100       | 0.03     | 0.099    | 0.1                 | 21.68             | [4]   |
| 4       | Thymoquinone    | TQMIP-G              | DPV    | 0.2-100        | 0.03     | 0.09     | 1.01                | 20.94             | [5]   |

### 5.3. Recommendations

Based on the performance and outcomes of the developed electrodes, several potential applications in the pharmaceutical and agricultural sectors are proposed:

- a) The pharmaceutical industry can benefit significantly from these sensors, as they offer a cost-effective alternative to traditional analytical methods. By addressing the limitations of existing systems, particularly in terms of expense and export-related compliance, these sensors provide a practical solution for quality monitoring and standardization.
- b) The molecularly imprinted polymer (MIP)-based sensors can be effectively employed for real-time, in-situ detection of key phytochemicals such as piperine and thymoquinone in various natural samples. This capability ensures precise monitoring of active constituents directly at the source without requiring elaborate sample preparation.
- c) Given the well-documented therapeutic properties of the targeted compounds in this study, the proposed sensing tools have strong potential in the herbal medicine and healthcare industries. They can support quality control, standardization, and product enhancement, paving the way for higher value products and improved consumer confidence in herbal formulations.
- d) Looking to the future, the LC-PSTAT system holds great promise for a wide range of applications, such as Agricultural sector, Pharmaceutical industry, and Food industry: The LC-PSTAT system represents a significant advancement in bioanalytical detection. Its

combination of affordability, portability, and high performance offers tremendous potential for application across a range of industries.

e) These versatile and user-friendly sensing technologies hold promise for expanding into broader industrial use, especially in applications where rapid, reliable, and cost-efficient analysis of bioactive compounds is essential.

### 5.4. Future scopes of research

To further extend and refine the developed electrochemical sensing platform for broader applicability and practical deployment, several future research directions are proposed.

- **Transition to disposable electrode platforms:** To enhance commercial viability in pharmaceutical and agricultural applications, future work may focus on adapting the sensors to disposable formats such as paper-based electrodes or screen-printed electrodes. These platforms would eliminate the need for surface regeneration, improve repeatability, and increase user convenience.
- **Extension to other bioactive spice constituents:** Although the present work focuses on piperine and thymoquinone, the same molecularly imprinted polymer (MIP) strategy can be extended to fabricate sensors for other bioactive compounds found in spices with health-promoting properties. Further optimization could also improve sensitivity for detecting lower concentrations of target analytes.
- **Integrated multi-analyte working electrode array:** The sensing platform can be advanced by designing an integrated array of multiple working electrodes on a single substrate, where each electrode is independently functionalized with an MIP selective to a specific spice biomarker. The array can operate using a common reference and counter electrode, enabling simultaneous or sequential detection of multiple analytes within a single electrochemical cell.
- **Multiplexed data acquisition and signal processing:** The existing low-cost potentiostat (LC-PSTAT) can be further enhanced by incorporating a multiplexing or channel-switching module to selectively address individual working electrodes in the array. This would allow multi-channel electrochemical measurements without significant increases in system complexity or cost.
- **Machine learning–assisted multi-analyte analysis:** Machine learning techniques can be employed to analyze the multi-channel electrochemical data generated by the

electrode array. Such approaches would enable deconvolution of overlapping signals, improved selectivity, and accurate quantification of multiple spice constituents in complex matrices.

- **Enhanced instrumentation and user interface:** Incorporation of a dedicated data acquisition module with a user-friendly digital display can further simplify operation and improve accessibility. Integration of wireless communication technologies such as Bluetooth or Wi-Fi would enable remote monitoring, data storage, and real-time analysis, particularly beneficial for industrial and field-based applications.
- **Broader application of MIP-based sensing:** Beyond spice and phytochemical analysis, the developed MIP-based sensing framework can be extended to other applications, including clinical diagnostics, targeted drug delivery monitoring, and detection of specific biomolecules in complex biological or environmental samples.

### 5.5. Conclusion

This thesis introduces a molecularly imprinted polymer (MIP)-based electrochemical sensing approach for the quantitative detection of specific phytochemicals, namely piperine and thymoquinone present in spices. Although the research is still at an early stage, it has laid a strong foundation for a novel strategy in the evaluation of bioactive plant metabolites. The sensors developed are affordable, lightweight, and portable, making them highly suitable for on-site applications. This portability opens the door for broader adoption, allowing spice producers to monitor product quality more effectively, and enabling consumers to assess quality before making purchases.

The simplicity and efficiency of the sensor fabrication technique outlined in this work have been validated for two key compounds and can be extended to detect other structurally similar molecules. The approach offers a promising tool for enhancing quality assurance in herbal and spice-based industries. Overall, this MIP-based sensing methodology holds potential to significantly advance quality control practices for medicinal plant products and natural health commodities.

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06/03/2026