Development of a Dedicated Electronic Tongue for the Detection of Taste-Affecting Compounds in Black Tea

Thesis submitted

By

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2025

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INDEX NO- 246/23/E

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LIST OF PUBLICATIONS

Journals

[1] **Moulick M,** Das D, Nag S, Tudu B, Bandyopadhyay R, Roy RB. Molecularly imprinted polymer-based electrode for tannic acid detection in black tea. IEEE Sensors Journal. 2023 Feb 2;23(6):5535-42.

[2] **Moulick M,** Nag S, Das D, Das D, Bandyopadhyay R, Pramanik P, Roy RB. Detection of theophylline using samarium oxide nanoparticles ingrained graphite electrode. Nano Life. 2023 Jun 24;13(02):2350007.

[3] **Moulick M,** Das D, Nag S, Pramanik P, Roy RB. Gadolinium oxide modified molecular imprinted polymer electrode for the electrochemical detection of theophylline in black tea. Journal of Food Composition and Analysis. 2025 Jan 1;137:106994.

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[5] **Moulick M,** Nag S, Das D, Hazarika AK, Sabhapondit S, Roy RB. A Molecular Imprinted Bi-polymer graphite electrode decorated with $NiCo_2O_4$ nano-cubes for rapid detection of theaflavin in black tea. IEEE Sensors Journal. 2025 Apr 3.

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[7] **Moulick M,** Chowdhury S, Das M, Saha S, Nag S, Naskar J, Modak A, Roy RB. A Customized Electronic Tongue by Developing an Array of Molecular Imprinted Polymer-Based Voltammetric Electrodes for Tea Quality Evaluation. IEEE Sensors Journal. 2025 Dec 8;26(2):1562-8.

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[1] **Moulick M,** Nag S, Das D, Tudu B, Bandyopadhyay R, Roy RB. Detection of Tannic Acid using Nd_2O_3 Modified Graphite Electrode. In 2022 2nd International Conference on Emerging Frontiers in Electrical and Electronic Technologies (ICEFEET) 2022 Jun 24 (pp. 1-5). IEEE.

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[3] **Moulick M,** Chowdhury S, Das M, Saha S, Bandyopadhyay D, Nag S, Roy RB. Development of an electronic tongue with MIP sensors for black tea quality evaluation. In2024 IEEE Calcutta Conference (CALCON) 2024 Dec 14 (pp. 1-4). IEEE.

Other Publications

[1] Kundu S, Gangopadhyay D, **Moulick M**, Bhattacharyya Banerjee M, Roy RB. Discrimination of different Citronella essential oil samples depending on citronellal using QCM based Sensor. American Journal of Electronics & Communication. 2023 Jul 3;4(1):25-8.

[2] Modak A, **Moulick M,** Roy RB. Efficient Near Infrared Spectroscopy Based Feature Selection of Tannic Acid for Black Tea Evaluation. In International Conference on Computational Intelligence in Communications and Business Analytics 2024 Jan 24 (pp. 149-157). Cham: Springer Nature Switzerland.

STATEMENT OF ORIGINALITY

I, Madhurima Moulick, registered on 5th April, 2023, do hereby declare that this thesis entitled "**Development of a Dedicated Electronic Tongue for the Detection of Taste-Affecting Compounds in Black Tea**" contains a literature survey and original research work done by the undersigned candidate as part of Doctoral studies. All information in this thesis has been obtained and presented under 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 7%.

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This is to certify that the thesis entitled "Development of a Dedicated Electronic Tongue for the Detection of Taste-Affecting Compounds in Black Tea" submitted by Madhurima Moulick, who got her name registered on 5th April 2023 for the award of Ph.D. (Engineering) degree of Jadavpur University, is absolutely based upon her own work under the supervision of Prof. Runu Banerjee Roy, and that neither her 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 inspirations…

-Mr. Nemai Moulick and Mr. Debashish Lahiri.

-My Parents and entire family.

ACKNOWLEDGEMENTS

The aspiration to become an academic and the love for teaching were the driving forces behind pursuing a doctorate in philosophy. As a student at this university, I have been extremely fortunate to have access to the facilities and infrastructure provided by the Department of Instrumentation and Electronics Engineering (IEE), Jadavpur University, Kolkata.

I would like to express my gratitude to Prof. Ratna Ghosh (IEE Dept.) for giving me the first exposure in the field of research. My heartfelt gratitude to my supervisor Prof. Runu Banerjee Roy, for her guidance and motivation throughout this journey. I would also like to extend my sincere gratitude to Prof. Rajib Bandyopadhyay, Prof. Bipan Tudu and Prof. Prolay Sharma of the Department of Instrumentation & Electronic Engineering at Jadavpur University for their immense support throughout the process.

I owe a great deal of gratitude to Dr Panchanan Pramanik, retired professor from the Department of Chemistry at IIT Kharagpur, who guided me with his immense knowledge in the field of chemistry for my research work. I sincerely thank Dr. Dipankar Mandal, Institute of Nano Science and Technology, Mohali, for FTIR analysis; Dr. Ramdhan Majhi, Indian Institute of Chemical Biology, Kolkata, for HPLC analysis; Department of Physics, Jadavpur University, Kolkata, for FESEM and FTIR analysis; UGC DAE Consortium, Kolkata Centre for XRD amenities, for their incredible technical assistance. I would like to thank the entire team of Tocklai Research Institute, Jorhat, Assam for their unconditional support during my research period.

This thesis would not have been completed without the support of my friend Mr. Souvik Nandy and his team at the Asian School of Tea and Nostealgia. They have been the driving force behind my research in this field. As a tea enthusiast, Mr. Nandy has been silently helping me with the completion of this entire research work, providing me with information on different samples of tea in the present market.

I wish to express my appreciation to my laboratory mates, Mrs. Shreya Nag, Ms Debangana Das, Mrs. Mahuya Bhattacharya, Mrs. Samhita Dasgupta, Mr. Dipan Bandyopadhyay, Mr. Srikanta Acharya, Mr. Deepam Ganguly, Mr. Sumit Kundu and Mr. Jeet Naskar.

Lastly, would like to extend my gratitude towards my family for their unconditional support during this journey.

ABSTRACT

Black tea is a globally consumed beverage around the world, generating a substantial revenue worldwide. Black tea is composed of a number of compounds, which have a positive effect on the consumer when consumed up to the therapeutic limit, else can lead to health complications. Till now, tea tasting is based on the tea-taster's score and is purely subjective and varies with the individual's state of mind. Thus, to obtain a quantitative measurement of the different taste-affecting compounds in black tea, an effective instrumental approach is the need of the hour. This research work is aimed to rapidly detect the presence of different analytes in black tea. Three distinct molecules, tannic acid (TAN), theophylline (TH), and total theaflavin (TF), were chosen as the target molecules. Tea mainly consists of two different types of compounds- polyphenols and methylxanthines. The presence of the TAN (polyphenol) determines tea astringency, TH (a methylxanthine derivative) is responsible for bitterness as well as astringency. The total theaflavins (TF) in black tea are responsible for the aroma and flavour of tea. The employment of nano-crystals of metal and rare-earth metal oxides and molecularly imprinted polymer (MIP) based electrodes is explained for the selective determination of the analytes. To comment on the morphological variations of the electrode materials, Fourier transform infrared (FTIR) spectroscope, field emission scanning electron microscope (FESEM), and an ultraviolet visible (UV-Vis.) spectroscope have been used. To begin with, an electrochemical sensing system has been reported, consisting of a modified graphite electrode (Nd_2O_3@GP) for the detection of TAN in black tea. To enhance the performance of the electrochemical system, MIP technology was used. This MIP based electrode showed a limit of detection (LOD) 37 nM and the electrode displayed an accuracy of 96.315%.

The next work discusses two different approaches for the detection of TH. Initially, the analysis of voltammetric responses in real black tea samples, while modifying the bare graphite electrode with samarium oxide nanoparticles, is highlighted. Additionally, the thermally stable properties of the Gd2O3 NPs have been explored to enhance the MIP electrode's response. In contrast to the initial investigation, the later MIP-based electrode showed reasonable molecular selectivity, stable behaviour over a sufficiently long time and the LOD 2.004 μM was significantly improved. Compared to the standard HPLC protocol, the electrode demonstrated an accuracy of 91.3% in real samples of black tea.

In the following section, the detection of total theaflavins has been explored using a modified

graphite electrode and a MIP-based electrode whose surface has been modified by $NiCo_2O_4$ nanoparticles. The MIP-based electrode showed a LOD of 13.89 μM with satisfactory repeatability, with an RSD value of 0.71%. This electrode shows a prediction accuracy of 92.6% when immersed in real samples of tea.

Towards the end of the thesis, an attempt was made to successfully detect two analytes, epicatechin (EC) and TH, using a single CuO modified electrode. This CuO@GP electrode demonstrated a linear range of 50.0–100.0 μM for both analytes, with a LOD of 4.0 μM for EC, 27.0 μM for TH. Recently, an approach was made to develop an e-tongue based on MIP-based electrodes. This array of MIP sensors was able to successfully detect the traces of TAN, TF and TH in a single tea sample. DPV responses from the tea sample were obtained and subjected to PCA clustering, which shows a satisfactory segregation among the three different analytes in a black tea sample with a separability index (SI) of 32.4305. Further, this same setup was used to discriminate among five different samples of black tea. In this latest approach, the Gradient Boost algorithm has been used to develop a robust cognitive model, which showed R^2 value of 0.967. The t-SNE algorithm on the fused data successfully discriminated among the five different samples of black tea. Addition of a few more specific sensors, targeting other major tea bio-markers to this array, may be considered for further study. The developed system is poised to be used as a taste profiling instrument on a day-to-day basis in the tea industries.

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LIST OF ABBREVIATIONS

CPE	carbon paste electrodes
MIP	molecular imprinted polymer
XRD	X-ray diffraction
FTIR	Fourier transform infrared
FESEM	field emission scanning electron microscope
TAN	Tannic acid
TH	theophylline
TF	Total theaflavins
E-tongue	Electronic tongue
WE	Working electrode
RE	Reference electrode
CE	Counter electrode
LSV	Linear sweep voltammetry
CV	Cyclic voltammetry
PV	Pulse voltammetry
NPV	Normal pulse voltammetry
DPV	Differential pulse voltammetry
SWV	Square wave voltammetry
L-DOPA	L-Dopamine
AA	Acrylamide
LOD	Limit of detection
EGDMA	Ethylene glycol dimethacrylate
PLSR	partial least square regression
PCR	principal component regression
MAA	methacrylic acid
CAF	caffeine
Nd2O3@GP	neodymium oxide nanoparticle modified graphite
Pt	platinum
PXRD	powder x-ray diffraction
TEM	transmission electron microscopy
NiHCF-AFCNT/GCE	glassy carbon electrode modified with nickel hexacyanoferrate on

	amino-functionalized carbon nanotubes
MIP_TAN	MIP infused in graphite paste
AN	acrylonitrile
MMA	methyl methacrylate
UV-vis	Ultraviolet-Visible spectroscopy
NIP	Non imprinted polymer
ABS	acetate buffer solution
CBS	citrate buffer solution
PBS	phosphate buffer solution
BW	Before washing
AW	After washing
RSD	Relative standard deviation
CAT	catechin
GA	Gallic acid
LV	Latent variable
RMSE	root mean square error
HPLC	High-performance liquid chromatography
Sm_2O_3NPs	Samarium oxide nanoparticles
PCA	Principal component analysis
Gd_2O_3@TH-MIP-CPE electrode	Gadolinium oxide modified MIP
LOOCV	leave-one-out cross-validation
TiO_2@GP	Titanium oxide modified graphite electrode
DCM	dichloromethane
MIP_TF/$NiCo_2O_4$	$NiCo_2O_4$ modified MIP electrode
CuO@GP	Cupric oxide nanoparticles infused graphite electrode
MSE	mean-squared error
t-SNE	t-Distributed Stochastic Neighbour Embedding

CHAPTER 1 *Introduction and scope of the thesis*

The aim of this thesis is exemplified in this chapter. The daily consumption of tea worldwide emphasizes on an in-depth research work to monitor its quality for beneficial consumption. Several traditional methods, their advantages and disadvantages are thoroughly discussed.

CHAPTER 1

Introduction and scope of the thesis

1.1 Introduction

Tea has long attracted the global population as the second-most consumed beverage after water due to its health benefits [1]. The production, marketing, and consumption of any consumable item depend solely on the crop quality. Over the last decade, serious research work has been carried out to improve the quality profiling of tea. Tea has numerous compounds, which have a multidimensional influence on its taste, flavour, and aroma. Depending on the tea processing methods and the degree of fermentation of the tea leaves, they can be broadly classified into green, black, and oolong tea. Black tea, due to its fermentation process, can retain and improve its flavor over time. Additionally, it has a long history suggesting its contribution to the prevention of some cancers, cardiovascular diseases and treatment of diabetes mellitus in clinical trials [2]. All these positive benefits of black tea can only be achieved if the beverage is consumed in proper quantity. Due to its positive impact on human health and its role in shaping the tea industries, real samples of black have been chosen as the choice of real samples throughout the research work.

Despite the fact that tea plays crucial role in the world economy, it is extremely unfortunate that tea quality assessment is solely dependent on group of specialized panelists called ‘tea tasters’ [3]. The tea gradation done by these panelists is subjective and suffers from inconsistent gradation due to the influence of psychological attributes [4]. Traditionally, high-end facilities like spectrophotometry [5], capillary electrophoresis [6], and high-performance liquid chromatography (HPLC) [7] provide reliable information about the different tea biomarkers. These techniques require highly skilled labor, high cost, and sample preparation time, hence deployment of these techniques in remote tea industries is not feasible.

Due to the aforementioned hindrances faced in the usage of the traditional methods, electrochemical sensing was introduced as a wide avenue for the rapid detection of important constituents in tea. Important constituents like tannic acid are reported to be detected using simple carbon paste electrodes (CPE) in tea [8]. Simple CPE and electrodes fabricated by addition of different modifiers have been proposed by many researchers for the detection of

caffeine [9]- [13]. Methylxanthine derivatives like theophylline, can influence the taste and astringency of black tea to a certain degree, literature [14]-[15]. However, these electrodes lacked selectivity due to the absence of specific site recognition mechanism.

The present thesis titled 'Development of dedicated electrochemical sensors for the detection of taste-affecting compounds in black tea' involves the development of electrochemical sensors for the detection of three different types of taste influencing molecules present in black tea, namely flavonoids, polyphenols and methylxanthines which are mainly responsible for tea astringency and bitterness. This thesis reports the use of modified graphite electrodes and molecular imprinted polymer (MIP) technology, which enables the detection of specific target molecules in the real samples. The MIP technology involves the creation of structurally similar vacant sites on the polymer matrix for adsorption of target molecules. In this thesis the use of metal oxide (CuO, TiO_2 and $NiCo_2O_4$) and various rare earth metal oxides (Sm_2O_3, Nd_2O_3) have been widely explored due to their excellent electro catalytic properties [16]-[18].

In the pursuit of a low-cost, portable instrumental method for error-free evaluation and quantification of the taste of black tea, electrochemical sensors which can be directly immersed in the liquid samples, have been fabricated in this thesis work. The particle size, structural morphology of the imprinted electrodes was characterized using XRD technique, FTIR spectroscopy and FESEM. This introductory chapter briefly introduces the human gustatory system, the black tea processing method, the chemical composition of tea, the various tea evaluation techniques, and the literature survey on electrochemical detection of tea quality. This chapter ends with a brief overview of the subsequent chapters.

1.2 The human gustatory system

Taste buds are a cluster of specialized receptor cells, known as gustatory cells, protruding out of the mucous layer of the tongue. Each taste bud is a barrel-shaped structure consisting of many spindle-shaped receptor cells bundled in the epithelial sack [19]. On top of each taste bud, there is a minute opening called the taste pore, which allows the chemicals of the ingested food to interact with the receptor cells through the microscopic hair-like outgrowth called 'microvilli'. A series of biochemical reactions occur when the chemicals in food interact with the taste receptor cells in the salivary base, leading to the production of electrical potentials. The series of steps involving chemical and physical interactions of food

molecules with the receptor cells is termed as 'signal transduction pathways. Signal transduction is initiated when the molecular component of the chemicals with the taste receptors through binding and diffusion. The base of the receptor cells is synaptically connected to the nerve cells by many to one relationship. The electrical potentials generated as a response to the chemical stimuli are transmitted by the nerve cells to brain for perceptive interpretations.

Around 50-150 taste receptors make a single taste bud. The taste buds are spatially organized into functional groups called 'papillae'. The positioning of the papillae and taste buds are presented in **Fig. 1.1**. The various types of papillae found on human tongue are described in **Table 1.1.**

Table 1.1: *Types of taste papillae and their location on human tongue*

Type	Taste	Location
Fungiform papilla	Sweet and salty	Present at the apex of the tongue, found to be evenly spread over the tongue toward the salty taste
Foliate papilla	Sour	Posterior lateral edges
Circumvallate papilla	Bitter	Present on the lingual valet and at the rear portion of the tongue.

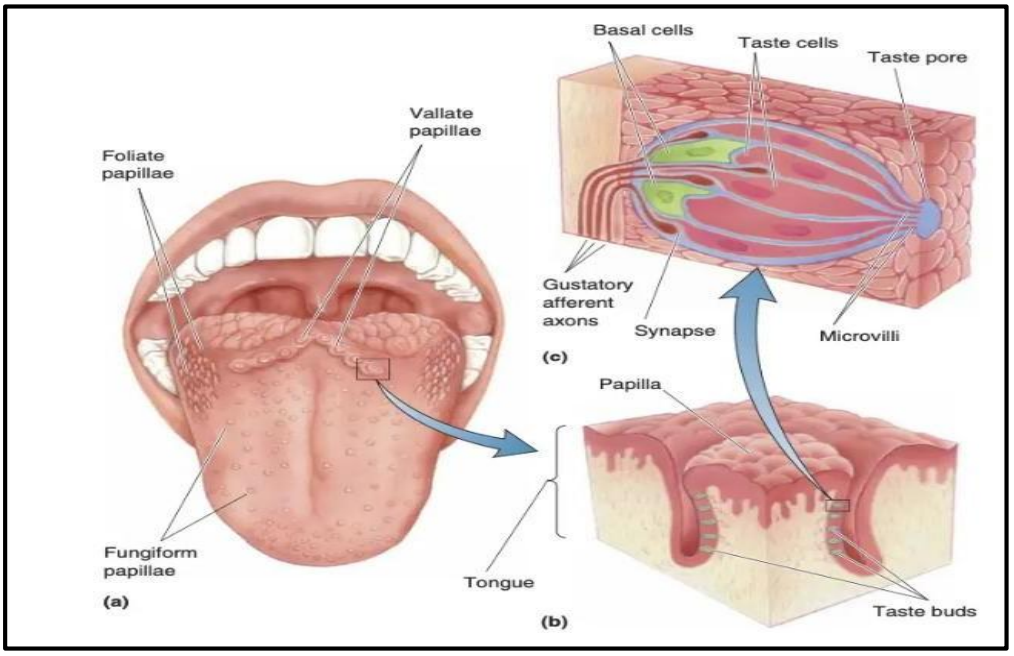

Fig. 1.1: *The human gustatory system*

1.3 Black tea processing and chemical composition

According to the manufacturing procedure, tea can be broadly classified into i) Unfermented green tea ii) Partially fermented oolong tea and iii) fully fermented black tea. **Fig 1.2.** portrays the manufacturing procedure for each of these categories.

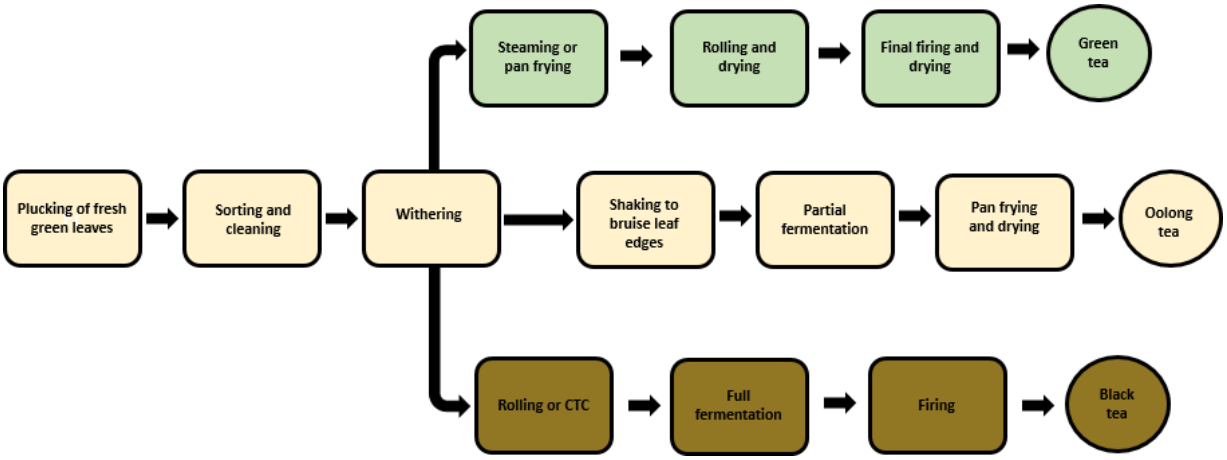

Fig. 1.2: *Tea processing flowchart*

Black tea is a more oxidized variety than the oolong and green teas. It contains more caffeine than the less oxidized varieties of tea samples. The algorithm for the processing of black tea involves the complete oxidation of the tea leaves. After plucking, the leaves are laid out to wither for around 8 to 24 hours, allowing the water to evaporate. The leaves are rolled in order to crack up the surface such that oxygen can react with the enzymes and the oxidation process begins. The leaves are left to be completely oxidized and eventually turn to a deep black colour. The stages of black tea manufacturing are described in detail below:

1.3.1 Plucking

The "two leaves and a bud" are the most preferable plucking configuration, that ensures that the green leaves collected from the tea plants produce desirable traits in consumable tea. Hand- picking is the choice of plucking when higher quality of tea is desired.

1.3.2 Withering

After plucking, the leaves are withered to remove their moisture content by spreading over a withering trough for 10 to 24 hours. The leaves are dried in sunlight, or by blowing warm air over them if the surrounding is humid. As the water content decreases, the leaves are now suitable to be rolled.

1.3.3 Rolling

The withered leaves are shaken, rolled and twisted to break the leaf cells. During this rolling stage, oils are released to give aroma to the tea. Tea leaves can be rolled by hand or machinery. For orthodox variety of tea, the withered leaves are rolled on rollers. The tea roller machine consists of three different parts. During rolling, the leaves are heated up due to chemical reactions taking place in the leaf-juice. After rolling, the first fines are segregated and the orthodox tea leaves are left for fermentation. For the CTC tea, the process of rolling is rigorous as the tea leaves are compelled to move between two cylinders, rotating in reverse directions with a minimal clearance between them. During this process, the various chemicals released remain on the tea surface for further processing.

1.3.4 Fermentation

It is a chemical process for the removal of oxygen. The tea leaves are spread in thin layers on trays in a controlled environment, where in they progressively change into darker tones. During this process, the chlorophyll in the leaves is enzymatically broken down, and tannins are released which mark the start of the oxidation process. During this course the colour of the green tea leaves change to bright copper or black. Depending on the degree of fermentation, oolong or black tea are produced. The extent of oxidation for the oolong tea is 60-70% and 100% oxidation is carried out for black tea production.

1.3.5 Drying

The moisture of the leaves is completely dried without burning the leaves and the oxidation process is stopped. The leaves then become ready for sorting and packing.

1.3.6 Sorting and packaging

Sorting is the procedure in which tea particles are separated while passing through different meshes. The sorted tea differs in price as well as quality from one another. Packaging is the process of preserving the finished tea using the most cost-effective but appropriate materials taking into consideration the demands of the end users.

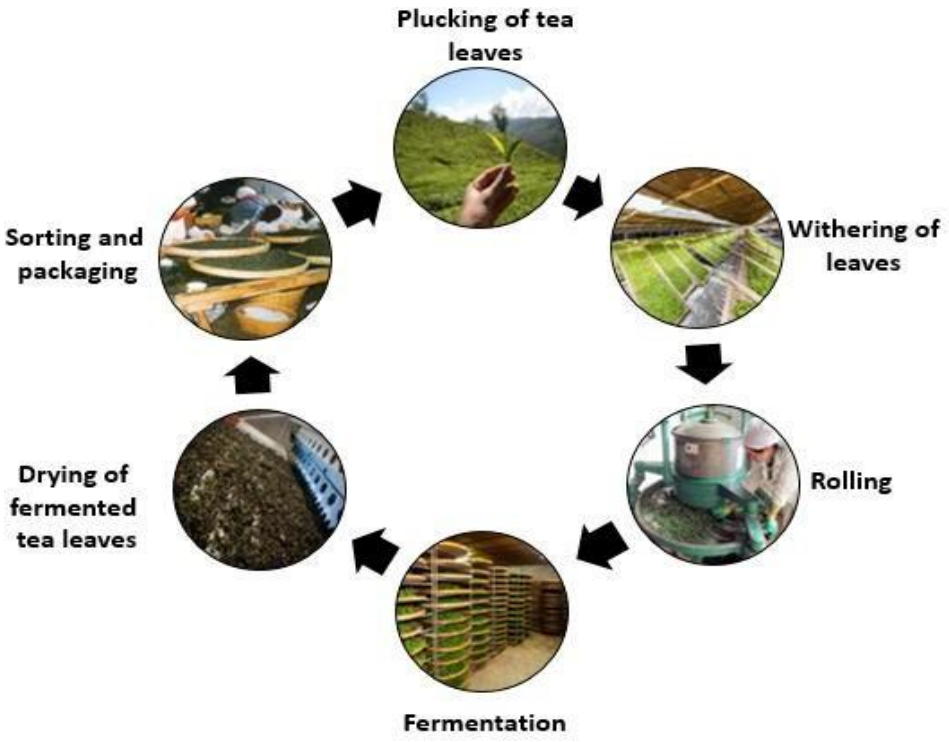

Fig. 1.3: *Stages in black tea processing*

1.3.7 Taste constituents in tea

The result of the intermixing of aroma, taste, and temperature is termed as flavor [20]. Tea comprises of around 500 compounds and their multidimensional influence varies the taste in different types of tea. Table 1.2, tabulates the different taste-affecting compounds in tea.

Table 1.2: *Taste-affecting compound found in tea*

Sl. No	Taste-affecting compound	Taste
1.	Theaflavin	Astringent
2.	Catechin	Strongly bitter
3.	Polyphenol	Astringent
4.	Amino acids	Brothy
5.	Caffeine	Bitter
6.	Thearubigin	Ashy and astringent
7.	Theophylline	Astringent

1.4 Tea quality evaluation techniques

1.4.1 Instrumental methods

Instrumental methods are regularly employed by the tea scientists for the determination of the quantity of non-volatile compounds of tea for its quality profiling. The concentrations of the different constituents of tea like the polyphenols, methyl-xanthanine derivatives (TH), caffeine, total theaflavins help in the overall quality assessment of tea. A pervasive literature survey highlights the use of traditional methods like HPLC, fluorometric method, spectrophotometry and colorimetry for the detection of TAN in beverages [21], [22], [23] and [24]. Some traditional methods for the detection of THP include surface-enhanced Raman scattering sensor [25], colorimetric aptasensor [26]. And HPLC-UV method [27]. The total theaflavins in black tea is responsible for imparting the tea liquor its orangish-brown colour and its quantitative presence in tea determines the tea astringency. Literature suggests the use of technologies like electrophoresis [28], pulse radiolysis [29] and flavognost method [30]. These instruments, although capable of estimating the quantity of the target molecules, required highly skilled professionals. The instruments were not portable as they were bulky in size. The high cost of maintenance and involvement of toxic solvents for sample preparation drew the need for low- cost, efficient, repeatable technologies for the fabrication of very responsive sensors for industrial applications.

1.4.2 Organoleptic sensation method

In tea terminology, organoleptic refers to tea's sensory attributes referring to its taste, aroma, texture, appearance and infusion. By engaging our sense organs, we can explore the complexities, nuances, and quality of different types of tea. The quality of dry leaves is awarded by placing the leaves on a white paper, sorting the leaves according to uniformity, texture and aroma exuded from them. The quality of infused tea leaves is adjudged depending on their aroma and colour. The aroma emitted from the hot-infused leaves is assigned as “delicate”, “fruity”, “burnt”, “smoky” and “sour”. Consumers classify tea based on their overall taste and aroma, which is highly subjective. Even the dependence on tea taster’s score does not give a quantitative profiling of tea. It has been indicated that the quality of consumables and beverages is highly influenced by the chemical components present in them, thus instrumental analysis of tea quality based on their chemical constituents is more reliable and accurate. Human tea tasters generally assign a score after tasting the tea liquor.

This type of quality profiling of tea is highly subjective and depends on an individual's taste parameters, mood, mental health etc. Thus, the scores obtain suffer inconsistency.

1.4.3 Electronic tongue

An electronic tongue can be defined as a sensory-electrical system, that is biologically inspired, and in which responses from the working electrodes are recorded and statistically interpreted using intelligent models. A complex chemical matrix of innumerable compounds classically examines the analytes from the natural origins. The effectiveness of using specific electrochemical sensors is restricted by the number of chemical compounds constituting the analytes from naturally occurring sources. In these circumstances, fabricated electrodes with conjunctional affinities obtain information about most of the compounds but with unique levels of sensitivities. To extract the required quantity from the electrical signatures collected from the electrodes, multivariate statistical methods are employed. The functional schematic of the electronic tongue (e-tongue) is presented in **Fig. 1.4**

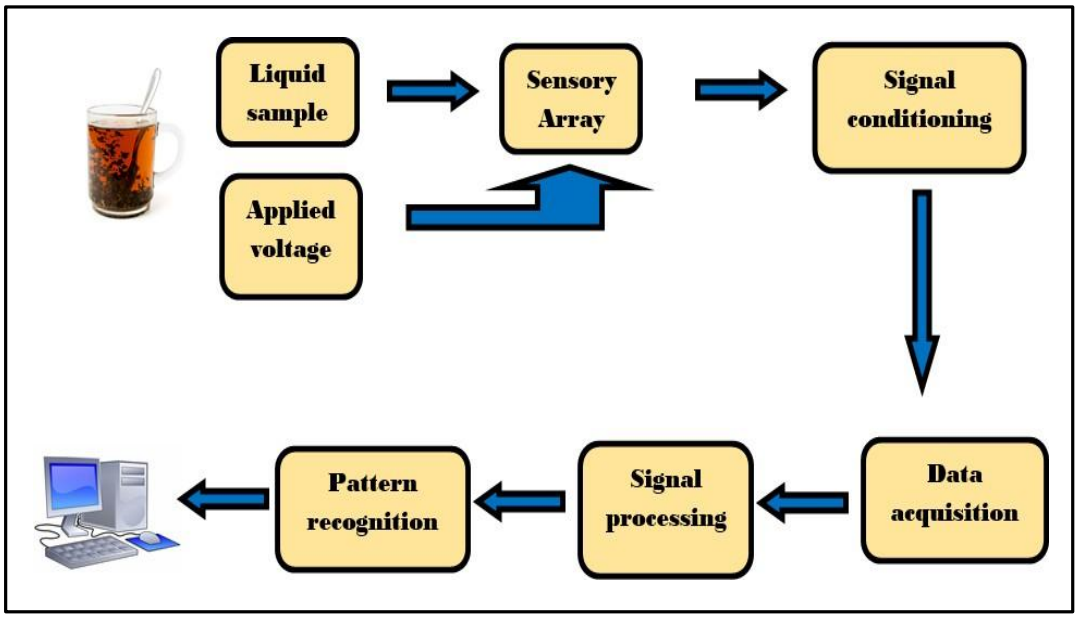

Fig. 1.4: *The schematic of an e-tongue*

1.4.3.1 Brief description of E-tongue

Evaluation of tea quality and determination of its grade can be carried out by e-tongue with the aid of electronic taste sensors and intelligent pattern analysis techniques. The analysis using an e- tongue is rapid and responsive, owing to the simple sample preparation and it also conveys a lot of information about the constitution of multi-component beverage

and food samples. The array of sensors mimics the human taste buds. Fig. 1.4. shows the functional block diagram of the e- tongue. The various components present in the e-tongue are:

- ***Sensor Array***

  Various types of chemical sensors based on different working principles are used in an e-tongue. A brief description of the different sensors used in an e-tongue is summarized in section 1.5.

- ***Signal conditioning***

  It establishes a link between the sensor array and the pattern recognition modules, the two crucial elements in an artificial taste-sensing instrument. Integrating the pattern analysis algorithms with the underlying chemical transduction mechanisms involves the application of a number of electronic circuits. Firstly, the response of the sensor array is recorded and converted into electrical signals. Subsequently, these signals undergo analog conditioning to enhance their information content. The analog signal is then sampled and digitized for further use. Finally, the sampled signal is digitally pre-processed to make it suitable for pattern analysis.

- ***Data acquisition***

  The signals from the sensor array after conditioning is digitized to be further processed for online/offline analysis. This data is directly fed into the data acquisition module of the e-tongue system.

- ***Signal processing***

  After the acquisition of the data, the signal is processed for the extraction of important information from the responses and the preparation of this information for multivariate pattern analysis. Signal processing is a critical step as it can impact the performance of the subsequent pattern analysis module.

- ***Pattern recognition***

  The electronic tongue is developed as a model for the human tongue, comprising various stages between the liquid sample and its recognition, namely, interaction, signal generation, processing and identification.

1.4.3.2 Principle of operation of E-tongue

1.4.3.2.1 Potentiometric

The electric potential developed across the interface of the electrode and sample is measured with respect to the potential of a reference electrode under the absence of current. Potentiometric e-tongue comprises of an electrode array composed of different sensing membranes producing a broadly selective response when subjected to a mixture of chemicals [31-33].

1.4.3.2.2 Voltammetry

The most widely accepted amperometric technique involves the principle of voltammetry, where the current across the WE are measured within a specified potential window [36]-[37]. **Fig. 1.5** shows the three-electrode setup in an electrochemical process.

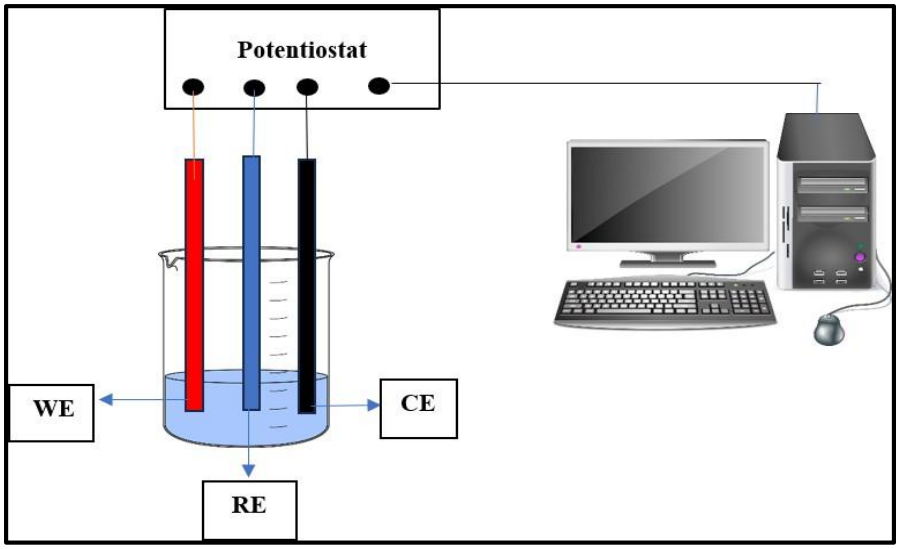

Fig. 1.5: *Three electrode-based electrochemical cells*

The applied potential is varied within a specified frame to keep the electroactive species at the surface of the WE. The quantitative information about the target analyte can be obtained in terms of the redox peak current due to molecular adsorption at the surface of WE. Unlike potentiometry, RE comes into play to maintain a reference potential against the WE with the help of a power source or potentiostat. The potentiostat bears an analogy with the controlled voltage source in an electrical system. It controls the voltage applied across the WE by imposing redox reactions at the interface of the electrode and thus produces a current. The obtained current possesses useful information about the reaction kinetics of the overall system.

Traditional voltammetric approaches are described below:

A. *Linear sweep voltammetry (LSV)*

The current generated in the electrochemical cell is studied with reference to the linearly varied potential between the RE and WE with respect to time. Factors like the scan rate, reactivity of the electro active species and electron transfer rate dominate LSV [35].

B. *Cyclic voltammetry (CV)*

It is the most widely accepted electrochemical measurement principle, where potential is varied linearly with time [35]. After achieving the saturation potential level, the potential of the WE take a reverse ramp to reach the initial potential level.

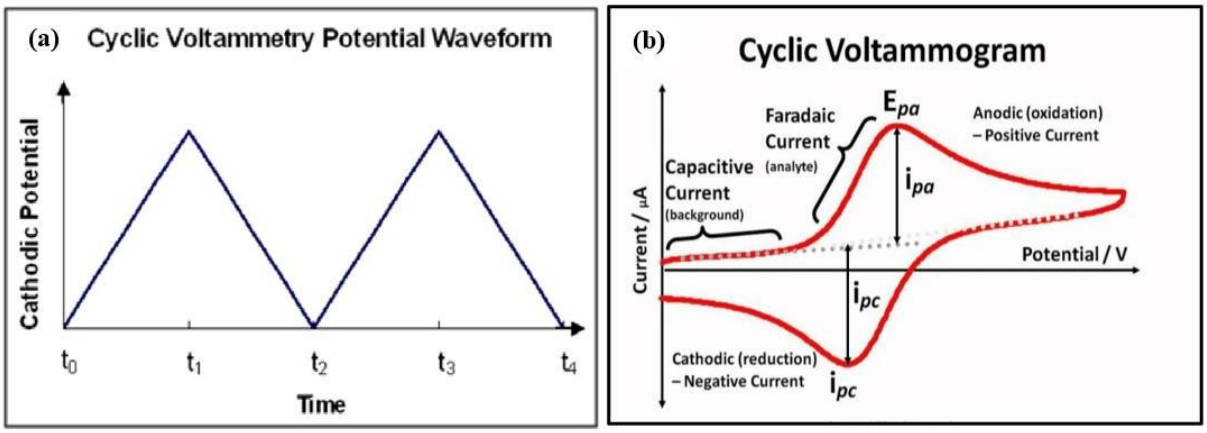

Fig. 1.6: *(a) Cyclic voltammetric pulse waveform (b) A typical CV response*

Fig. 1.6 (a) shows a typical cyclic voltammogram and **1.6 (b)** shows the anodic (*Ia*) and cathodic (*Ic*) currents respectively, corresponding the underlying redox reactions. As seen, the reaction is triggered by a triangular waveform [36]. The oxidation current increases with the input potential during the forward scan (t_o-t_1). During this period, oxidation takes place, on further application of the voltage, the current value decreases to an almost constant level. During the reverse weep (t_1-t_2), the cathodic peak current is investigated by means of reduction of the oxidized components.

C. *Pulse voltammetry (PV)*

PV operates about a potential pulse based on the deviation in the rate of charging and faradaic currents. The Faradaic current decays at a slower rate in comparison to the charging current, which decays itself exponentially with a function of $1/(\text{time})^{1/2}$. Barker and Jenkin developed this voltammetry technique [37], and it detects analytes

up to a 10^{-8} M concentration level. There are two classifications of this voltammetry.

- ***Normal pulse voltammetry (NPV)***

  It comprises of a series of monotonically increasing potential pulses that trigger the output current at the termination of each pulse, allowing a period time for the charging current to decay. The duration of the pulse time (t) in response to the potential (E) ranges from 1-100 ms with the time interval being 0.1 s to 5 s approximately. The voltammogram is represented as the sampled current response on the vertical axis vs a stepped pulse on the horizontal axis.

- ***Differential pulse voltammetry (DPV)***

  A typical DPV process is illustrated in Fig. 1.7. NPV and DPV have some similarities, the difference lies in the shape and incrementally increased amplitude of the applied excitation voltage. It is seen that the baseline potential remains maintained after each change in application of the subsequent pulse and the current corresponding to it is sampled before and after the pulse [38]. For a quantitative investigation of the target molecule, the difference in the two values of the sampling current is recorded. DPV appears to be a better and sensitive approach for the detection of lower concentration limits.

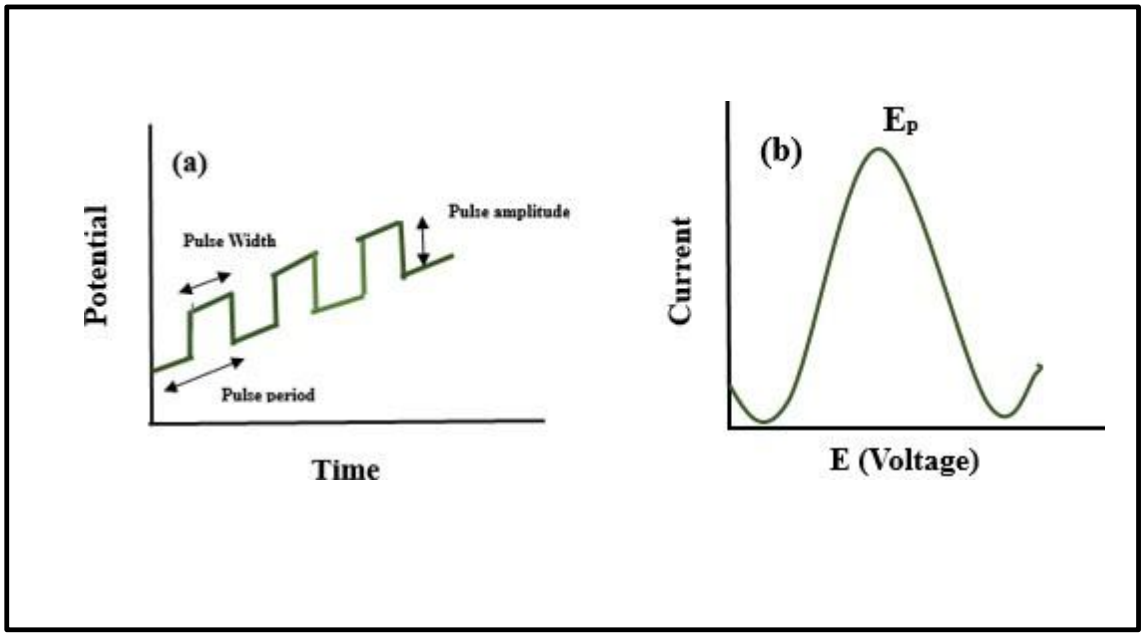

Fig. 1.7: *(a) Differential pulse waveform (b) A typical DPV response*

- ***Square wave voltammetry (SWV)***

  Identical short square wave pulses of amplitude (E_{sw}) are imposed on a staircase shaped waveform in such a manner that the square wave's forward pulse matches the staircase's phase [39]. The resultant current, is calculated from the difference between the forward and reverse currents.

1.5 Sensor array of the e-tongue

Different types of sensors based on different working principles can be employed in the sensor array, and the subsequent sections summarize the different electrochemical sensors briefly. The transduction methodology of any electrochemical sensing is based on the networking of the target compound with the recognition element decorated over the support material. Table 1.4 summarizes the different electrochemical sensors used in an e-tongue.

1.5.1 Noble metals

Previous reports suggest the use of array of noble metals like gold, platinum and iridium for the overall quality gradation of tea. The use of these noble metals proved to be highly expensive and they provided overall estimation of the taste index of tea similar to our human gustatory sensation [68].

1.5.2 Carbon paste electrode (CPE)

Noble metal electrodes, although, provided satisfactory analytics but were quite expensive, thus, a more cost-effective approach was the fabrication of carbon paste or bare graphite electrodes. Its easy sample preparation technique and cost-effectiveness led to the popularity of such electrodes.

1.5.2.1 Modified carbon paste electrodes

To increase the electrode efficacy, the surface of the working graphite electrodes can be modified using nanoparticles of metal oxides as well as rare earth metal oxides. Metal oxides like TiO_2 and CuO nanoparticles have been used to decorate the surface of electrodes to improvise the thermal and chemical stability [101]-[102]. Rare earth metal oxides like CeO_2, and Nd_2O_3 induced excellent electro-catalytic performance due to increased surface area on account of an increment in the number of active sites leading to molecular adsorption

followed by escalated electron transfer [103]. Owing to their exceptional electron transfer rate and chemical kinetics, these nanocomposites have been impregnated on the surface of the working electrodes for higher current oxidation efficiency.

1.5.3 Molecular imprinted polymer (MIP) technology

Experimental limitations like reduced specificity of the working electrodes, necessitated the acceptance of the molecular imprinted polymer (MIP) technology for improved affinity and reproducibility. An efficient and responsive technology for the fabrication of highly selective sensors, MIP serves as a gateway for the development of highly specific electrochemical sensors. MIP materials are synthesized by co-polymerization of functional monomers and cross linker around a template molecule [40]. The target molecules are subsequently leached off the polymer matrix under specific circumstances, thus active sites structurally similar to the target template is created. A substrate that is structurally identical to the formed cavities, is thereby adsorbed to the site. The schematic for the MIP procedure is illustrated in **Fig. 1.8.** The different types of MIP electrodes used in the detection of important analytes present in different beverages and consumable food products is summarized in **Table 1.5.**

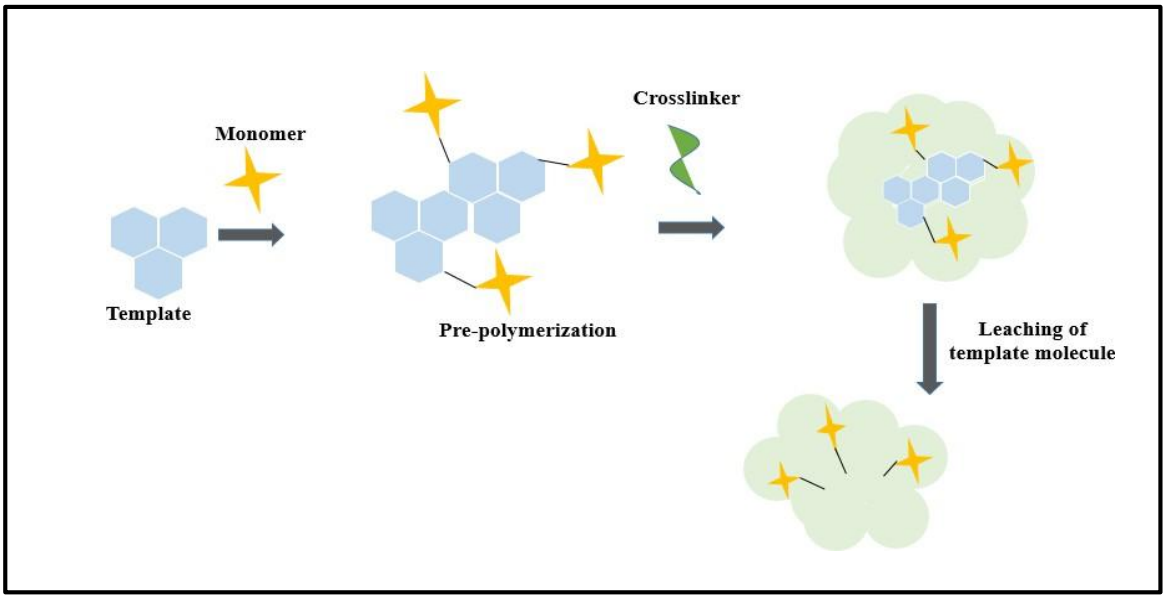

Fig. 1.8: *Schematic of MIP process*

Three simple steps can be followed for the synthesis of MIP [41]:

1. **Association:** Covalent and non-covalent bonds can congregate the monomers around the target molecule.

2. **Polymerization:** Various polymerization techniques can be employed to generate cross-linked monomers to trap the target molecules.
3. **Elution**: By engaging the correct elution reagent, the trapped molecules in the polymer matrix are released. Hence, cavities, with structurally similar fit are created, hence adsorption in feasible.

There two crucial elements that help in shaping the fundamental principle that underlie molecular recognition process [42]. These are:

- The template-mediated pre-organization of the polymer's complementary functional groups.
- Imprinting of a complementary shape-selective cavity to the template.

Due to their exceptional flexibility, adaptability and mechanical stability MIPs have proved to be suitable towards any target analyte due to the extensive accessibility of practical monomers. The MIP approach may be used in biosensors to replace unstable biological molecules like antibodies and enzymes. **Table 1.3** highlights the benefits of MIP sensors over organic biomolecules.

Table 1.3: *Comparison between MIP sensors with natural biomolecule-based sensors*

Sl. No.	Sensor-based on natural biomolecules	MIP sensors
1.	Not very stable	MIP sensor stability is quite satisfactory with low or high pH.
2.	Pricey receptors and enzymes	Inexpensive and simple to use
3.	A lack of compatibility with miniaturization and micromachining.	Polymers and micromachining technologies are compatible
4.	Performs badly in other solvents except water.	They can react in organic solvents.

Any polymer matrix bed can generate active cavities by a number of chemical processes like non-covalent imprinting, imprinting with sacrificial spacers, non-covalent imprinting and imprinting with the use of covalent bonds. The sections below give an elaborate view on the types of imprinting forms.

1.5.3.1 Imprinting techniques

1.5.3.1.1 Covalent imprinting

In this technique, using a chemical process which is independent of polymer formation, the target template and minimum one polymerizable segment is congregated by means of reversible covalent bonds to create a template-monomer unit. At the point of inception, a pre- polymerization derivative is created using the target and monomer, followed by the polymerization technique. After successful polymerization, the template is extracted off, and the covalent linkage between the template and polymer is cut. An identical bond is re-established on rebinding the template into the MIP [43]. The covalent imprinting process is portrayed in the schematic below in **Fig. 1.9**.

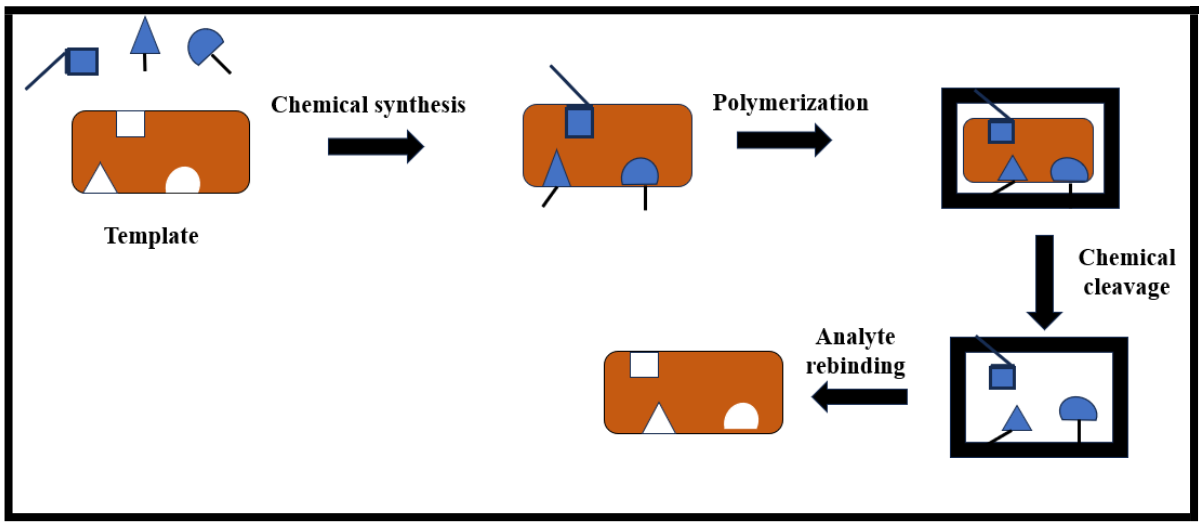

Fig. 1.9: *Schematic for covalent imprinting*

D. Imprinting with readily reversible covalent bonds

In this type of covalent bonding uses techniques for quick reversible binding reactions, like boronate ester, ketal, and Schiff's base development, to promote the design of the template- monomer binds. The hydrolysis of the organization molecule from the polymer under sensitive liquid conditions is necessary for catalyzing the binds. The number of configurations to be imprinted is restrained by the assistant requirements of covalent methods, namely 1,2- and 1,3-diols (boronate ester and ketal), aldehyde (acetal and Schiff's base), ketone (ketal), and amine (Schiff's base). This strategy's main aim is that the bounce back stage is intermediate to the as prepared polymer, in which each of the active sites almost are identical to one

another. The resistance faced in any type of covalent etching mechanism is the prerequisite for congregation of the functional monomers.

E. Covalent imprinting with boronate esters

For the imprinting of any carbohydrate or starch component the boronate ester strategy is one of the smooth reversible covalent techniques. This method was first put into practice by Gunter Wulff et al., for imprinting the subordinates of mannose, galactose, and fructose, sialic acid, castasterone, and L-Dopamine (L-DOPA) [45]-[48]. Various studies have reported that combination boronate esters to create MIPs for fluorescent detection [49]-[51]. This technique has been explored for engraving mono alcohol forms and the ones with segregated distinct hydroxyl groups owing to the use of boron ophthalide-based monomers [52].

F. Covalent imprinting with Schiff's bases

The chemistry of Schiff's base (imine) includes the assembly of an essential amine and a carbonyl molecule. This is a beneficial process for imprinting amine- or aldehyde-bearing templates. This method has been implemented with success to imprint amino acid derivatives [53].

G. Covalent imprinting with acetals and ketals

An in-depth analysis of the mono- and di-ketone templates with a polymerizable diol was used as the binding group for the MIP's readiness in [54]-[56]. In [57] it has been thoroughly examined if a series of cyclic hemiacetals could function as mono-alcohol-hindering groups.

1.5.3.1.2 Non-covalent imprinting

The challenges faced in covalent imprinting are overcome in the non-covalent imprinting process. In the 1980s, Mosbach's team popularised it and developed a usable process for engineering imprinted receptors in engineered polymers [58]. Fig. 1.10, shows the non-covalent imprinting technique [59]. Various interactions like dipole-dipole forces and Van der Waal's forces the template-monomer units are generated in appropriate solvents. The template is extracted out of the polymerized test unit and can rebound using the identical interaction forces.

In this imprinting process, segments of the polymer units having useful functional groups start layering on top of the template molecule post the development of a pre-polymerization complex between the monomers and template. As polymerization proceeds, the structures created change their shape depending on the quantity of the monomer added, resulting in greater affinity towards the target analyte in the cavities until a complete polymerized unit is formed. This imprinting can be done using one unit or combination of different monomers.

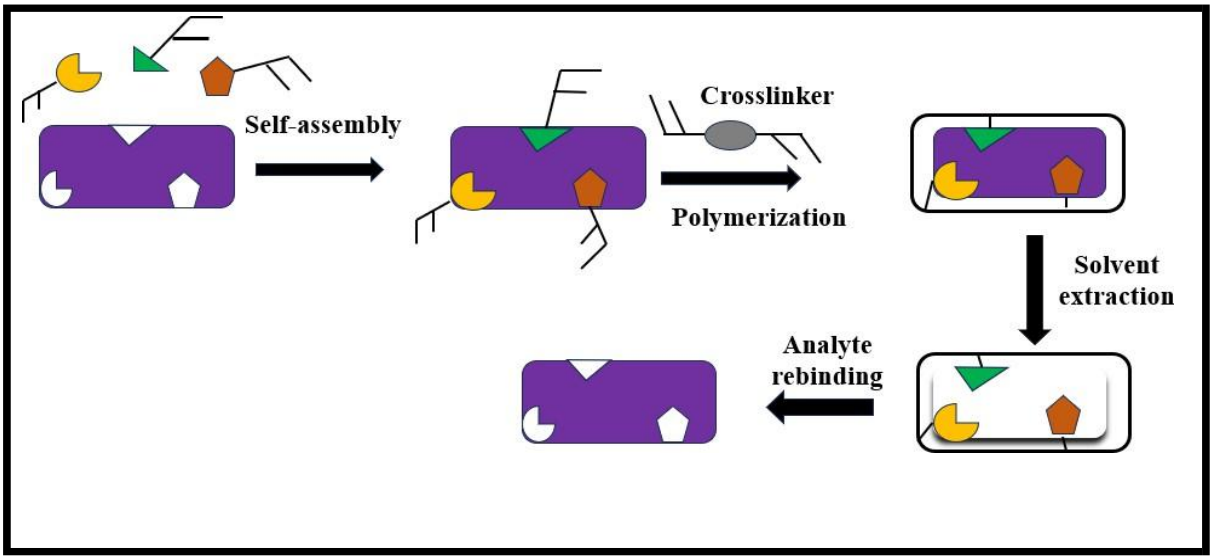

Fig. 1.10: *Schematic for non-covalent imprinting*

H. *Non-covalent imprinting with single monomer*

This approach is the easiest-used strategy and widely used in previous research. The presence of the polymerized complex and the linkage between the monomers and the template in the presence of the crosslinker must be a point of consideration. The monomer has the potential to alter the equilibrium from the ideal template-monomer complexes, whereas the template plays a crucial role in the improvisation of the usable receptors. The self-associative properties of the monomer, may arise some problems for e.g. carboxylic acids have a proclivity to form a dimer. The potential interactions of the monomer template complex with the initiators should also be accounted for.

I. *Non-covalent imprinting with a combination of monomers*

A combination of different potential monomers can be deployed to improve selectivity. It is very intriguing to combine the potential interactions of different monomers [60]. For the bonds formed between the template and the monomers to be

efficient, they must be stronger than interactions between bonds themselves. In [61], it is seen that the combination of basic 2- vinylpyridine and the acidic hydrogen-bonding in MAA were utilized to synthesize better MIPs for the detection of amino acid derivatives compared to the materials synthesized with either monomer solely.

1.5.3.1.3 Semi-covalent imprinting technique

Although, non-covalent imprinting is the most widespread technology, it is compromised by the heterogeneity of the binding site distribution. Semi-covalent imprinting technology is a hybrid approach of both the non-covalent and covalent imprinting technologies, in which the potential functional monomers and templates are covalently linked during the polymerization and rebinding stages. This approach overcomes the drawbacks of covalent imprinting by including covalent attachment during polymerization and hydrogen bond formation during recognition.

1.5.3.1.4 Imprinting with sacrificial spacers

The cross-linking group performs a dual function during the polymer synthesis; it links the template to the monomer as well as acts as bridge between the template and the polymer matrix.

This reduces the flocking during the rebounding process [62]-[64]. In [65], Salicylate (2 hydroxybenzonate), has been reported to be used as a spacer between the target's primary amine group and the residual component of polymerizable MAA.

In this thesis, the non-covalent imprinting approach has been the choice as it not only facilitates the preparation of template-monomer complexes but also the removal of the template from the polymer using a suitable reagent is also easier.

1.6 Literature survey on tea quality detection using electrochemical sensors

This section provides a tabular insight into the various electrochemical sensors used to determine tea quality. Various literature suggests the use of electrochemical sensors based on noble metals as working electrodes for the detection of tea quality, classification of different types of tea, tabulated in **Table 1.4**. A significant number of research activities related to the electrochemical transduction mechanism pertaining to specific molecules present in the black tea is summarized in **Table 1.5.**

Table 1.4: *An overview of the various electrochemical sensors for tea quality analysis*

Sl. No	Operational electrodes	Application	Ref. no.
1.	Made of three noble metals; iridium, platinum and rhodium	Classification of nine different types of tea samples	[66]
2.	Seven potentiometric sensors	Discrimination of four different grades of green tea	[67]
3.	Wires of Au, Ir, Pd, Pt and Rh	Classification of nine tea samples with five different taster's score	[68]
4.	Noble metals, glassy carbon electrode and conducting polymer	Discrimination of Indian black tea varieties.	[69]
5.	Platinum and glassy carbon electrodes	Discrimination of tea with various quality grades	[70]
6.	AFW/Nf/GCE	Selective and sensitive determination of theophylline urine samples	[71]
7.	Graphene/GCE	Detection of gallic acid in tea	[72]
8.	CPE modified with 1,4 benzoquinone	Detection of caffeine	[73]
9.	Silica gel modified carbon paste electrode	Detection of tannic acid	[74]
10.	Screen-printed carbon sensors /coupled with a mobile PalmSens	Detection of gallic acid	[75]
11.	GCE/Chloramine T SPE/Chloramine T PtE/Chloramine T	Sensing of tea polyphenols	[76]
12.	Polyepinephrine/glassy carbon electrode	Detection of gallic acid in black tea sample	[77]
13.	GCE/CdSe	Determination of theophylline	[78]
14.	GCE	Catechin detection	[79]
15.	PDDA-MWCNT	Detection of caffeine	[80]
16.	Nafion–Gr/GCE	Detection of caffeine	[81]
17.	Boron-doped diamond electrode (BDD)	Catechin determination	[82]
18.	Porous pseudo-carbon paste electrode Polypyrrole-modified carbon paste electrode SBA-15 modified CPE carbon paste electrode	Detection of tannic acid	[83]
19.	Pt|PEDOT-modified electrodes	Detection of epicatechin	[84]
20.	CPE modified with Cobalt oxide nanoparticles	Caffeine detection	[85]

Table 1.5: *An overview of the various electrochemical sensors used in detection of specific analytes based on MIP mechanism*

Sl. No	Target molecule	Electrochemical sensor used	Principle of operation	Ref. no.
1.	CAT	MIP electrode	CV and DPV	[86]
2.	TF	MIP electrode	CV and DPV	[87]
3.	EGCG	MIP modified with $Ni(OH)_2$ nanopetal	CV and DPV	[88]
4.	EC	Quercetin imprinted polymer graphite electrode	CV and DPC	[89]
5.	EGCG	MIP sensor based on electrochemical polymerization of β-CD and EGCG	CV, DPV	[90]
6.	CAF	MIP electrode	CV and DPV	[91]
7..	Amino acid	MIP electrode	CV	[92]
8.	EC	Amine Functionalized MWCNTs modified MIP electrode	CV and DPV	[93]
9.	TH	MIP electrode	Thermogravimetric analyzer	[94]
10.	Vanillin	MIP based capacitive sensor	Parallel plate capacitance analysis	[95]
11.	Curcumin	MIP electrode	CV	[96]
12.	Quercetin	MIP based 3D Pd nanoparticles-porous graphene-carbon nanotubes composite modified GCE	CV, DPV, EIS	[97]
13.	Glucose	Graphene oxide MIP electrode	CV	[98]
14.	Haemoglobin	Magnetic MIP electrode.	CV and DPV	[99]
15.	Amoxicillin		CV and DPV	[100]

Ivarsson et.al. [66], developed an e-tongue based on noble metals and used three different waveforms to segregate nine different forms of tea. Multivariate data analysis ((MVDA) and principal component analysis (PCA)) were employed to evaluate current

responses. It was inferred that the best separation was obtained on merging LAPV and staircase voltammetry.

Palit et. al., [68] developed a voltammetric e-tongue consisting of an array of noble working electrodes, along with a counter and reference electrodes, which could declare tea-taster like scores for different samples of black tea. The system described in this approach is portable and affordable by the tea industries, but lacked the selectivity required to detect the specific taste affecting components present in black tea.

The authors proposed electrochemical impedance spectroscopy (EIS) for classifying of Indian-origin black tea. The impedance responses from noble electrodes, glassy carbon electrodes and polyaniline and polypyrrole working electrodes dipped in tea infusions were measured. The correlations between the frequency-specific impedance response of the different working electrodes and the respective chemical concentrations which depend on sample variability, manufacturing process and brand type have been recorded in [69].

An electrochemical approach based on uthenium tris (2, 2′) bipyridyl (Ru(bpy)33+) modified boron-doped diamond electrode has been reported in [82]. The authors have used this electrode for the detection of catechins using electrochemical autoxidation. They have thoroughly investigated the effects of different pH values, ions like Cu^{2+} and Fe^{2+} ions and presence of ascorbic acid. Sensitive amperometric response was recorded which covered a linear operating range from 0.3268 μM to 0.1591 mM.

A novel method for the determination of CAF using a CPE modified with cobalt oxide nanoparticles is explained in detail in [85]. The modification of the surface of the CPE enhanced the sensitivity of the electrode. The electrochemical behavior of the fabricated electrode was studied using CV. The fabricated electrode showed a LOD of 0.016 μmol L^{-1}.

Trishita et.al.[87], reported the detection of total theaflavin in black tea using a MIP electrode. A stable and repeatable electrode was fabricated by creating recognition sites for TF molecule. The monomer used was acrylamide (AA) and EGDMA was used as the cross-linking agent. The sensor showed a linear working range of 20–100 μM, with a limit of detection (LOD) 14 μM. The fabricated electrode was used to measure the TF content in real tea samples and showed a prediction accuracy of 94%.

Trishita et. al. lucidly describes a procedure for the synthesis of a MIP electrode

modified with $Ni(OH)_2$ nano-cubes for the selective determination of EGCG in green tea samples. The DPV response in [88] showed a range of linearity from 10 nM -100 nM and a LOD of 7 nM. The author had thoroughly investigated the analytical parameters like repeatability, reproducibility and stability.

In literature [89], *Das et.al.* , employed the MIP technology for fabricating a cost-effective and selective electrode for the detection of EC molecule in green tea. AA was copolymerized with EGDMA and optically inactive quercetin (Q) was chosen as the template. The fabricated electrode showed two linear working ranges from 1 µM to 100µM and 100µM to 500 µM respectively. Real sample analysis was performed using the partial least square (PLSR) and principal component regression (PCR) models that portrayed accuracies of 94.54 % and 94.41% respectively.

In the work by *Alizadeh et al.* [91], a voltammetric sensor for CAF detection was fabricated using the MIP scheme. The MIP material was synthesized using methacrylic acid (MAA) along with AIBN as a cross-linking agent. From the calibration curve, a linear operational range of 6×10^{-8} to 2.5×10^{-5} mol $^{L-1}$ was obtained. The MIP electrode showed a higher recognition ability than its non- imprinted counterpart. The sensor showed successful determination of CAF in spiked beverages and tea samples.

1.7 Objectives and outline of thesis

The common practice of tea gradation by tea tasters involves a lot of inaccuracy and the traditional methods like gas chromatography, HPLC are complicated and needed expertise, the tea industries are constantly searching for intelligent and responsive instruments for quality profiling of tea. These aforementioned challenges paved way for an alternative technique for electrochemical detection of taste attributing analytes in black tea. The current approach concentrates on the use of electrochemical sensors, specially modified graphite electrodes (CPE) and MIP based sensors for the detection of some important taste affecting molecules like tannic acid, theophylline, total theaflavins and epicatechin in black tea. Concerning the development of the sensors, some important factors like the effects of pH, buffer, concentration variation, and scan rate have been thoroughly explored to justify the sensors' performance under optimum conditions. The following points summarize the goals of this thesis:

- Proper optimization of the monomers, modifiers, cross-linking agents, and initiators for the development of the selective sensors for TAN, TH, and TF

- Investigation and analysis of the sensor materials in terms of structural variations.
- Thoroughly investigate the electrode's performance and study of electro-analytic factors like repeatability, reproducibility, and stability.
- Fabrication of an e-tongue based on MIP sensors for quality profiling of tea.

The entire thesis has been systematically arranged into seven chapters, highlighting in detail the goals mentioned above.

Chapter 1 elaborates on the thesis's purpose and intricately exemplifies its various goals. The human gustatory system has been described elaborately indicating the different taste papillae located on the tongue. An introduction to the origin of tea and the various important taste biomarkers in tea have been tabulated in this section. This segment of the thesis throws light on the principle of electrochemical transduction. A brief discussion of traditional detection methods of taste molecules has been included. This section illustrates the mechanism of MIP-based electrochemical sensors and how they can be employed to overcome the numerous hindrances faced by traditional techniques. Different imprinting techniques and their classification have been incorporated. A pervasive literature survey on various electrochemical techniques for tea quality profiling has been tabulated in this segment.

Chapter 2 explores the rapid electrochemical detection of tannic acid in black tea using two distinct approaches. In the first approach, cyclic voltammetric responses were recorded in tannic acid using a neodymium (III) oxide nanoparticles modified graphite electrode, as the working electrode. The working principle was based on a three-electrode seup. Platinum and Ag/AgCl served as the counter and reference electrodes. The exceptional electrocatalytic properties of Nd_2O_3 nanoparticles contributed to the increase in the electrode's efficiency. However, due to lack of selectivity and to further explore the reproducibility and repeatability of the sensors, the MIP based approach served as a practical solution. The inexpensive and simple use of the polymers along with the generation of structurally similar recognition sites played an essential role in the second approach. The MIP_TAN displayed a wide linear operational range from 0.1 to 500 µM and a LOD of 37 nM. It showed satisfactory repeatability. The sensor was also inferred as highly reproducible. The MIP_TAN was successfully deployed in real samples of black tea and showed an accuracy of 96.31% when compared to standard HPLC values.

Chapter 3 presents two different approaches for the quick and responsive electrochemical detection of theophylline using the principle of voltammetry. As the first step, voltammetric responses in theophylline were recorded by modifying the graphite electrode using rare earth metal oxide to promote the electrode's efficacy. Due to the promising electroanalytical properties of Sm_2O_3 nano-cubes, they have been the choice of modifier in this approach. This electrode was employed for the successful segregation of two varieties of black tea samples using PCA and showed a satisfactory separability index. Moreover, the Sm_2O_3@GP, showed moderate electroanalytical characteristics. Till the earlier approach, the capacity to recognize a particular template, was restricted. A constructive solution to the aforementioned hindrance was the development of recognition cavities on a polymer matrix. Thus, MIP based sensor was the choice in this regard. Moreover, to further improve the current sensitivity of the sensor, the MIP material was further embedded with grains of gadolinium oxide (Gd_2O_3) nano-composites. CV and DPV responses were recorded using Gd_2O_3@TH-MIP-CPE in black tea samples. Compared to the preceding research, this modified MIP electrode demonstrated significant repeatability and had the potential to predict the quantity of theophylline in real black tea samples. Compared to the standard HPLC technique, the Gd_2O_3@TH-MIP-CPE demonstrated accurate analysis of the black tea samples.

Chapter 4 puts forth the fabrication of a MIP-based sensor modified with Ni2Co2O4 nanocomposites. The proposed electrode was based on a co-polymer of acrylic acid and methacrylic acid and imprinted with TF template. The synthesized material was characterized by FTIR and SEM analysis. The MIP_TF2/$NiCo_2O_4$ showed an improved linear operational range of 80-1000 µM with a LOD of 13.89 µM, as compared to previous literature.

Chapter 5 proposes an approach to fabricate a graphite electrode modified with CuO nanoparticles for the simultaneous detection of epicatechin and theophylline from real samples of black tea. The electrode CuO@GP showed a satisfactory linear range of operation and LOD of 4.0 μM for epicatechin and 27.0 μM for theophylline respectively. An attempt was taken to fabricate a MIP based electrode for the simultaneous detection of TAN and TH, however, due to the complex structure of TAN molecule, satisfactory results were not obtained as the TH molecules present in tea got adsorbed in the elaborate structural cavity of TAN. The second section presents an attempt towards the development of an electronic tongue by coupling an array of highly selective electrodes for the overall quality profiling of

tea. The system consists of a switching circuit for to transfer the signal from one working electrode to the next one, keeping the reference and counter electrodes unchanged. This approach also successfully segregated the three analytes present in the same tea sample using PCA analysis. The study was further extended to the successful clustering of five different samples of black tea based on TAN, TH and TF content in each of the samples.

Chapter 6 shows a general outline of the work done and highlights the concluding remarks. The important points and weaknesses of the proposed framework have been examined here with certain suggestions and a few proposals are presented which may be considered as future opportunities for the research work.

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CHAPTER 2

Crafting of electrochemical sensors for the detection of tannic acid in black tea

This chapter explores the fabrication and employment of modified graphite electrodes and MIP based electrodes for the detection of tannic content in real samples of black tea. Detailed discussions are provided on the electrochemical properties of the electrode material, experimental parameters for TAN detection and real sample analysis using PLSR model.

Publication Outcomes

- Moulick M, Nag S, Das D, Tudu B, Bandyopadhyay R, Roy RB. Detection of Tannic Acid using Nd2O3 Modified Graphite Electrode. In2022 2nd International Conference on Emerging Frontiers in Electrical and Electronic Technologies (ICEFEET) 2022 Jun 24 (pp. 1-5). IEEE.
- Moulick M, Das D, Nag S, Tudu B, Bandyopadhyay R, Roy RB. Molecularly imprinted polymer-based electrode for tannic acid detection in black tea. IEEE Sensors Journal. 2023 Feb 2;23 (6):5535-42.

CHAPTER 2

Crafting of electrochemical sensors for the detection of tannic acid in black tea

2.1 Introduction

It has been highlighted in section 1.3.7 of chapter 1 of this thesis, that tea quality is largely dependent on the quantitative presence of polyphenols. One of the major components responsible for tea astringency is tannic acid. It is a polyphenol with antioxidant, anticarcinogenic, and anti-inflammatory properties [1]. Black tea has the highest concentration of tannic acid of all the various kinds of tea. The tannic content in black tea remarkably impacts its taste and flavour. It renders tea its dry texture and gives a bitter flavour to the tea liquor. It is reported that the acceptable dose of tannic acid as an additive is 10–400 μg [2]. Literature revealed the detection of tannic acid in tea, fruits and other beverages using HPLC [3], inhibited electroluminescence [4] and fluorometric methods [5]. Another effective approach employing graphite electrodes from waste Zn-Cu cells have been reported in [6]. To use these techniques, highly skilled manpower, complicated sample preparation and high costs are involved. Several other approaches, such as spectrophotometry and colorimetry [7] and [8], have been previously used for the determination tannic acid in lupus and tea have been reported. These processes may become time consuming and were not cost-effective for real time sample scanning. To circumvent these challenges electrochemical approaches have been adopted.

Studies on electrochemical analysis, reported so far using several conventional electrodes, have limitations like lack of selectivity, slow electron transfer rate, and a low sensitivity. Pre-treated pencil graphite electrodes have been employed to detect tannic acid in tea infusions in [9]. Electrochemical detection of tannic acid in tea using a modified glassy carbon electrode has been reported in [10]. Literature [11] described the detection of tannic acid using an electrochemical sensor and a point-of-test paper-based chromogenic strip. These approaches had drawbacks like stability, selectivity, low recovery rates. Thus, it was necessary to develop robust and specific sensors with greater selectivity towards the concerned molecule. In this chapter, two different electrochemical approaches have been explored for the sensor development of tannic acid in black tea, which are detailed in section 2.2 and 2.3.

2.2 Nd_2O_3 modified graphite electrode for detection of tannic acid

In this work, simple and affordable neodymium (III) oxide nanoparticles embedded carbon electrode (Nd_2O_3@GP) has been developed for the detection of tannic acid. Various metal oxide nanoparticles like ZnO, ZrO_2, MnO_2 have previously been used in electrochemical sensors, however despite having excellent electrocatalytic characteristics of rare earth metals, Nd_2O_3 nanoparticles have not been used frequently in this field [12]. A three-electrode setup has been used to assess the performance of the Nd_2O_3@GP electrode using cyclic voltammetry (CV). The CV responses reveal that the developed electrode is successfully able to trace the presence of tannic acid. The linear working range of the electrode is found to be 5 to 200µM. The limit of detection (LOD) was found to be 0.7µM. This electrochemical procedure can thus be used in the detection of tannic acid in various food and beverages consumed in our daily life.

2.2.1 Experimental section

2.2.1.1 Chemicals and Reagents

Tannic acid (TAN) was procured from Nice Chemicals Pvt Ltd., India. Graphite powder (99%) was procured from Sigma Aldrich, USA. Paraffin oil was procured from Merck, India. India. Neodymium nitrate hexahydrate (98%) was obtained from Sigma Aldrich, USA. Millipore water (Resistance= 18MΩ) was used to prepare testing solutions.

2.2.1.2 Apparatus and Devices

To delve into the electrochemical response of the Nd_2O_3@GP electrode a potentiostat/galvanostat along with a three-electrode setup was used. Platinum (Pt) was chosen as the counter electrode and Ag/AgCl as the reference.

2.2.1.3 Experimental Setup

Th experimental setup is shown in Fig 2.1. It shows the three-electrode working system along with the potentiostat used for TAN detection.

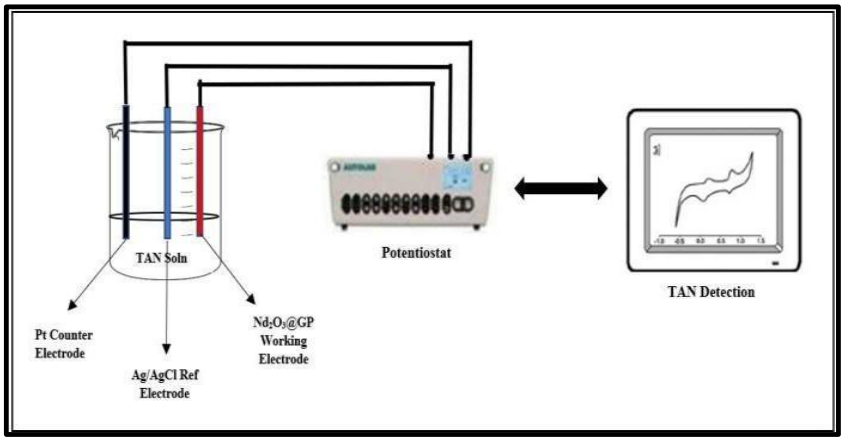

Fig. 2.1: *Illustration of the experimental setup.*

2.2.1.4 Synthesis of Nd_2O_3NPs

Nanocomposites of Nd_2O_3 was synthesized using sol-gel method [12]. 0.438 g of Nd $(NO_3)_3.6H_2O$ was dispersed in 10-15 mL of Millipore water. 10 mL of homogeneous mixture of 20% (v/v) poly(ethylene glycol)-400 was added to the prepared solution. Subsequently, 0.1 M NaOH was added dropwise. The obtained white gel was filtered by washing it thrice using Millipore water. The residue was thoroughly dried at 60 °C. The powder was further calcined at 600 °C for 4 h to get the Nd_2O_3 nanoparticles.

2.2.1.5 Characterization of the prepared Nd_2O_3NPs

The powder x-ray diffraction pattern (PXRD) and transmission electron microscopy (TEM) images of the synthesized Nd_2O_3NPs are shown in Fig.2.2 and 2.3. The TEM image obtained conveys the information that the synthesized material is of nano size. The average particle size of the Nd_2O_3 material is 14.25 ± 6.12 nm

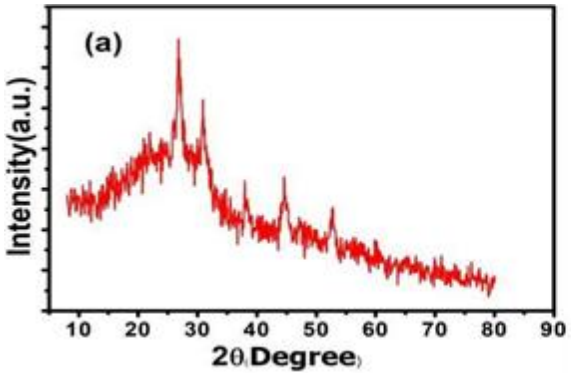

Fig. 2.2: *PXRD pattern of Nd_2O_3NPs*

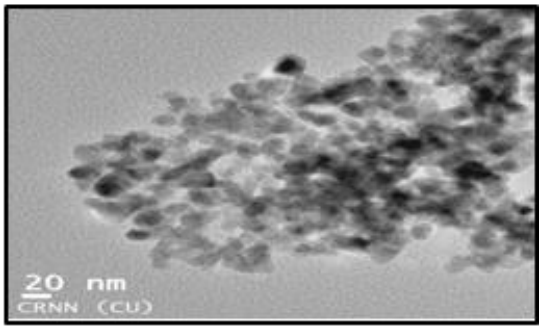

Fig. 2.3: *TEM image of Nd_2O_3NPs*

2.2.1.6 Preparation of the Working Electrode

To prepare the Nd_2O_3@GP electrode, Nd_2O_3NPs was mixed with graphite powder in three different ratios 1:9, 2:8 and 3:7. The highest peak current response was obtained in the first case. The above mixture was ground using a mortar and pestle. 0.15 ml paraffin oil was added dropwise. The mixing process continued till we obtained a fine paste. This fine paste was then introduced into a glass capillary with an inner diameter of 2mm. To establish electrical contact a copper connecting wire was inserted into the tube. Fig.2.4. shows the prepared working electrode.

Fig. 2.4: *Nd_2O_3@GP working electrode*

2.2.2 Results and discussions

2.2.2.1 Comparative Study of the Performance of the Nd_2O_3@GP Electrode

The electrochemical behaviour of the Nd_2O_3@GP was studied and represented. The CV response of the working electrode in the range of -0.2V to 1.0V was first obtained in the presence of 0.1M Phosphate buffer solution of pH7(PBS7). Fig.4, shows the CV responses, without the TAN molecule, no peak is obtained. With the addition of the TAN molecule, the response was again recorded in the same range. As shown in Fig.2.5, TAN was detected by the sensor and an oxidation peak was determined at 0.42V with a peak current of 10.48µA.

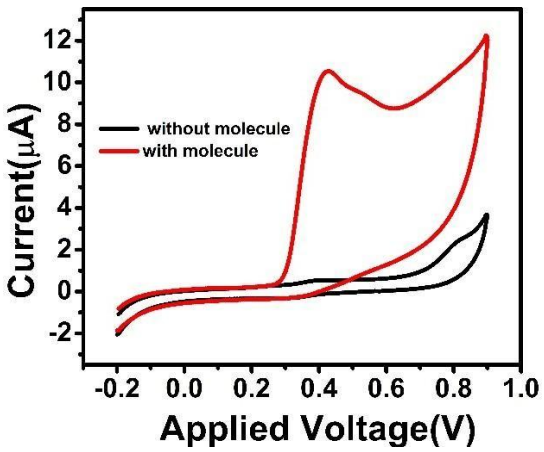

Fig. 2.5: *CV response of Nd_2O_3@GP with and without TAN molecule*

2.2.2.2 pH optimization

Phosphate buffer solutions of pH levels 5, 6 and 7 were evaluated using CV response as shown in Fig.2.6. The best response is obtained using PBS7. Hence, all the further experimentation work was carried out in the presence of PBS7. It was also observed that on increasing the pH of the buffer solution above 7, the oxidation peak completely vanished. This is in accordance with a previously reported work [13].

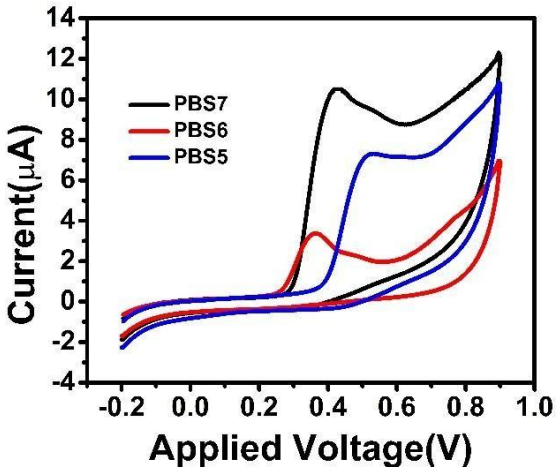

Fig. 2.6: *CV responses in the presence of 0.1M PBS (pH 7,6 and 5)*

2.2.2.3 Effect of Scan Rate Variation

The Nd_2O_3@GP electrode was subjected to various scan rates and the effect on the oxidation peak current of TAN was carried out. The cyclic voltammograms were obtained in the potential range of -0.2V -1.0V for different scan rates varying from 25mV/s to 100mV/s. The CV responses in Fig.2.7(a). show the increase in oxidation peak current with the increase in scan rate. The linearity plot of the scan rate variation in Fig.2.7 (b). depicts the regression

equation for oxidation peak current as, y=0.1732x+0.25; R^2 =0.93. Hence, it can be concluded that it is an adsorption-controlled process [14].

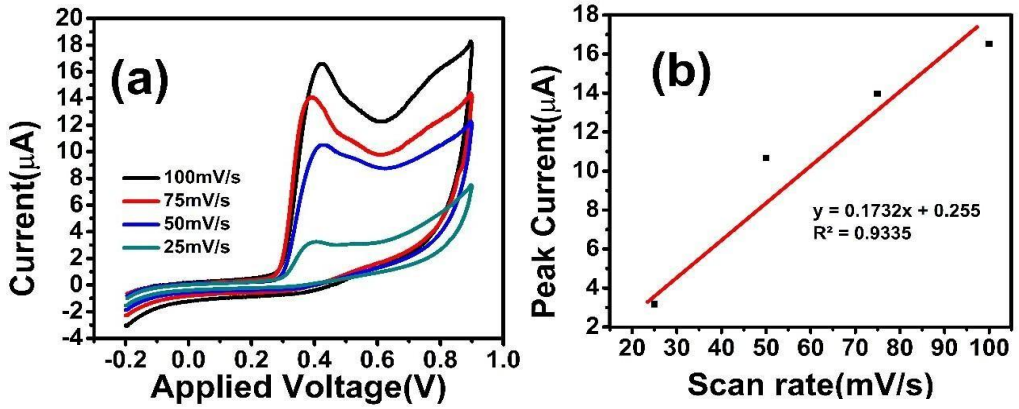

Fig. 2.7: *(a) Peak Current vs. scan rate variation (b)Linear variation of oxidation peak current with scan rate in 0.1M PBS7*

2.2.2.4 Effect of Concentration Variation

The CV responses of Nd_2O_3@GP electrode were recorded for several concentration levels of TAN. The responses were noted in the range of -0.2 - 1V for different concentrations of TAN in the presence of 0.1M PBS7. Fig.2.8 (a), shows the increase in the peak current in the range of 0.3 - 0.4V with the increase in the TAN concentration from 5 to 200µM. Fig.2.8 (b) shows the variation of oxidation peak current with respect to concentration change and the resultant linear equation obtained is y=0.096x+1.568; R^2=0.984. An increasing concentration of TAN molecule results in a linear increase in oxidation peak currents. This may be due to a surface- controlled adsorption phenomenon at the electrode surface. The calibration curve and graphical representation for single- layer adsorption is shown in Fig. 2.8 (c). The LOD is calculated using the formula 3σ /m and the result obtained is 0.7µM. Here, σ represents the standard deviation of the oxidation peak current values as documented at the lowest concentration range (5µM) and m signifies slope obtained from the plotted calibration graph [15]. A tabular comparison of the present work with previously reported literature has been incorporated in Table2.1.

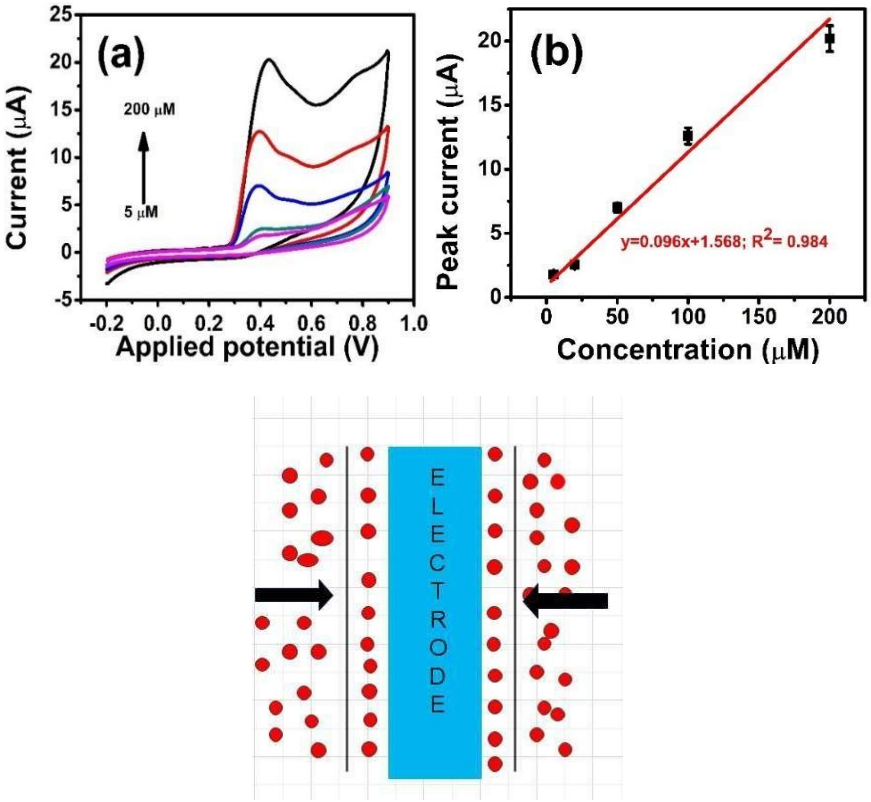

Fig. 2.8: (a) *CV responses for TAN molecule of varying concentrations ranging from 5 to 200µM using Nd_2O_3@GP in 0.1M PBS7* ***(b)*** *Linear variation of peak current vs. concentration* ***(c)*** *Single layer adsorption of TAN molecules ● on the surface of Nd_2O_3@GP*

2.2.2.5 Study of selectivity using Nd_2O_3@GP

Electrochemical behaviour of Nd_2O_3@GP was also studied in the presence of gallic acid and malic acid apart from TAN. The peak current response was recorded in Fig.2.9. The maximum peak current was observed in the presence of TAN compared to malic acid and gallic acid reflecting the highly selective behaviour of the Nd_2O_3@GP electrode.

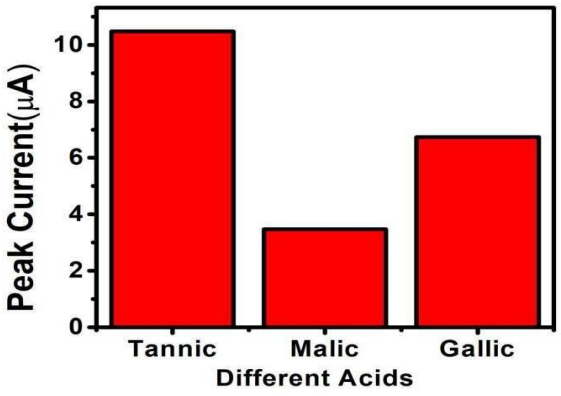

Fig. 2.9: *Peak Response of Nd_2O_3@GP for different acids*

Table 2.1: *Comparative study of obtained results with previously reported literature*

Electrode Material	LOD (µM)	Ref No.
NiHCF-AFCNT/GCE[a]	1.0	16
Microbial sensor	8.0	17
Nd_2O_3@GP	**0.7**	**This work**

[a]NiHCF-AFCNT: glassy carbon electrode modified with nickel hexacyanoferrate on amino-functionalized carbon nanotubes

2.2.3 Summary

The current approach encompasses around the fabrication of an inexpensive modified graphite electrode that established a linearity of 5 to 200µM and a satisfactory LOD 0.7µM. The results obtained during this research work was found comparable with the previously reported literature and showed an improved LOD as demonstrated in Table 2.1. In the days to come this sensor can be employed to obtain voltammetry responses towards real-time samples. To replace the expensive fluorometric methods and HPLC technique, this approach employing chemometrics may be found highly appreciable. Neodymium oxide (Nd_2O_3) has been chosen as a modifier due to its excellent adsorption property in the solution.

This work has been published as cited *"Moulick M, Nag S, Das D, Tudu B, Bandyopadhyay R, Roy RB. Detection of Tannic Acid using Nd_2O_3 Modified Graphite Electrode. In 2022 2nd International Conference on Emerging Frontiers in Electrical and Electronic Technologies (ICEFEET) 2022 Jun 24 (pp. 1-5). IEEE."*

2.3 Fabrication of a MIP-based electrode for tannic acid detection in black tea

The previous research work delved into the electrocatalytic effect of the rare earth material neodymium (III) oxide for detecting tannic acid [18]. The shortcomings of the previously adopted technology were limited linear operational range, detection limit, and template molecule recognition ability. A constructive solution to the template recognition ability was explored using easily available cost-effective polymers. Polymers with exceptional recognition capacity, profound hydrogen bonding play an influential role in electrocatalytic activities [19]. MIP based electrochemical sensors have emerged to be an excellent respite in this context. In the aforesaid MIP technique, the target analyte bonds either covalently or noncovalently in a network of monomers and cross-linkers. It can be defined as a simple recognition tool that produces empty pockets similar in structure to the target molecule on the surface of the polymer [20]. After the completion of polymerization, the template molecule is leached out by washing with a suitable reagent, thus leaving a polymer network with analyte pocket. For any type of analyte, the fundamental adsorption technique lies in the molecular recognition mechanism [21]. The MIP technology is thus adopted as it shows improved stability, repeatability, and reproducibility. As its working principle is based on a lock and key mechanism, a high degree of adsorption of the target molecules has been shown. Successful determination of catechins, caffeine, epigallocatechin-3-gallate, and gallic acid has been achieved previously in our research laboratory using the MIP technology [22], [23], [24], [25]. The present work reports the development of an acrylic acid (AA)-based MIP infused in graphite paste (MIP_TAN) electrode to detect and estimate the TAN in black tea, shown in Fig.2.10. The electrochemical performance of this developed electrode has been studied in detail in terms of sensory factors such as repeatability, stability, reproducibility, and wide linear range of operation.

The novelty of this of procedure lies in its easy mechanism leveraging the site recognition technique and quantitative detection of molecular presence in sample and can be assured along with qualitative measurement. Furthermore, this electrode can be fabricated quite easily, which is another advantage of the MIP technology. Moreover, this electrode can directly be immersed into any solution without any prior preparation, making it easy for industrial applications.

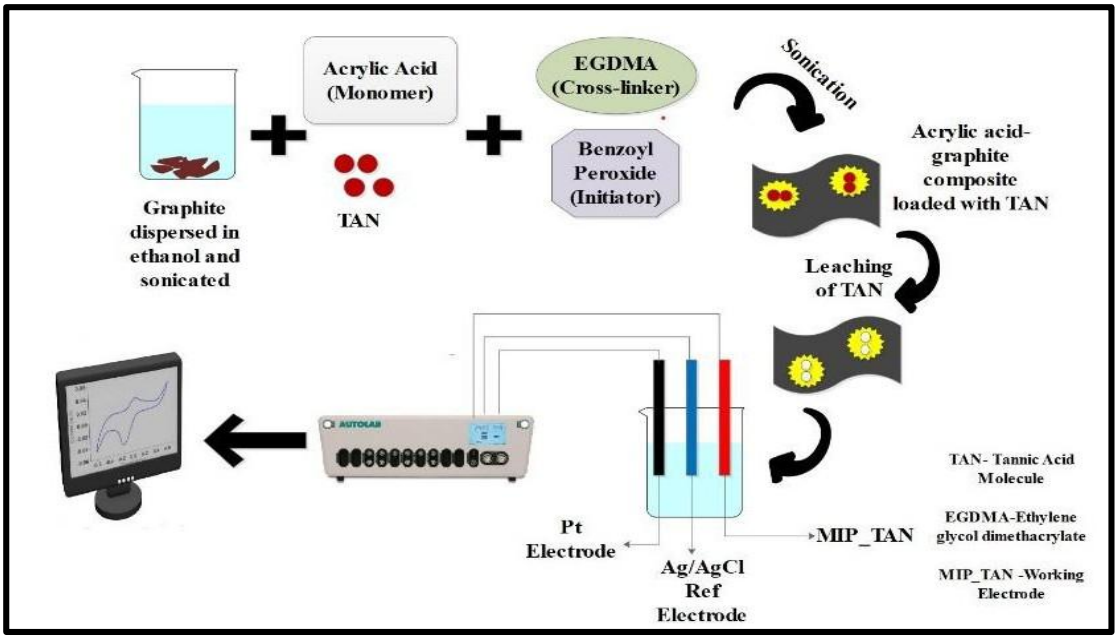

Fig. 2.10: *Schematic representation of MIP_TAN synthesis for detection of TAN in real black tea samples*

2.3.1 Experimental

2.3.1.1 Chemicals required

TAN was procured from Nice Chemicals Pvt Ltd. India. Graphite powder (99%), acrylic acid (AA), acrylonitrile (AN), methyl methacrylate (MMA), benzoyl peroxide, ethylene glycol dimethyl acrylate (EGDMA) was procured from Sigma Aldrich, USA. Paraffin oil, Dopamine, Caffeine, Aluminium, and Magnesium were purchased from Merk. The procured chemicals were pure and had analytical grade, thus no purification was further required. The solutions were prepared with Millipore water (R= 18MΩ). The experiments were carried out at normal room temperature.

2.3.1.2 Experimental Procedure

Potentiostat/GalvanostatPGSTAT101 (Metrohom Autolab) comprising a three-electrode configuration was used. This system comprises a counter electrode made of Platinum (Pt), an Ag/AgCl reference electrode and the MIP_TAN as the working electrode. The polymer materials synthesized were characterized by Ultraviolet-Visible (UV-Vis) Spectroscopy. The UV Spectrophotometer was employed to acquire spectral statistics in the range of 250 to 450 nm. The surface morphology of the synthesized polymer material was studied by a ZEISS EVO 18 Scanning Electron Microscope operational at an acceleration voltage of 15 kV.

2.3.1.3 Synthesis of MIP and NIP Materials

The polymer was prepared in the laboratory itself. Initially, graphite in the form of powder (0.95g) was suspended in ethyl alcohol (20 ml) using ultrasonication for 45 minutes. Following this, AA monomer (0.05g) and the target molecule, TAN (0.05g), were successively added and sonicated. Next, 400 µL of EGDMA (cross-linker) was put into the above solution in addition to 1 mg of benzoyl peroxide followed by sonication for another 35 minutes. The solution was next polymerized by constantly heating in a water bath at an optimized temperature of 30◦C for half an hour. After the preparation of the polymer, it was rinsed with distilled water. The target molecules were washed out for 24 hours for leaching the TAN molecules, producing the MIP substance. The prepared sample was dried and collected for the fabrication of MIP_TAN. The methodology for preparing the NIP material is similar to MIP except for the use of TAN.

2.3.1.4 Fabricating the MIP_TAN Electrode

With 0.3g of the prepared MIP material, a fine paste was prepared using few drops of paraffin oil. The paste was stuffed into a capillary tube with an internal radius of 1.25 mm. An electrical junction was created using a conducting wire inserted into the other end of the capillary. The NIP graphite-based polymer of acrylic acid (NIP_TAN) electrode was devised in the same technique using NIP material.

2.3.2 Results and discussions

2.3.2.1 Detection Principle of TAN Using MIP_TAN

TAN contains phenolic hydroxyl and carbonyl functional groups. There is an intermolecular hydrogen bonding between AA and TAN through the respective carbonyl and hydroxyl functional groups. EGDMA, a crosslinker is slowly added to the mixture containing graphite and AA burdened with the target molecule, TAN. This process of polymerization proceeds with the addition of benzoyl peroxide, an initiator. Due to cross-linking, the AA polymer became immutable to fit in the TAN molecules. Following the template washing method, TAN close to the polymer surface was removed, etching distinct pockets for TAN near the surface.

2.3.2.2 Monomer optimization

Three monomers, namely, AA, AN, and MMA were used simultaneously to synthesize the MIP materials before optimizing the final monomer for the experiment. The performance of the prepared electrodes was assessed using CV operating between -0.2 V to 1 V, giving a prominent peak at 0.3 V in the presence of 0.1M Phosphate Buffer of pH 7 (PBS-7) at a scan rate of 0.05 V/s. The highest oxidation current was obtained using AA imprinted polymer material due to TAN detection as shown in Fig.2.11, which is approximately 2.01 and 10-times MMA and AN imprinted electrode material. This interplay between the template and monomer particles during the pre-polymerization enhances the behaviour of recognition sites and affects the electrochemical response of the imprinted polymer material, synthesized in the laboratory. The dipole-dipole interactivity between TAN and the AA molecules in the pre-polymerization network has actuated the template recognition phenomenon and improved the performance at the MIP_TAN electrode surface [23]. Therefore, AA has been optimized to serve as a suitable monomer for the detection of TAN molecules.

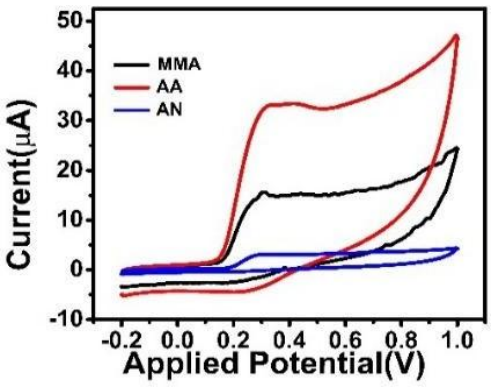

Fig. 2.11: *CV response of different monomers in the presence of 0.1M PBS7*

2.3.2.3 Buffer and pH optimization

The responses of the MIP_TAN electrode were studied in various supporting electrolyte/buffer compositions like phosphate buffer saline (PBS), acetate buffer solution (ABS), and citrate buffer solution (CBS) using CV, shown in Fig.2.12 (a). PBS of different pH levels 5,6 and 7 were evaluated using CV. The highest peak current value was obtained using PBS7, Fig.2.12 (b). All the subsequent experiments were carried out using PBS7.

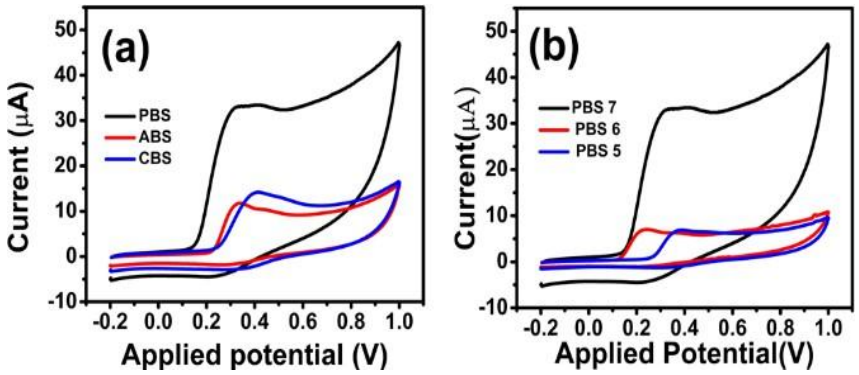

Fig. 2.12: *CV responses (a) Buffer Variation and (b) pH variation of PBS.*

2.3.2.4 Material Characterization by Spectroscopy

The removal of TAN was reassured by employing UV-Vis Spectroscopy. The spectrum appended below in Fig.2.13, illustrates the absorption spectra of the synthesised polymer sample before washing (BW) the TAN molecule and after washing (AW) the TAN molecule (AW). A small amount of the synthesized polymer material (before template wash) was suspended in Millipore water and sonicated for 15 mins. In the following step a part of the prepared polymer was next thoroughly washed. The polymer material was slowly washed at regular intervals using Wattman's filter paper for 24 hours using millipore water. To ensure successful removal of the analytes the residual polymer material after template wash was dispersed in Millipore water and sonicated for 15 mins. Both these solutions were next subjected to UV-Vis spectroscopy analysis. The spectral response clearly shows a heightened peak around 275 nm for TAN absorption, however, the peak vanished after TAN particles were leached out [26], thus, confirming the successful template removal.

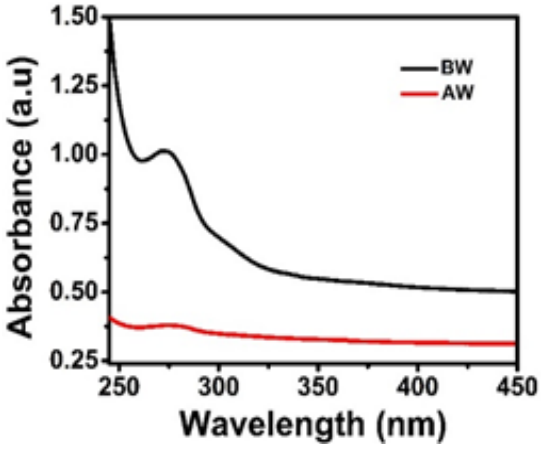

Fig. 2.13: *UV-Vis spectra before (BW) and after analyte extraction (AW)*

2.3.2.5 SEM Analysis

The SEM results in Fig.2.14, suggest that the surface of the synthesized MIP material is uneven and wrinkled in appearance due to continuous etching of the TAN molecules which have in turn created pockets for the adsorption of the required analyte. The smooth surface of the NIP material suggests that an extraction process was absent.

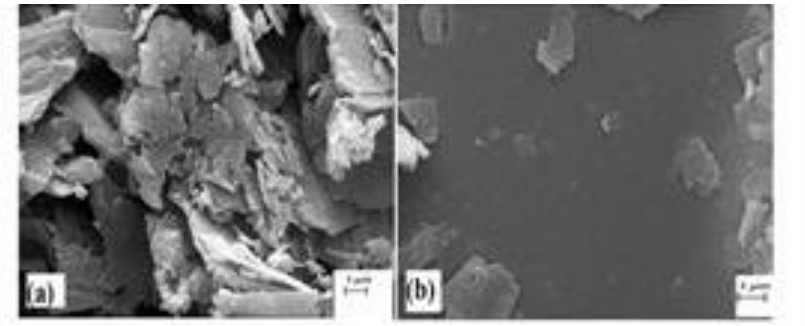

Fig. 2.14: *SEM visuals (a) MIP and (b) NIP materials.*

2.3.2.6 Comparison of MIP_TAN and NIP_TAN electrodes

The electrochemical behavioral pattern of MIP_TAN was evaluated. A comparison of the oxidation of TAN at the MIP_TAN and NIP_TAN electrodes were carried out by recording the cyclic voltammograms in the potential window of -0.2 to 1.0 V in presence of 0.1M PBS7 at a scan rate of 0.1V/s. Fig.2.15 shows the elevated oxidation peak for MIP_TAN in comparison to NIP_TAN. The oxidation peak current for MIP_TAN (34.4 µA) is almost thrice as compared to NIP_TAN (10.2µA), which shows that molecular recognition sites are present in the synthesized material. It can be inferred that there has been an effective absorption of TAN and thus greater sensitivity and the oxidation peak current were observed.

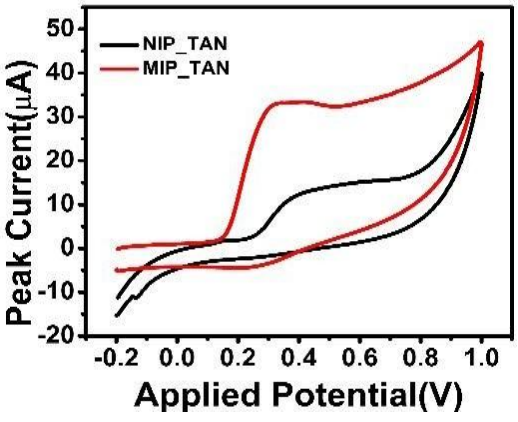

Fig. 2.15: *Voltammograms of MIP_TAN and NIP_TAN.*

2.3.2.7 Scan Rate Variation

The scan rate was varied in the range of 5 mV/s -100 mV/s. From Fig.2.16. it is inferred that the peak current (I_{tan}) due to the oxidation of TAN bears a linear relationship with the increment of scan rate(v) with R^2 =0.95, concluding the phenomenon of the oxidation of TAN as a surface-controlled behaviour [27]. A linear relation between anodic current and scan rate can be interpreted from Fig.2.16. The above linearity is expressed by the linear regression equation (1)

$$I_{tan} = 0.3116v + 1.1689 \; R^2 = 0.95 \quad (1)$$

The quantity of electrons transferred during this oxidation process is evaluated using the mathematical relationship (2).

$$I_p = \frac{nFQv}{4RT} \quad (2)$$

n=electrons transferred, F= Faraday's constant, Q=charge consumed v=scan rate, R =the universal gas constant and T= standard experimentation temperature respectively. The numerical value of n is calculated as 0.91 which is approximately unity. A linear dependence of the oxidation peak potential (Ep) on the logarithm of the scan rate (log v) is presented in Figure 2.16(c).The linear regression equation is represented in equation (3).

$$E_p = 0.0433 log v + 0.2233; R^2 = 0.95 \quad (3)$$

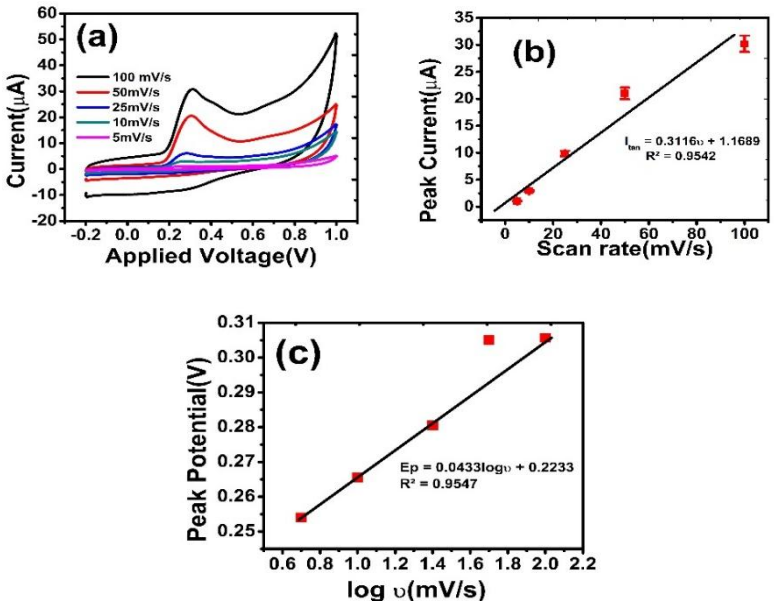

Fig. 2.16: *(a)Change of peak current with scan rate variation (b)Peak current vs scan rate (c) Peak potential vs log of scan rate.*

2.3.2.8 Variation of Concentration and Limit of Detection

The effect of various concentrations of TAN at the surface of the MIP_TAN electrode was assessed using DPV. The responses for different concentrations of TAN were measured in the linear range of -0.1 to 0.7 V. Fig .8. shows a linear gradation in oxidation peak current (I_{tan}) with an increment in the concentration of TAN (C_{tan}) from 0.1µM to 500µM at a scan rate of 0.05V/s. From Fig.2.17. it is observed there are two distinct linear portions in the graph. Langmuir adsorption isotherm behaviour explains the presence of two distinct slopes in the calibration curve [28] which explains the monolayered adsorption of analytes followed by multi-layered adsorption of the analyte TAN by the first linear region formed. The initial linear region lies between 0.1 -50µM which obeys the regression equation (4). The next linear segment lies between 50 to 500µM which follows the equation (5).

$$I_{tan} = 0.3114C_{tan} + 22.063 \; R^2 = 0.951 \quad (4)$$

$$I_{tan} = 0.1568C_{tan} + 29.389 \; R^2 = 0.991 \quad (5)$$

Using, LOD=3σ /m, the value was determined as 37nM. Here, σ= standard deviation of oxidation peak currents recorded at the least determined concentration of TAN and m=slope of the calibration plot [22].

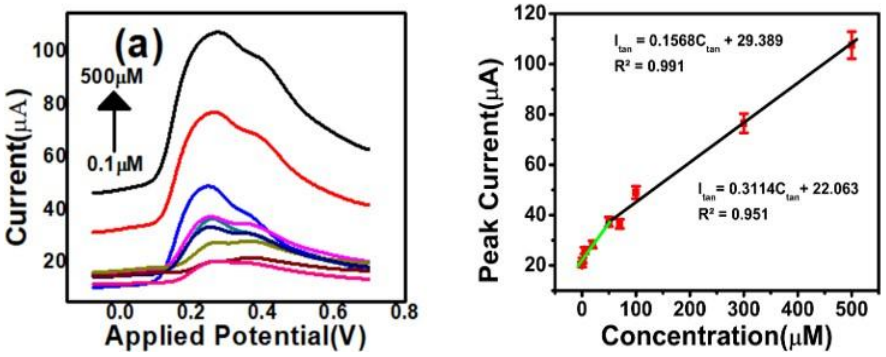

Fig. 2.17: *(a) DPV responses show the change in peak current vs. concentration and (b) linear variation of peak current vs. concentration.*

2.3.2.9 Repeatability Reproducibility and Stability of MIP_TAN Electrode

The analytical characteristics of the MIP_TAN electrode was assessed by the factors-reproducibility, repeatability and stability at concentrations of 20µM and 200 µM respectively in the presence of PBS-7. Three successive DPV measurements were carried out using the MIP_TAN sensor. Fig.2.18 (a) and (b) shows that repeated measurements with

MIP_TAN give a %RSD =1.14 and %RSD=1.11 respectively in the two linear ranges. Reproducibility is also accounted for in the proposed work, as shown in Fig. 2.18 (c) and (d). Three discrete electrodes were assembled by the abovementioned method and three DPV readings were recorded. An acceptable reproducibility of the electrode was observed with% RSD=1.46 and 1.66. The MIP_TAN exhibited a stable response for a period of forty days, wherein DPV measurements were recorded at an interval of 20 days under normal room temperature, with %RSD=1.75 and 1.76. This is shown in Fig. 2.18 (e) and (f). It was observed that the oxidation peak had dropped around 3% on the 40th day due to the ageing effect of the electrode.

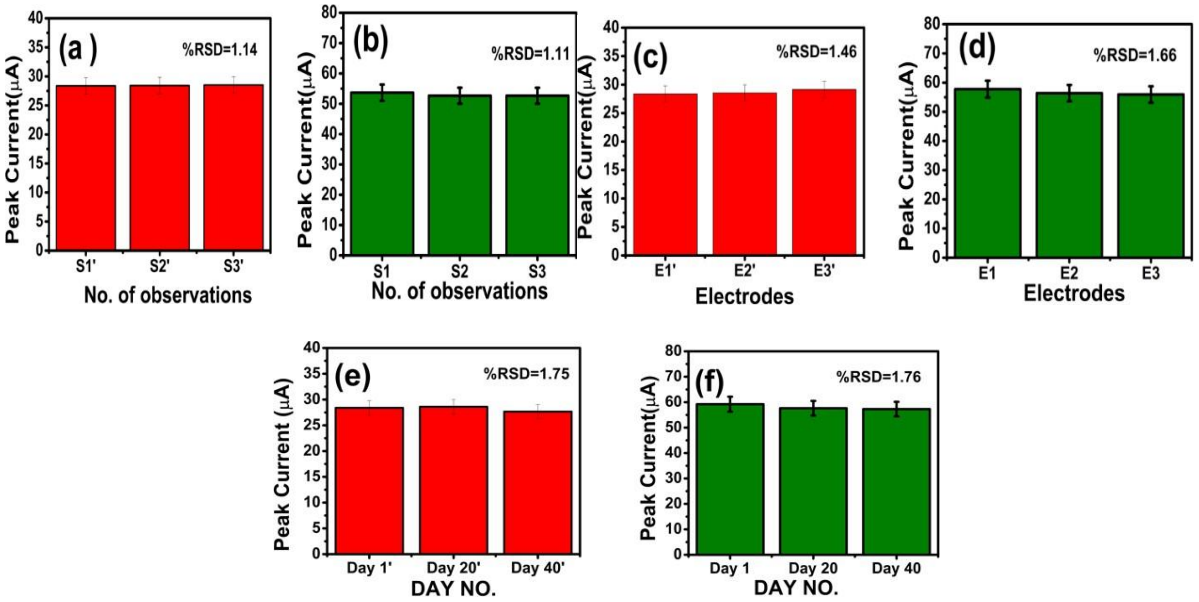

Fig. 2.18: *(a) Study of repeatability at 20 µM (b) Study of repeatability at 200µM (c) Study of reproducibility at 20µM (d) Study of reproducibility at 200µM (e) (e) Stability of MIP_TAN electrode at 20 µM (f) Stability of MIP_TAN electrode at 200 µM*

2.3.2.10 Study of various interferents

To study the sensitivity of MIP_TAN in the presence of interferent species along with the target analyte (TAN), the fabricated electrode was exposed to two categories of interferent species namely organic compounds and cations in the presence of TAN. The organic interferent species were dopamine and caffeine, the cations were aluminium and magnesium. The reason that the authors have considered cations as possible interferents is because in the proposed work the real sample taken is black tea. Literature [29] states that black tea contains Al and Mg at mg/g levels, so in order to study the sensitivity of the MIP_TAN in the presence of these metal ions, the authors have chosen the cations as the

interferent species. Previous literature [4] also suggests that most metal ions do not interfere with the determination of TAN. The responses were obtained in the presence of 5-fold concentrations of each interferent species with 100 µM of the analyte. Fig. 2.19 clearly demonstrates the effect of each of these species when present along with the target analyte on the MIP_TAN. The bar plot depicts the relative change in the responses of the electrode in the presence of the interferent species in comparison to the target analyte only. The change in signal was within a tolerable limit of ±5%.

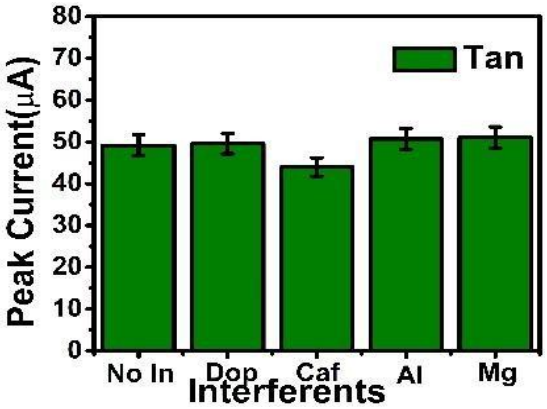

Fig. 2.19: *Influence of interferent species on peak current responses in 0.1M PBS7 at MIP_TAN.*

2.3.2.11 Selectivity of MIP_TAN

To study the selectivity profile of MIP_TAN, the electrode was used to record the electrochemical responses when exposed to TAN, catechin and gallic acid. The peak currents were recorded in each case and it was seen that the highest current is obtained in case of TAN(34.4 µA) as compared to CAT (around 19.2µA) and GA (15 µA). This shows the highly selective nature of the fabricated electrode. Fig.2.20, below shows the selectivity profile of the electrode.

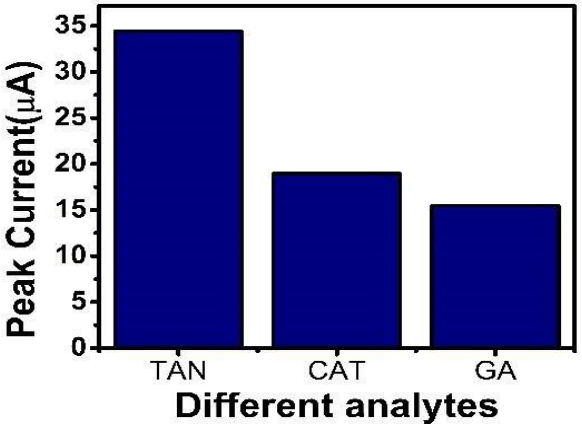

Fig. 2.20: *Selectivity profile of the fabricated electrode MIP_TAN*

2.3.3 Real sample analysis using PLSR

The MIP_TAN electrode was employed to estimate the content of TAN in four different samples of black tea, using the PLSR model to comment on its practicality. For the preparation of the experimental solution, a simple procedure as explained in [25] was used. In the potential range of 0.0 – 0.8V, ten repetitive DPV responses were recorded for the individual tea samples.

Henceforth, using MATLAB (2017b), the data matrix [328 x 40] obtained after infusion of MIP_TAN in the different black tea samples, was subjected to PLSR.

The obtained matrix [328 x 40] was divided into two subsets – training and testing subsets in the ratio 70:30. The capability of MIP_TAN to predict the TAN content was studied using PLSR and LOOCV methods The performance of the developed model was estimated by correlation coefficient(R2) and root mean square error (RMSE). The prediction accuracy of the PLSR model was 96.315% in the black tea samples. The latent variable (LV) is chosen corresponding to the lowest value of RMSEC. The RMSEC value 0.043 is the lowest for 7LVs. A comparative table between the HPLC data and predicted TAN values by MIP_TAN in different black tea samples is given in Table 2.2. The bar plot in Fig. 2.21 justifies an acceptable level of agreement between the actual and predicted TAN content in the different samples of black tea.

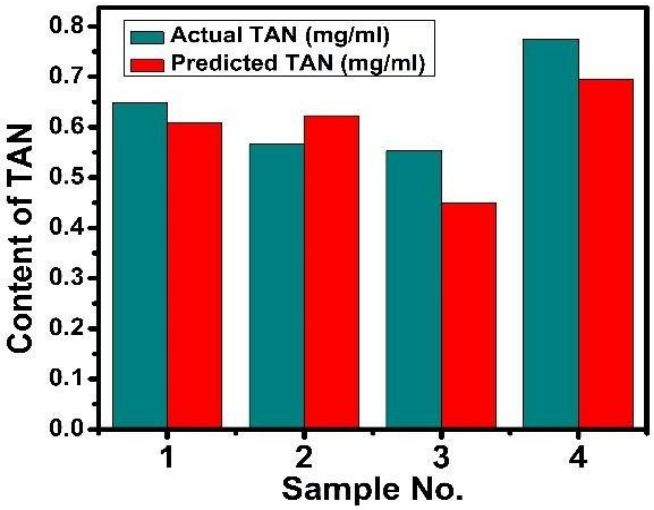

Fig. 2.21: *Bar plot between actual and predicted TAN content in black tea*

Table 2.2: *Comparison of actual vs predicted TAN content*

Sample No	Actual TAN (mg/ml) (HPLC)	Predicted TAN (mg/ml) PLSR	Prediction Accuracy (%)
1	0.649	0.638	98.305
2	0.566	0.583	96.996
3	0.553	0.541	97.830
4	0.775	0.714	92.129
	Average Prediction Accuracy	**96.315%**	

2.3.4 Comparison of the present method with previous techniques

A deep survey into literature reveals a number of different techniques used previously for the detection of TAN, given in Table 2.3. The MIP_TAN electrode shows two linear ranges of operations. The various methodologies used were fluorescence and anodic strip voltammetry. Some of the work reported low value of LOD however, the processes involved in preparation of the materials are either time consuming or expensive. The MIP_TAN electrode shows an LOD of 37 nM, additionally the method of preparation of the electrode material is inexpensive and easy.

Table 2.3: *Comparative study of TAN detection*

Electrode	Linear Range(µM)	LOD(µM)	Technique	Ref. No
Si-gel/CPE	0.001-1	0.0003	ASDPV	[30]
Pr-Tu-GC	2 - 42	0.6	DPV	[31]
PPCPE	-	0.01	ASV	[32]
a-CQDs	0.001-0.2	0.006	Fluorescence	[33]
MIP_TAN	**0.1-500**	**0.037**	**CV and DPV**	**This work**

2.3.5 Summary

The proposed treatise presents an elegant mechanism for the fabrication of a MIP electrode for the determination of TAN in black tea samples. The sensor has a wide linear range of operation from 0.1 to 500µM with an LOD 37nM under an optimized experimental setup. The MIP_TAN represents good repeatability and reproducibility. The electrode shows good stability over a period of 40 days. With real samples, a comparative finding between the HPLC analysis and the predicted TAN using MIP_TAN is presented as well. The average

prediction accuracy using PLSR was obtained as 96.315 %. Thus, an easy-to-use, simple and cheap sensor for the determination of TAN is devised, which can be employed for the quantitative estimation of TAN in black tea.

2.4 Conclusion

Monitoring the daily consumption of TAN is essential to prevent negative health impacts. Also, the content of TAN in black tea has a remarkable influence on its taste and flavour. In this chapter, initial effort was given to find the cyclic voltammetric responses with a Nd_2O_3 modified graphite electrode for tannic acid detection in black tea, and it portrayed a wide linearity range and significant LOD. To further enhance sensor performance, the MIP technology has been attempted to fabricate an electrode with a greater affinity towards the target molecule. MIP_TAN has shown a linear range of performance from 0.1 to 500 µM with an LOD of 37 nM. To predict the content of TAN, a partial least square regression model (PLSR) was constructed utilising DPV responses of MIP_TAN and HPLC as reference data. The prediction accuracy of PLSR was obtained as 96.315%. The electrode was examined on real samples of black tea which produced satisfactory results. To conclude, the MIP_TAN electrode can serve as an methodical sensor for the detection of tannic acid in black tea.

This work has been published as cited *"Moulick M, Das D, Nag S, Tudu B, Bandyopadhyay R, Roy RB. Molecularly imprinted polymer-based electrode for tannic acid detection in black tea. IEEE Sensors Journal. 2023 Feb 2;23 (6):5535-42."*

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CHAPTER 3

Fabrication of electrochemical sensors for the detection of taste-affecting analytes in black tea: Theophylline

This chapter discusses the learning process underway regarding the rapid electrochemical detection of theophylline in black tea samples using two different approaches. The fabricated electrodes illustrated satisfactory results.

Publication Outcomes

- Moulick M, Nag S, Das D, Das D, Bandyopadhyay R, Pramanik P, Banerjee Roy R. Detection of Theophylline using Samarium Oxide Nanoparticles Ingrained Graphite Electrode. Nano Life. 2023 Jun 24;13(02):2350007.
- Moulick M, Das D, Nag S, Pramanik P, Roy RB. Gadolinium oxide modified molecular imprinted polymer electrode for the electrochemical detection of theophylline in black tea. Journal of Food Composition and Analysis. 2025 Jan 1; 137:106994.

CHAPTER 3

Fabrication of electrochemical sensors for the detection of taste-affecting analytes in black tea: Theophylline

3.1 Introduction

In chapter 2 of this thesis, two different sensors have been successfully fabricated to detect tannic acid in real black tea samples. In this chapter, theophylline (1,3-dimethylxanthine) (TH), naturally found in black tea and green tea are chosen as the target analytes for detection. Tea leaves basically contain two different types of compounds-polyphenols and methylxanthines [1]. A methyl-xanthine derivative ($C_7H_8N_4O_2$) obtained from tea, TH acts as a muscle relaxant and bronchial dilator, can be used in the treatment of asthma and COPD caused by reversible airflow obstruction [2]. It has a therapeutic index 20-100 µM and an increment in the level can have consequences like restlessness, diarrhoea and hyperactivity of the heart. [3] High intake of methylxanthines has a negative effect on premenstrual syndrome and also fertility [4]. Thus, it is necessary to monitor the level of consumption of theophylline. As highlighted in section 1.4.1, different technologies that have been proposed for detecting and quantifying theophylline levels in beverages like chromatographic approaches, [5-6], electrophoresis and colorimetric aptasensor [7-8]. These conventional methods have good detection limit; however, they are time consuming and requires rigorous sample preparation procedures. Thus, a challenge remains to device a low cost, easy to use and highly sensitive sensor for detecting theophylline in real samples. Due to miniaturization, simple fabrication techniques and high repeatability, electrochemical sensors have proved to effective analytical tools. In a convincing literature, the authors have reported the development of an electrochemical sensor based on dopamine-melanine-gold nanoparticles modified glass carbon electrode for the detection of theophylline [9]. Despite possessing a proper bandgap for efficient and quick electron transfer and electrochemical inactivity, rare earth metals have not been widely used in modifying the surface of electrochemical sensors. In this overview, the electrochemical behaviour of two different fabricated electrodes is studied in detail using cyclic and differential pulse voltammetry. The details of sensor fabrication, material characterizations and also practicability of the sensors are elaborated in the sections 3.2 and 3.3.

3.2 Detection of theophylline using Sm_2O_3 nanoparticles ingrained graphite electrode

This section deals with the development of a reasonably efficient electrode for the detection of TH by a graphite electrode decorated with Sm_2O_3 nano-particles. It's electrochemical behaviour is studied in detail using CV and DPV. This electrode exhibits a range of 10-1000µM with a low detection limit of 5.69µM. The salient properties of the electrode are examined in terms of their repeatability, reproducibility and stability with RSD values 1.59%, 2.04% and 5.33% . The samples of black tea were taken and successfully segregated using the principal component analysis tool with a separability index (SI) of 13.14. This study distinctly demonstrates a significant improvement in electrochemical detection of TH in tea samples using samarium oxide nanoparticles infused in the graphite paste.

3.2.1 Experimental study

3.2.1.1 Reagents and chemicals used

Pure graphite powder (98% pure), paraffin oil (98% purity), Samarium nitrate hexahydrate $Sm(NO_3)_3.6H2O$ (99.99), poly (ethylene glycol)- 600, NaOH (97.0 %) were purchased from Aldrich, USA. The chemicals purchased were all of analytical grade. The testing solutions were made using Millipore water. During experimentation the temperature of the laboratory was maintained at standard room temperature.

3.2.1.2 Synthesis of samarium oxide nanoparticles (Sm_2O_3NPs)

The Sm_2O_3NPs were synthesized in our laboratory using sol-gel technique in a similar manner as stated in the reported work [10]. The SEM image of Sm_2O_3NPs is shown in Fig. 3.1. The SEM image confirms the presence of nanosized particles in the prepared material.

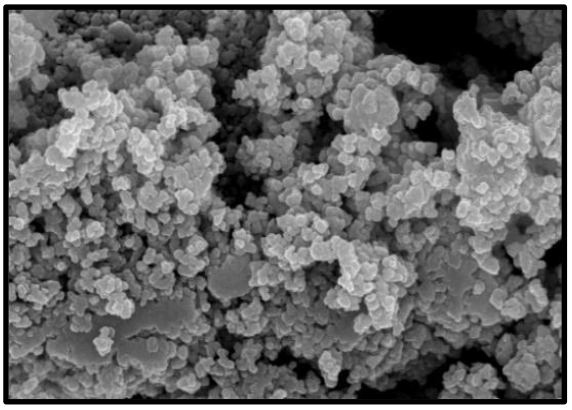

Fig. 3.1: *SEM image of Sm_2O_3 NPs*

3.2.1.3 Fabrication of Sm_2O_3@GP electrode

Three different electrodes were prepared varying the ratio of Sm_2O_3NPs and pure graphite. The ratios considered were 1:9, 2:8 and 3: 7 respectively. On recording the voltammograms, it is observed that the current response was maximum in the first combination. The nanoparticles were blended into a fine paste with graphite using paraffin oil as a strong binder. The prepared paste is inserted into a thin glass capillary tube. The electrical contact is further established using a copper wire.

3.2.2 Results and discussions

3.2.2.1 Detection Mechanism

The detection mechanism has been explained using the schematic given below. The molecule A undergoes oxidation, resulting in the formation of an unstable compound B. The compound B undergoes tautomerism to form the compound C. During the first stage of oxidation, the release of the two electrons is responsible for the voltammetric response.

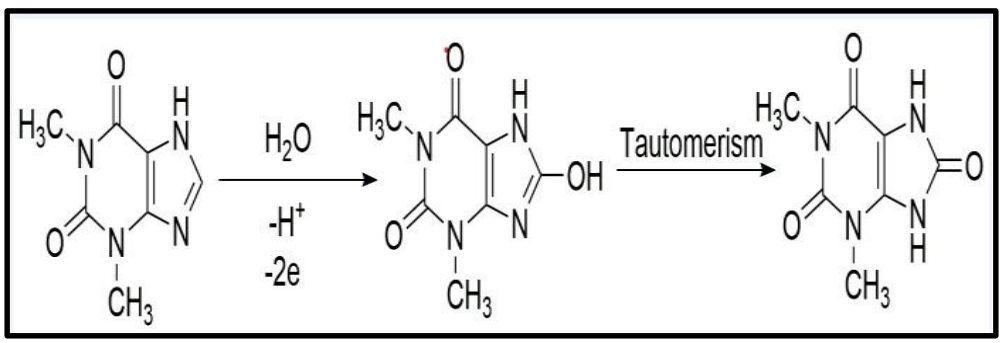

Fig. 3.2: *Schematic of the detection mechanism*

3.2.2.2 Comparison between CPE and Sm_2O_3@GP

The electrochemical response of the bare electrode (CPE) and modified graphite electrode, Sm_2O_3@GP, was analysed using CV. The CV response for Sm_2O_3@GP is shown in Fig. 3.4. It was seen that the response of the Sm2O3@GP was 2.07 times the bare graphite electrode. The bar plot in Fig. 3.3 illustrates the comparative responses. Hence, we can infer that on modifying the electrode surface by Sm_2O_3NPs the oxidation peak current got enhanced.

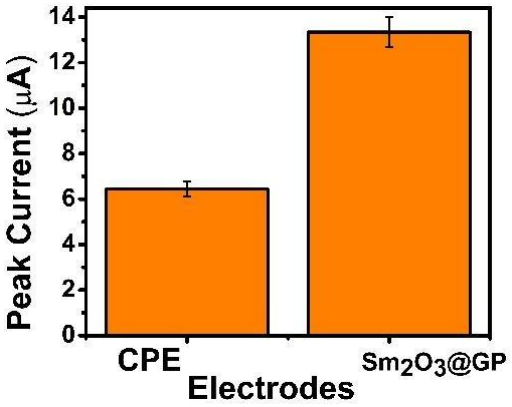

Fig. 3.3: *Bar plot showing the current responses of bare graphite electrode vs. Sm_2O_3@GP*

3.2.2.3 Optimization of buffer and pH

The prepared electrode Sm_2O_3@GP was immersed in 100µM solution of THP in the presence of acetate buffered saline (ABS), citrate buffered saline (CBS) and phosphate buffered saline (PBS). The best response was clearly obtained using PBS, shown in Fig. 3.4(a). The pH of the test solution can highly influence the electrochemical performance of Sm_2O_3@GP. So, to investigate the same, cyclic voltametric tests were carried out in 100µM of the test solution utilizing phosphate buffer solution of pH-5,6 and 7 namely. The responses are shown in Fig. 3.4(b). It is clearly evident that the electrode shows a sharp response with a current of 13.34µA, which is the highest among the three. Hence, PBS 6 is chosen for all further experimentation.

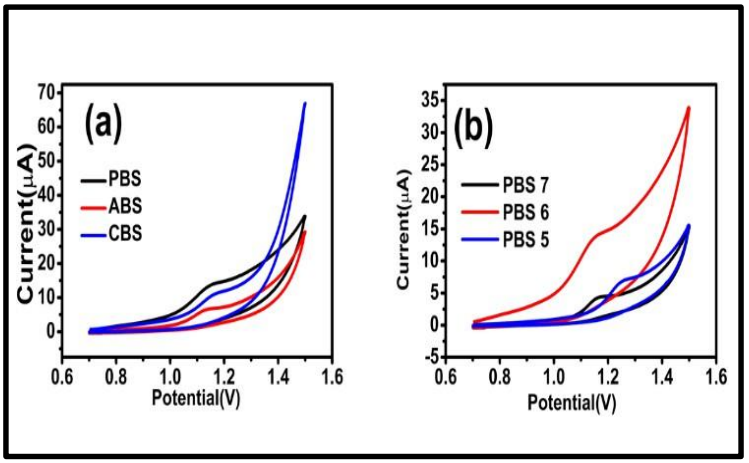

Fig. 3.4: *CV responses (a) showing the influence of buffer (b) influence of pH*

3.2.2.4 Influence of scan rate variation

The change in scan rate can highly influence the kinetics involved in an electrochemical process. In the proposed method, we have varied the scan rate from 1 mV/s to 200 mV/s for a clearer insight. Fig. 3.5 (a) shows the voltammograms obtained by varying the scan rates in the potential window 0.6 to 1.6 V, which demonstrates the gradual increment in the peak current with a linear change in scan rate variation. It is observed that on increasing the scan rate, the equilibrium of the electrode surface gets disturbed as a result the induced over voltage changes as a result there is a shift in potential. The linear graph given below in Fig. 3.5 (b) gives the regression relation as

$$y = 112.4x + 6.384 \; ; R^2 = 0.964 \tag{3.1}$$

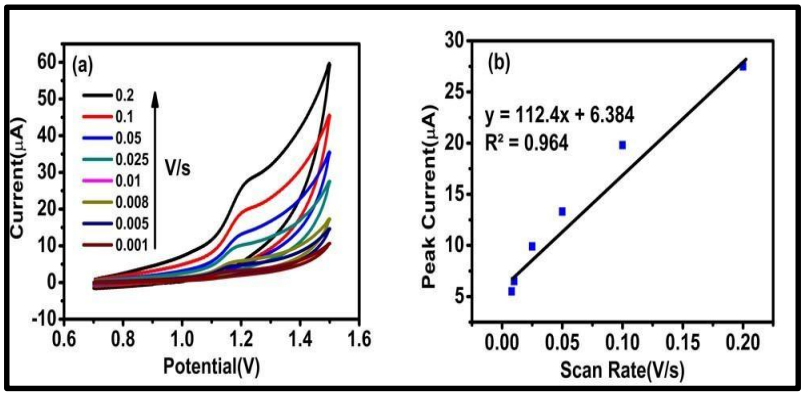

Fig. 3.5: *CV responses (a) Oxidation peak current vs. scan rate (b) linearity plot of current vs. scan rate*

3.2.2.5 Influence of concentration variation

To study the credence of oxidation current profile on change in concentration we have recorded the DPV responses. The concentration of the solution was varied from 10 to 1000 µM. The potential window was chosen between 0.6 to 1.6 V, the scan rate was fixed at 50 mV/s. Fig. 3.6(a) shows the dependent nature of the oxidation peak current on the concentration variation. The Fig. 3.6 (b) demonstrates the calibration curve and the linear regression equation obtained as

$$y = 0.0296x + 9.6734;\ R^2 = 0.951 \quad (3.2)$$

The obtained limit of detection (LOD) was as 5.69 µM, using the equation 3Sy/x/m. Here, Sy/x and m indicate standard deviation and the slope of the calibration curve. A comparative table is appended below which gives us an insight to the previous techniques used in the detection of THP. Table 3.1 below gives a comparative study of previously used techniques for THP detection.

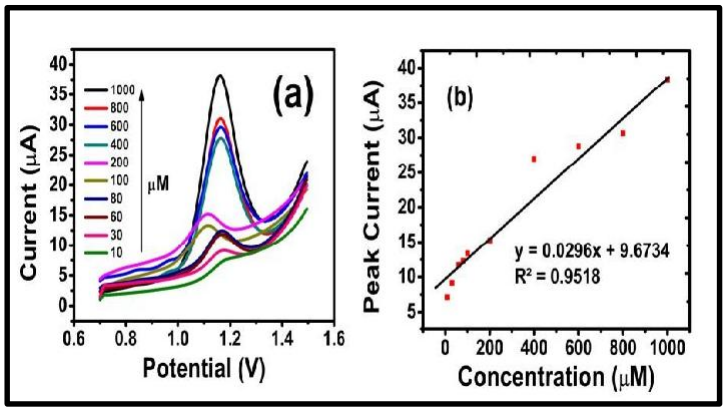

Fig. 3.6: *(a) DPV responses with change in concentration (b) linearity plot of current vs. concentration.*

Table 3.1: *Comparative study of various techniques used for the detection of TH*

Electrodes Used	Technique	Linear Range	LOD(µM)	Reference No.
Carbon Paste	SWV	0.02-1 mM	15µM	[11]
Glass Carbon	Chronoamperometry	10-100 µM	7.02 µM	[12]
Carbon Paste electrode modified with Kalonite	SWV	1-5 mM	190 µM	[13]
SPE	CV	55-110 µM	10 µM	[14]
Glass Carbon	DPV	0.01-320 µM	5.9 µM	[15]
Glass Carbon	DPV	0.56–893 µM	0.56 µM	[16]
Gold and Graphite electrode	Amperometry	20-600 µM	20 µM	[17]
Epoxy-Graphite electrode	Chronoamperometry	-	1 µM	[18]
Nafon:lead-ruthenium oxide pyrochlore chemically modifed Electrode	SWV	-	0.1 µM	[19]
Sm2O3@GP	**CV and DPV**	**10-1000 µM**	**5.69µM**	**This Work**

3.2.2.6 Study of repeatability, reproducibility and stability

The sensory characteristics of the Sm_2O_3@GP was studied in terms of its repeatability, reproducibility and stability for 100 µM THP solution in PBS 6 buffer. To study repeatability, three consecutive readings were taken which showed RSD 1.59% shown in Fig. 3.7 (a). For the reproducibility study three different electrodes were synthesized using the same procedure and showed RSD 2.04% as shown in Fig. 3.7 (b) Further, the same electrode was used for stability analysis. The results were recorded in an interval of 15 days which showed RSD 5.33% with a negligible variation in the peak current shown in Fig. 3.7 (c). This may be due to ageing effect of Sm_2O_3@GP.

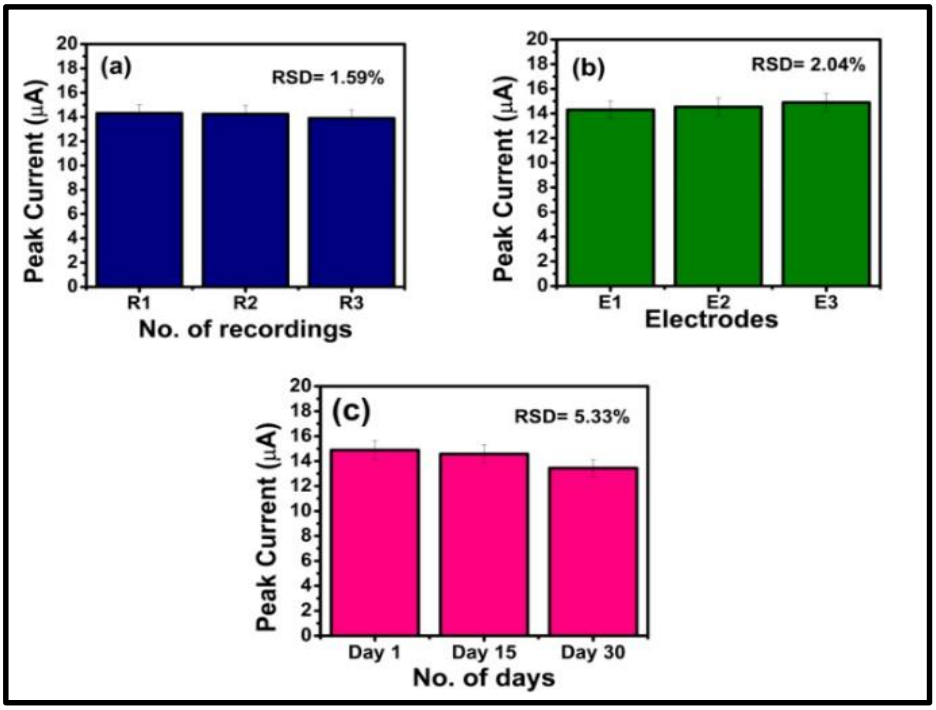

Fig. 3.7: *Bar Plots depicting (a) Repeatability (b) Reproducibility and (c) Stability of Sm_2O_3@GP*

3.2.3 Real sample analysis using PCA

Principal component analysis (PCA) was performed on two samples of black tea. The samples were purchased from the local vendor namely S1 and S2. Ten repetitions of each of the samples was taken. Fig. 11 illustrates the efficient clustering using PCA. The variation in the quantity of THP might be the reason behind the segregation of the two samples. The PCA score plot shows 97.79% and 1.63% of total variances. This depicts a separability index (SI) of 13.14.

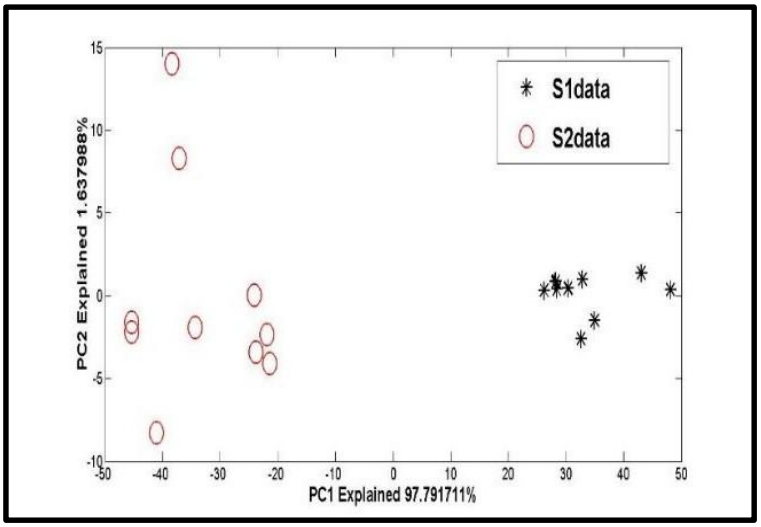

Fig. 3.8: *PCA Plot*

3.2.4 Summary

This approach illustrates the electrochemical detection of TH using Sm_2O_3 nanoparticles ingrained over graphite paste. An effective electrochemical sensor was thus developed. The fabricated electrode showed reliable sensory features in terms of repeatability, reproducibility and stability. This electrode showed a linear operational range of 10 to 1000 µM with an LOD 5.69 µM. This sensor also served as an efficient and repeatable instrument for discriminating two different samples of black tea based on TH content thus establishing its practicability in the tea sector.

This work has been published as cited *"Moulick M, Nag S, Das D, Das D, Bandyopadhyay R, Pramanik P, Banerjee Roy R. Detection of Theophylline using Samarium Oxide Nanoparticles Ingrained Graphite Electrode. Nano Life. 2023 Jun 24;13(02):2350007."*

3.3 Gadolinium oxide ingrained molecular imprinted polymer electrode for the electrochemical detection of theophylline in black tea

The electrocatalytic influence of a rare-earth metal samarium oxide for the quick determination of theophylline was explored in the previous work [20]. Modifications of electrode surface by micro and nano-sized particles enhances their electrochemical performances. However, challenges like limited linear range of operation, detection limit and recognition capability provoked the adaptation of using inexpensive polymers with extended stability. In this context, MIP based sensors paved a constructive pathway. MIP process involves the preparation of polymers with artificial recognition sites with a high degree of selectivity for the target analyte. Additionally, the coupling of nano-sized rare earth modifiers with the MIP material generates an enhanced electro-catalytic behaviour due to an increase in surface area resulting in increased number of active sites that causes additional molecular adsorption succeeded by amplified and fast electron transfer rate.

The present study reports a facile approach to fabricate a molecular imprinted polymer electrode modified with nano-composites of gadolinium oxide (Gd_2O_3@TH-MIP-CPE) to detect theophylline in variants of black tea samples. The novelty of the work resides in the adoption of MIP sensing technology for developing the specific sensor for TH detection coupled with Gd_2O_3NPs which shows a low limit of detection having value 2.004 µM and high selectivity towards the specific analyte, simplifying its usage in the detection of TH in real tea samples. Pre-treatment of the tea samples is not required, the electrode can be directly immersed in the tea liquor, making it a considerable advantage at the industrial level. The PLSR model was adopted to predict the quantity of TH in the unknown samples which showed an accuracy of 91.3 %.

3.3.1 Experimental study

3.3.1.1 Chemicals and reagents

Graphite powder, AA, EGDMA, TH, gallic acid (GA), tannic acid (TAN) and epicatechin (EC) were procured from Sigma Aldrich, India. Black tea samples were obtained from Asian School of Tea, Kolkata. Benzoyl peroxide was purchased from Sisco Research Laboratories Pvt. Ltd, India. The paraffin oil was obtained from Merck, India. Millipore water (R=18MΩ) was utilized throughout the experimentation. The entire process was carried out at standard temperature (25±2 ◦C). All reagents were used as received and did not

undergo any additional purification.

3.3.1.2 Synthesis of Gd_2O3 NPs

Gadolinium oxide (Gd_2O_3) (18 gm) was dissolved in excess nitric acid (HNO_3) (about 50 cc concentrated. HNO3) and heated over a water bath till most of the acid evaporates and solid gadolinium nitrate (Gd (NO3)3) appears. 100 cc distilled water was added and dissolved the crystalline Gd (NO3)3. In this solution, 60 cc mono-ethanolamine (C_2H_7NO) was added and it resulted in a white precipitate of gadolinium hydroxide. Consecutively, concentrated HNO_3 was added drop wise till the precipitate disappears. The clear solution was heated on a hot plate and gradually it becomes a brownish-fluffy mass. This black fluffy mass was ground using mortar pestle and heated in a furnace at 700ºC for four hours producing white fine powder of Gd2O3NPs. Particle size ranges from 40 to 50 nm confirmed from SEM.

3.3.1.3 Preparation of MIP and NIP materials

Commercial graphite powder of 0.95 g was dispersed in 20 ml of ethanol and sonicated for 35 mins. This was followed by the addition of 0.05 g AA and 0.05 g TH and further sonicated for 45 mins. 1 mg of benzoyl peroxide, a polymerization initiator and 400 µl of EGDMA were added to the above solution and sonicated. The solution was thoroughly dried in a water bath for 60 mins for polymerization to take place. The polymerized powdered sample was collected and the template molecule (TH) was leached out using distilled water for the creation of recognition sites. The NIP sample was prepared in the same way except for the addition of the template, TH.

3.3.1.4 Fabrication of Gd_2O_3@TH-MIP-CPE electrode

This convenient approach involves the development of a MIP material prepared by co- polymerization of acrylic acid (AA) and ethylene glycol dimethacrylate (EGDMA), followed by infusion of Gd2O3NPs, which yields increased oxidation efficiency and improved analytical features like stability, reproducibility and repeatability (Sato et al. 2009). Gd2O3 NPs and prepared MIP material were taken in the ratio of 1:9 and a total of 0.3 g of this material was blended properly using a mortar-pestle. Paraffin oil, a binder, was added drop-wise till a fine paste was obtained. The paste was then stuffed inside a capillary tube. Electrical contact was further established using a copper wire through the backside of the electrode.

3.3.1.5 Real sample preparation

Tea samples were purchased from Asian School of Tea. The testing solution was prepared similarly as reported in [21].

3.3.2 Results and discussions

3.3.2.1 Characterization of materials

3.3.2.1.1 UV-Vis spectroscopy

UV-Vis spectroscopy ensured complete removal of the TH molecule from the polymer matrix. It is observed from Fig. 3.8 that a visible peak is present around 265.5 nm before leaching the TH molecule, but the peak complete disappears on successful leaching of TH from the synthesized polymer matrix.

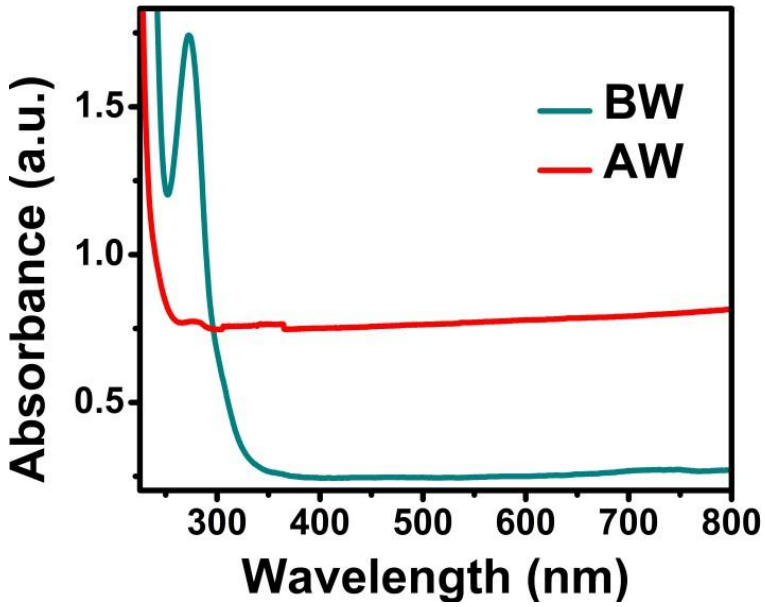

Fig. 3.9: *UV–Vis spectral response before and after analyte extraction*

3.3.2.1.2 SEM analysis of synthesized material

The surface morphology of the material prepared in the laboratory was studied using SEM. The SEM images in Fig. 3.10 (a), (b) and (c) throw light on the surface of the modified MIP material, the synthesized Gd_2O_3NPs and the NIP surface. The MIP surface displays a rugged morphology with presence of vacant sites due to the extraction of the template as compared to the NIP. The crystalline morphology of Gd_2O_3 NPs is evident from Fig 3.10 (b). The grain boundaries are clearly noticed and represent structural homogeneity.

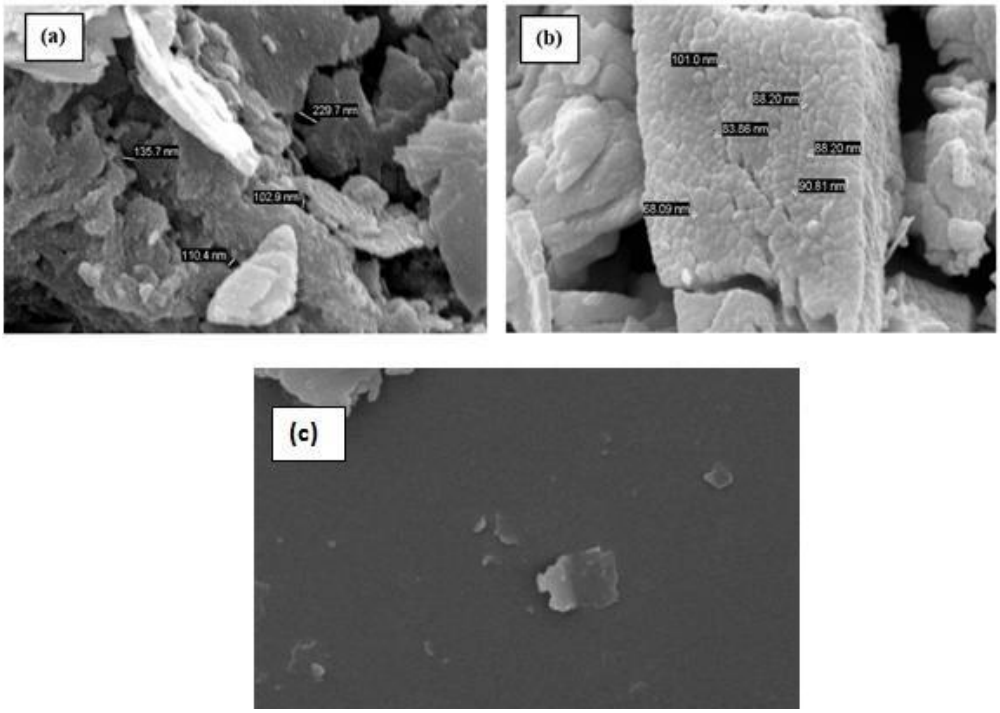

Fig. 3.10: *SEM image of (a) MIP-Gd_2O_3 (b) Gd_2O_3 NPs (c) NIP*

3.3.2.1.3 FTIR analysis of Gd_2O_3 NPs

The FTIR analysis of the synthesized Gd_2O_3 NPs is depicted in Fig. 3.11. The band around 603 cm^{-1} can be assigned to the Gd-O vibration of Gd_2O_3 [22]. The absorption bands around the range 3400-3500 cm^{-1} are due to the O-H stretching and C-O stretching accounts for the band around 1500 cm^{-1} [23].

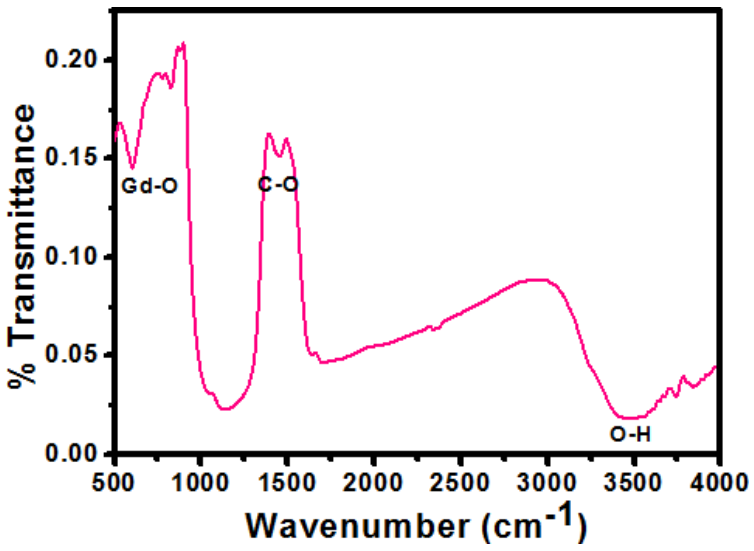

Fig. 3.11: *FTIR spectra of Gd_2O_3 NPs*

3.3.2.1.4 FTIR analysis of MIP Vs NIP material

High intensity transmission bands were observed in the FTIR spectral response around 1720 cm^{-1} due to C=O vibrations of the COOH group of AA. O-H bending vibrations were also observed at 1450 cm^{-1} with a comparatively lower intensity. The template TH was successfully ingrained in the polymer matrix as confirmed by the N-H stretching vibration of the amino group of TH in the range of around 1650 to 1550 cm^{-1}. The FTIR spectra of the NIP material do not show these vibrations associated with the N-H bonds, confirming the template molecule's absence. The results are in accordance with the research work [24]. The comparative FTIR analysis is shown in Fig. 3.12 below.

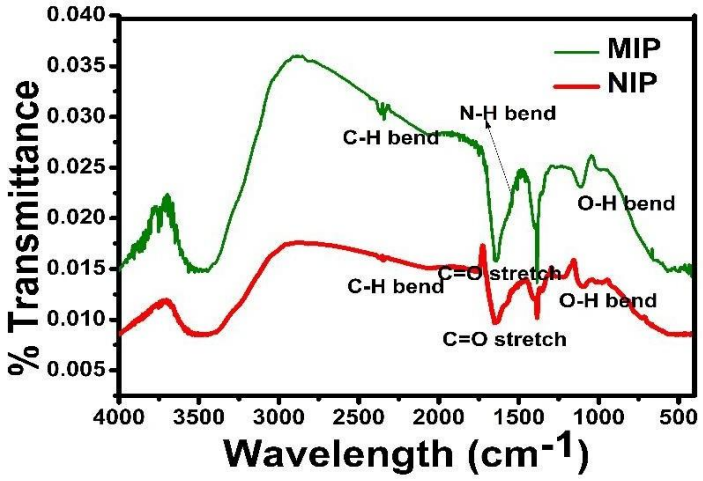

Fig. 3.12: *FTIR spectra of MIP vs. NIP*

3.3.2.2 Comparative Performance of different electrodes

An insight into the electrochemical behaviour of Gd_2O_3@TH-MIP-CPE clearly shows increased sensitivity of TH towards the fabricated electrode. It is clearly seen from Fig. 3.13 (a) that the oxidation peak current of TH is increased by 15.19 times and 7.33 times as compared to the carbon paste electrode (CPE) and modified graphite electrode (Sm_2O_3@GP), respectively. This enhanced electrocatalytic behaviour of Gd_2O_3@TH-MIP-CPE may be due to Gd_2O_3NPs which has increased the flow rate of electrons between the surface of the electrode and analyte TH. Further the electrochemical responses of the Gd_2O_3@TH-MIP-CPE electrode have been compared with the NIP electrode, which shows an enhanced performance in case of the MIP material (87.84 µA) due to the presence of the cavities which are structurally similar to TH molecule as compared to NIP (44.31 µA) shown in Fig. 3.13 (b).

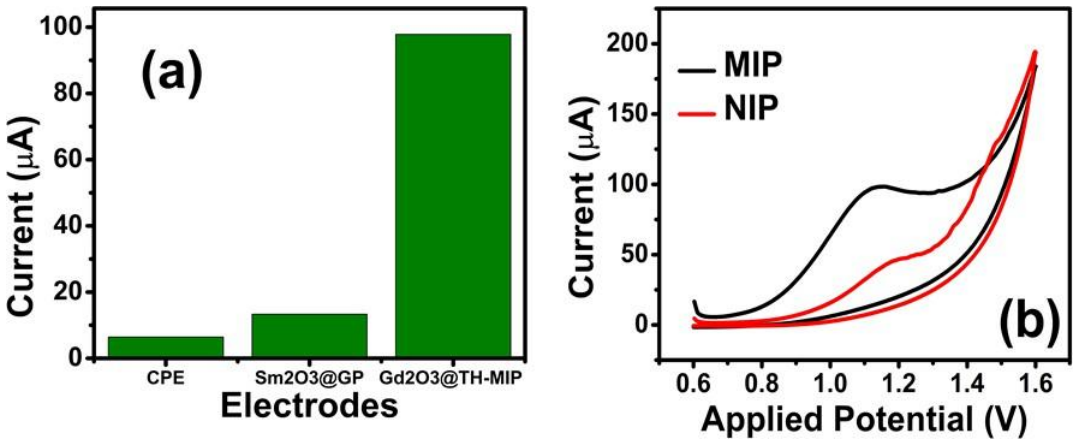

Fig. 3.13: *(a)Comparative study of synthesized electrodes (b) Comparison of MIP Vs NIP materials.*

3.3.2.3 Effect of buffer and pH variation

The electrolytic response of Gd_2O_3@TH-MIP-CPE was further investigated using various supporting electrolytes: phosphate buffer saline (PBS), acetate buffered solution (ABS) and citrate buffered saline (CBS). The optimal CV response was recorded in the presence of PBS in Fig. 3.14 (a). The pH value of the optimized supporting electrolyte, i.e., PBS was varied. As clearly visible in Fig. 3.14 (b), highest peak current of 97.84 µA was obtained in PBS 7. Thus, PBS 7 was optimized to be the test solution for further experimental work throughout the study.

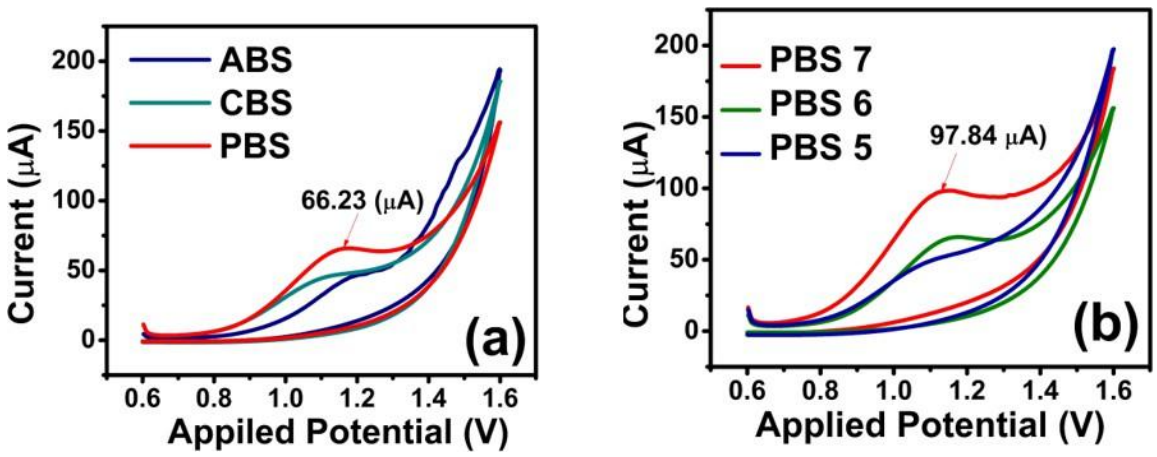

Fig. 3.14: *CV responses of Gd_2O_3@TH-MIP-CPE. (a) under the influence of various buffers (b) on variation of pH value of buffer*

3.3.2.4 Influence of Scan Rate Variation

The change in scan rates influences the electrochemical behaviour of Gd_2O_3@TH-MIP-CPE, which was thoroughly investigated by means of CV. The electrode was introduced in 100 µM of the testing solution in the presence of PBS 7 at different scan rates in the window 10 to 100 mV/s. The corresponding voltammograms were recorded as in Fig. 3.15 (a). It is observed that the oxidation peak current (I_{th}) is directly proportional to the scan rate, shown in (1).

$$I_{th=} 0.97v + 19.745 \; ; R^2 = 0.97 \quad (1)$$

The kinetics involving the oxidation reaction of TH has been analyzed. The quantity of electrons transferred has been derived from the equation (2)

$$I_p = \frac{nFQv}{4RT} \quad (2)$$

Where I_p =peak current, n denotes number of electrons, F stands for Faraday's constant, R stands for universal gas constant and T is the ambient temperature, Q is the charge consumed during the oxidation process and *v* denotes the scan rate. After calculation, n was obtained as 1.4 which is approximately close to unity.

The linearity between oxidation peak potential (Ep) versus the logarithm of scan rate (log *v*) is conveyed in Fig. 3.15 (c). The linear regression equation is shown as

$$E_p = 0.1813\log v + 0.8141; R^2 = 0.96 \quad (3)$$

The charge transfer coefficient α = 0.77 is derived from the slope of the equation (3) using the formula *2.3RT/(1-α) nF.* The value n = 1.4 and the presence of a single oxidation peak in the voltammogram indicate that the developed electrode exhibits a single-electron transfer process.

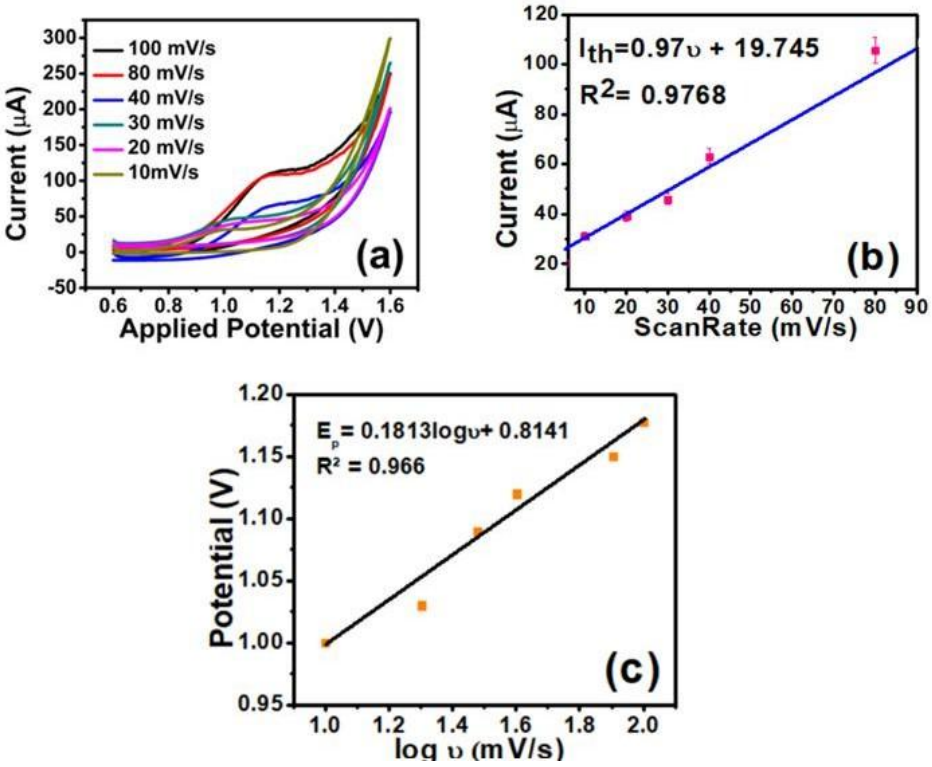

Fig. 3.15: *(a)Variation of peak current with scan rate variation. (b) Peak current versus scan rate. (c) Peak potential versus log of scan rate.*

3.3.2.5 Influence of concentration variation

The dependence of oxidation peak current (I_{th}) on increasing the concentration of TH has been elucidated in this work using DPV measurements. A significant increase in the current is observed on increasing the concentration of TH from 50 to 900 µM as observed in Fig. 3.16 (a).

The calibration curve of TH shows the linear change of peak current with an increase in the concentration. The electrode exhibits a linear range from 50 µM to 900 µM. The linear regression equation follows as

$$I_{th} = 0.163\ C_{th} + 47.49;\ R^2 = 0.92 \quad (4)$$

LOD=3σ/m is utilized to calculate the LOD, where 'σ' denotes the standard deviation of oxidation current recorded during the least concentration and 'm' signifies the slope from the calibration plot. The value of σ= 0.109, was calculated using standard deviation calculator for three consecutive readings of peak current (45.77, 45.82,45.98 µA) of the lowest concentration i.e. 50 µM. The LOD of the fabricated sensor is found to be 2.004 µM.

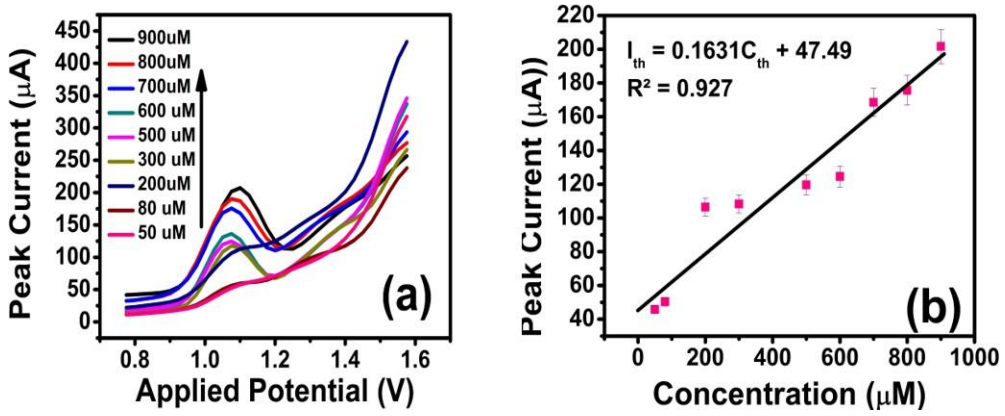

Fig. 3.16: *(a) DPV responses indicating change in peak current with variation of concentration (b) linear variation of oxidation peak current*

3.3.2.6 Study of Repeatability, Reproducibility and Stability

The analytical characteristics of Gd_2O_3@TH-MIP-CPE were studied at a concentration of 100 µM of the target analyte solution and satisfactory results were yielded. A relative standard deviation %RSD of 1.19 was reported for three consecutive readings. This concluded the highly repeatable characteristic of the fabricated electrode. The highly reproducible nature of the electrode material has been investigated by fabricating three similar electrodes under environmental similar conditions. These electrodes were directly placed in the analyte solution and the voltammograms were recorded. The electrodes showed a good reproducibility with a %RSD of 0.92. Further trials were carried out for a month with intervals of 15 days. The electrode delivered a stable performance having %RSD of 1.015. It was observed that the oxidation peak current had decreased to 95.92 µA on day 30 of the experiment due to electrode ageing effect. This decrease accounts for 1.96% as compared to day 1 of the experimentation.

3.3.2.7 Study of effect of different interferent species

Gd_2O_3@TH-MIP-CPE was subjected to different categories of interferent species namely organic compound (GA), metallic (Na^+) and non-metallic (Cl^{1-}) ions in the presence of TH stock solution, time to time to analyse the change in the electroanalytical behaviour in the electrode. The authors recorded CV signatures in the presence of five folds of each of the interfering agents in the presence of 100µM of the target analyte solution as shown in Fig. 3.17. The relative signal fluctuation was within a tolerance limit of ±6%.

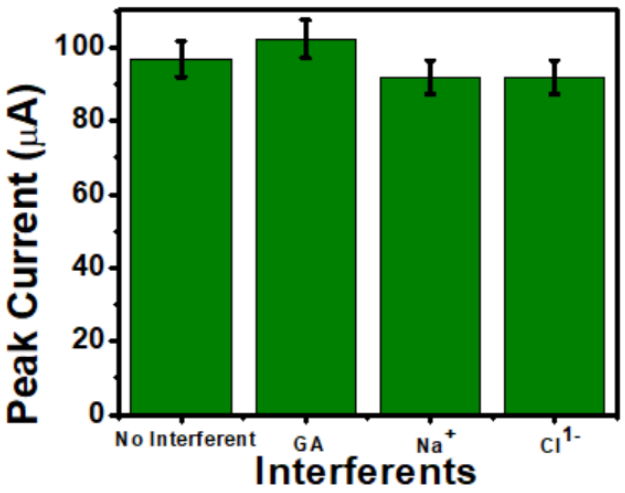

Fig. 3.17: *Study of the effect of various interferent species on the electrode.*

3.3.2.8 Selectivity profile of Gd_2O_3TH-MIP-CPE

An insight into the selectivity profile of the fabricated electrode shows highly selective performance towards TH as shown in Fig. 10. The effect of selectivity on each of the analytes TH, EC, GA and TAN were investigated. Gd_2O_3@TH-MIP-CPE was directly placed in 100 µM solutions of TH, EC, GA and TAN. During experimentation, it was concluded that the electrode displayed maximum selectivity for TH solution. Thus, from the bar plot in Fig. 3.18 it can be concluded that Gd_2O_3@TH-MIP-CPE is highly selective for the detection of TH analyte.

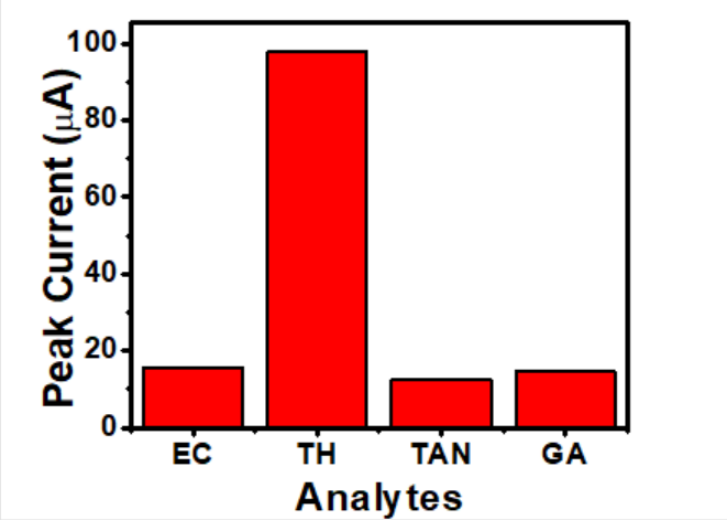

Fig. 3.18: *Selectivity Profile*

3.3.2.9 Comparative Study of the proposed method with pre-existing literature

The proposed method for the detection of TH has been compared with previous techniques. The reports have been tabulated in Table 3.2. A quick insight into the tabular comparison shows us that the fabricated electrode, Gd_2O_3@TH-MIP-CPE, shows a comparatively lower detection limit compared to some other reported techniques due to modification of the electrode surface with Gd_2O_3 NPs. The linear working range of the electrodes have not been mentioned precisely in [25]-[26]. Further, the selectivity study of the fabricated electrodes has not been discussed vividly in some of the papers.

Table 3.2: *Literature survey on previously used techniques for TH detection*

Electrodes Used	Technique	Linear Range (µM)	LOD(µM)	Reference No.
GCE modified with MnO2 nanoparticles	DPV	0.01-320	5.9	[27]
Carbon Paste electrode modified with graphene and EFTA	Chronoamperometry	10-100 µM	7.02	[28]
Carbon Paste electrode	SWV	1-5 mM	190 µM	[29]
Carbon fiber	Microchannel	6-600	4.0	[25]
Au	CV	0-50	3.2	[26]
MIP	DPV		1.0	[30]
Ag-THO-MIP	SERS	-	3.5	[31]
Gd_2O_3@TH-MIP-CPE	CV and DPV	50-900	2.004	This work

3.3.3 Real Sample Analysis Using Partial Least Square Regression (PLSR)

Five different samples of black tea, obtained from The Asian School of Tea (Kolkata) were tested using Gd_2O_3@TH-MIP-CPE to determine the quantity of TH present in each of the discrete samples taken using the PLSR model. The preparation of the black tea liquor was done using similar method as stated in [28]. 200mg (dry weight) of each of the black tea sample was weighed on a scale and put in boiling water. After infusion for a couple of minutes, the liquor was filtered and 20 mL of the liquor was subjected for DPV analysis. A total of ten repetitive DPV measurements were recorded for each of the samples. Thereafter, the data matrix [159×50] recorded, by the infusion of Gd_2O_3@TH-MIP-CPE in the black tea samples and was subjected to PLSR modelling using MATLAB (2017b). High-performance Liquid Chromatography (HPLC) has been performed and quantitative analysis of

theophylline content in various black tea samples are analyzed by developing regression model, partial least square regression.

The data matrix and the HPLC values were partitioned into training and testing subsets in the ratio of 80:20. The predictability of the Gd_2O_3@TH-MIP-CPE was studied PLSR model incorporated with leave-one-out cross validation (LOOCV) technique. The PLSR model had a prediction accuracy of 91.3% when the electrode was employed to measure TH content in five black tea samples. The RMSEC value 0.7377 is the lowest for 20 latent variables (LVs). A table of comparison has been incorporated below.

Table 3.3: *Tabular comparison of actual vs. predicted TH content from PLSR model*

Sample No	**Actual TH (mg/ml) (HPLC)**	**Predicted TH (mg/ml) PLSR**	**Prediction Accuracy (%)**
1	0.0027	0.0028	97%
2	0.0011	0.0010	91%
3	0.0010	0.0009	90%
4	0.0004	0.00039	97.5%
5	0.0026	0.0021	81%
	Average Prediction Accuracy		**91.3%**

3.3.4 Summary

The present research work incorporated the molecular printing technology and modified the surface of the MIP material using nano-composites of Gd_2O_3 for the detection of TH in black tea samples. Gd_2O_3@TH-MIP-CPE demonstrated a linear working range from 50 to 900 µM, with an LOD of 2.004 µM. In terms of its analytical behaviour, the electrode has proven to be repeatable and reproducible. The electrode reflects a stable behaviour too. Moreover, the electrode validation was performed using PLSR model with standard HPLC data. The average prediction accuracy was 91.3%. Thus, in this facile approach, not only was the synthesis of MIP material was focused upon, the ability of the electrode to quantify TH in different samples of black tea was highlighted.

3.4 Conclusion

In this work, two different electrodes were developed for the rapid detection of TH in real samples of black tea. In the first investigation, analysis of the voltammetric responses in real samples of black tea while modifying the bare graphite electrode with samarium oxide nanoparticles to increase the electrode's efficacy, is highlighted. The electroanalytical features of Sm_2O_3 have been thoroughly explored to enhance the electrochemical performance of the fabricated sensor. The challenges faced in the previous process was limited molecular recognition ability and the detection limit. A constructive solution to these hindrances was the adoption of MIP technology. Electrochemical sensors based on molecular imprinted acrylic acid polymer modified with rare earth oxide was an alternative solution. The thermally stable nature of the Gd_2O_3 NPs contributed to the improved response of the electrode. In contrast to the initial investigation, the later alternative showed reasonable molecular selectivity, stable behaviour over a sufficiently good period of time and the LOD was significantly improved as compared to previous techniques cited in Table 3.2. Compared to the standard HPLC protocol, the electrode demonstrated an accuracy of 91.3% in real samples of black tea.

This work has been published as cited *"Moulick M, Das D, Nag S, Pramanik P, Roy RB. Gadolinium oxide modified molecular imprinted polymer electrode for the electrochemical detection of theophylline in black tea. Journal of Food Composition and Analysis. 2025 Jan 1; 137:106994."*

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CHAPTER 4

Development of dedicated electrochemical sensors for the detection of total theaflavins in black tea

This chapter presents two different approaches for the electrochemical detection of total theaflavins in black tea samples. The fabricated electrodes illustrated accurate analysis of these samples. The electrodes demonstrated satisfactory electroanalytic characteristics.

Publication Outcomes

- Moulick M, Nag S, Das D, Roy RB. A Novel Graphite Electrode Modified with Titanium Oxide Nanoparticles for the Detection of Theaflavin in Black Tea. In National Conference on CONTROL INSTRUMENTATION SYSTEM CONFERENCE 2023 Oct 6 (pp. 519-527). Singapore: Springer Nature Singapore.
- Moulick M, Nag S, Das D, Hazarika AK, Sabhapondit S, Roy RB. A Molecular Imprinted Bi-polymer graphite electrode decorated with NiCo2O4 nano-cubes for rapid detection of theaflavin in black tea. IEEE Sensors Journal. 2025 Apr 3.

CHAPTER 4

Development of dedicated electrochemical sensors for the detection of total theaflavins in black tea

4.1 Introduction

Theaflavin is a significant constituent in tea and is responsible for its quality profiling in terms of its astringency, as highlighted in section 1.1 of the thesis. In chapter 3, detection of a methylxanthine derivative (TH) has been highlighted. Two different sensors modified with rare earth metal oxide nano-crystals were fabricated for the detection of TH is black tea samples. Along with TH, another very important compound responsible for tea astringency i.e. theaflavin has been focused on in this chapter. The changes in astringency profile of black tea, influenced by TF contribute towards its quality [1] which has a powerful impact on human taste sensation. It has been established that the content TF can be positively correlated with the liquor's brightness, along with its astringency [2]. A persuasive literature survey reveals various health benefits of TF including being antiallergic, anticarcinogenic, antifungal, and neuroprotective effects [3]-[8]. Methods like capillary electrophoresis [9], pulse radiolysis [10], high-performance liquid chromatography (HPLC) [11], and flavognost method [12] although accurate, they require high-end instrumentation facility, human expertise and are time- consuming and costly. The hindrances mentioned above opened the avenue for electrochemical approaches. In literature [13], the researchers employed the cyclic voltammetry principle using hanging mercury drop electrodes (HMDE) for detecting TF in different types of black tea. In the article [14], the authors quantified TF using an electronic tongue consisting of five noble metals. However, electrode's limited operational range and current sensitivity and expense led a pathway towards the fabrication of modified graphite electrodes. The use of metal oxides and rare earth oxides nano-crystallites have successfully improved the electrode's efficacy as seen in literatures [15]-[16]. Thus, in this chapter, the authors have taken the opportunity to discuss two different approaches towards electrode development i.e. a modified graphite electrode and, further to increase the electrode's molecular recognition ability, MIP technology coupled with diffusion of metal nano-cubes has been explored in sections 4.2 and 4.3. This chapter will portray a journey towards the development of a cost-effective and optimum sensor for the detection of total theaflavins in black tea for its quality profiling.

4.2 Detection of theaflavin in black tea using TiO_2 nano- cubes modified graphite electrode

In this section, a graphite electrode has been fabricated by modifying its surface using nano- cubes of TiO_2. Voltammetric techniques (CV and DPV) have been used to examine the electrochemical behaviour of the TiO_2@GP electrode. The electrode exhibits a linear working window of approximately 20 to 500 µM, an LOD 20.27 µM. The efficacy of the electrode was established by its capability of successfully discriminating the various samples of black tea using PCA.

4.2.1 Experimental section

4.2.1.1 Chemicals and Reagents

Paraffin oil , ammonia, Poly (ethylene glycol)-400 and hexafluorotitanic acid (60 wt.% in H2O) were all acquired from Merck, India. Analytical grade graphite amorphous powder was obtained from Sigma-Aldrich company. No pre-treatment is required and the experimentation solutions were made in Millipore water of resistance of 18MΩ. All experimentation was carried out at ambient room temperature.

4.2.1.2 Measurement Setup

A galvanostat, along with a three-electrode configuration, was used. The reference electrode used was Ag/AgCl and platinum was used as a counter electrode. The block diagram of the setup is given below in Fig.4.1.

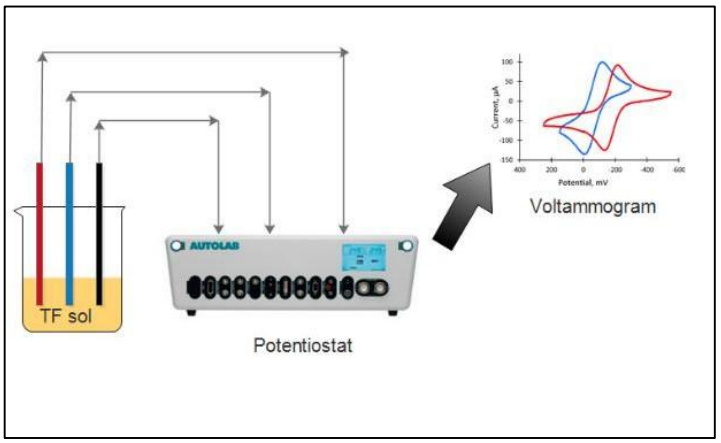

Fig. 4.1: *Experimental setup*

4.2.1.3 Synthesis of TiO_2 nps

Nano-sized TiO_2 crystals were prepared by the sol-gel process as [17]. Nanoparticles were obtained after calcining the white powder-like sample at a temperature of around 450 ^oC.

4.2.1.4 FESEM of TiO_2 nps

Surface morphological characterisations of TiO_2 nps were obtained using the JEM6700F field emission scanning electron microscope, i.e., FESEM. Fig.4.2, shows TiO_2 nps are cubical in shape.

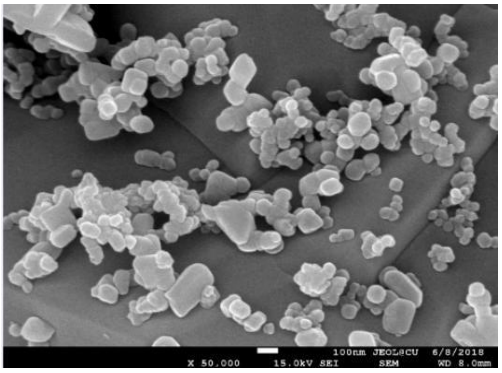

Fig. 4.2: *FESEM image of TiO_2 nano-cubes*

4.2.1.5 Preparation of TiO_2@GP

TiO_2 nano-cubes (30 mg) and graphite powder (270 mg) was thoroughly mixed in the ratio 1:9, and finely ground, and converted into a paste using paraffin oil. The obtained paste was inserted into a glass capillary tube of inner diameter 2.5mm, electrical attachment was established using a copper wire. A graphite electrode was also prepared similarly.

4.2.2 Results and discussion

4.2.2.1 The Electrochemical performance of TiO_2@GP Vs. Graphite electrode

The electro-chemical behaviour of TiO_2@GP was studied using CV in the potential window of 0.0V to 0.8V. Oxidation peaks were absent in the absence of TF, however, after introducing 100 µM of TF stock solution, a distinct peak was clearly observed around 0.4V; thus, the detection of TF was established as depicted in Fig. 4.3(a).

ON introduction of TiO_2 nano-cubes, there was a sharp increment in the oxidation current around 1.95 times of the bare graphite electrode. This is due to the increased exchange rate of electrons on the electrode-analyte surface.

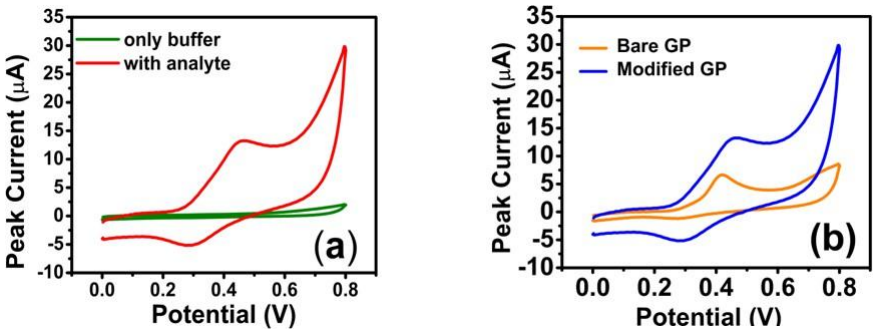

Fig. 4.3: *(a) CV plot of TiO_2@GP in the presence of TF (b) Comparison of the electrochemical behaviour of TiO_2@GP vs. Bare Graphite Electrode*

4.2.2.2 Buffer Variation

TiO_2@GP was tested in the presence of buffers of pH 6 – Acetate Buffered Saline (ABS), Phosphate Buffer Saline (PBS) and Citrate buffer Saline (CBS). To conclude , the peak oxidation current response was obtained using ABS 6, hence it was optimized to carry out all further experimentation.

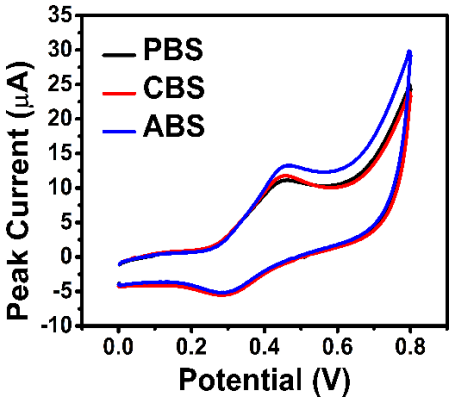

Fig. 4.4: *CV responses of TiO_2@GP in different buffer solutions*

4.2.2.3 Scan rate variation

The electro-chemical behaviour of TiO_2@GP for different scan rates was investigated by CV in the potential window of 0 to 0.8 V. An increase in the peak current was noted in the potential window of 10 to 200 mV/s, shown in Fig. 4.5 (a). Fig. 4.5 (b), which demonstrates a linear regression equation as $I_{TF} = 0.1346v + 4.3925$; $R^2 = 0.96$.

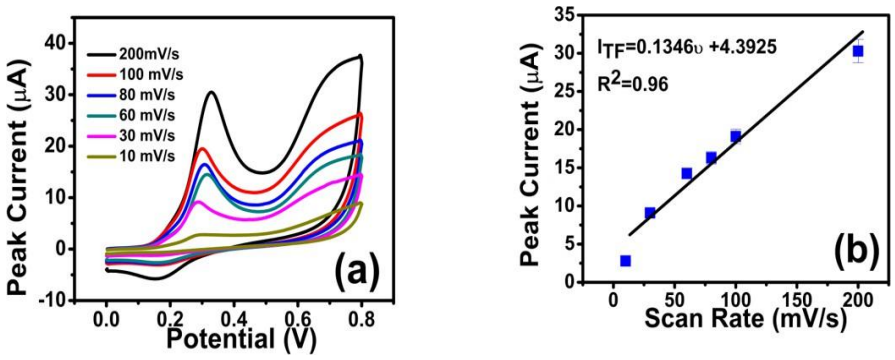

Fig. 4.5: *(a) CV response of change in peak current vs scan rate (b) Linearity between peak current vs. scan rate*

4.2.2.4 Concentration variation profile

The electrochemical behaviour of TiO_2@GP at different concentrations ranging from 20 to 500µM, were analysed using DPV. Fig. 4.6 (a) demonstrates the relative increment in current with the steady increase in the analyte's concentration. A linear regression relation was obtained between current and different concentrations as follows $I_{TF} = 0.0293C_{TF} + 8.8101$; $R^2 = 0.93$. The Table 1, given below, shows a comparative study of different techniques used in the detection of TF.

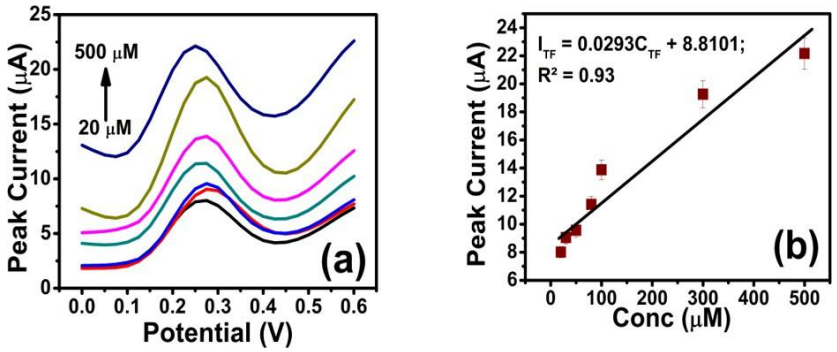

Fig. 4.6: *(a) DPV response showing variation of oxidation current with varying concentrations of TF (b) Linearity between peak current vs. concentration*

Table 4.1: *Comparative study of the performance of TiO_2@GP with previously reported literature.*

Method	LOD (µM)	Conc. (µM)	Ref
HPLC and Solid phase extraction,	Around 25	-	18
HPLC	0.02	0.88-2.66	19
MLAPV	-	-	20
TiO_2@GP	**20.27**	**20-500**	**This work**

4.2.3 Real sample analysis

Liquor from three tea samples was prepared. 200 mg of (dry weight) tea leaves were soaked in boiling hot water. 20 ml of each of the three prepared liquors was filtered properly and subsequently subjected to DPV analysis using TiO_2@GP. Ten consecutive DPV responses were taken for each liquor. PCA was performed on the obtained data sets. PCA transforms the data samples into a reduced information-rich dataset . Fig. 4.7, demonstrates an effective clustering of the samples of tea. The PCA score plot shows 97.76 % and 1.58% of the total variance.

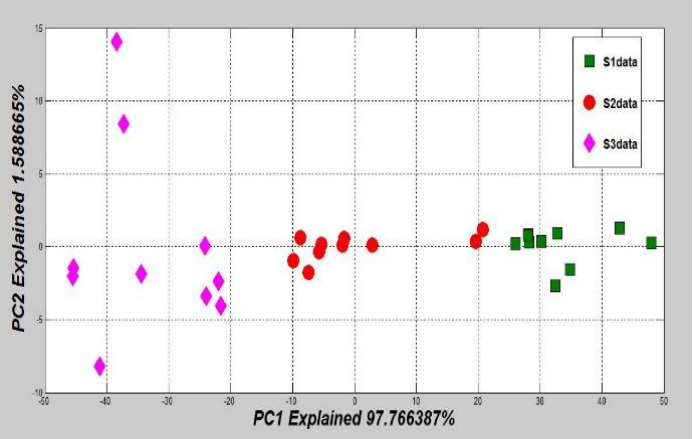

Fig. 4.7: *PCA plot*

4.2.4 Summary

This piece of research, serves as an easy electrochemical detection of TF. The electrode showed satisfactory linear range of operation and an LOD of 20.27 µM. The authors intend to develop an array of electrochemical sensors to detect different analytes in the same sample of black tea.

This work has been published as cited *"Moulick M, Nag S, Das D, Roy RB. A Novel Graphite Electrode Modified with Titanium Oxide Nanoparticles for the Detection of Theaflavin in Black Tea. In National Conference on CONTROL INSTRUMENTATION SYSTEM CONFERENCE 2023 Oct 6 (pp. 519-527). Singapore: Springer Nature Singapore."*

4.3 A Molecular Imprinted Bi-polymer graphite electrode impregnated with $NiCo_2O_4$ nano-cubes for rapid detection of theaflavin in black tea

In section 4.2 of this chapter, a TiO_2 modified graphite electrode for the detection of total theaflavins in black tea has been thoroughly explored. The excellent electrocatalytic features of TiO2 were explained in detail. The fabricated electrode was successfully able to discriminate among the different samples of black tea based on their TF content. However, hindrances like molecular recognition capability, lower detection limit and linear operational range were the factors which lead the pathway towards the fabrication of a MIP-based electrode. Further to improvise on the electrode's efficacy, the MIP material was modified using nano-cubes of NiCo2O4. The proposed electrode was prepared from a co-polymer of acrylic acid and methacrylic acid and imprinted with theaflavin template. Analyses of six samples of black tea from Tocklai Research Institute, Assam, have been carried out by comparing the TF content in each of the black tea samples with the standard HPLC values using the PLSR technique, which exhibits an accuracy of 92.6%.

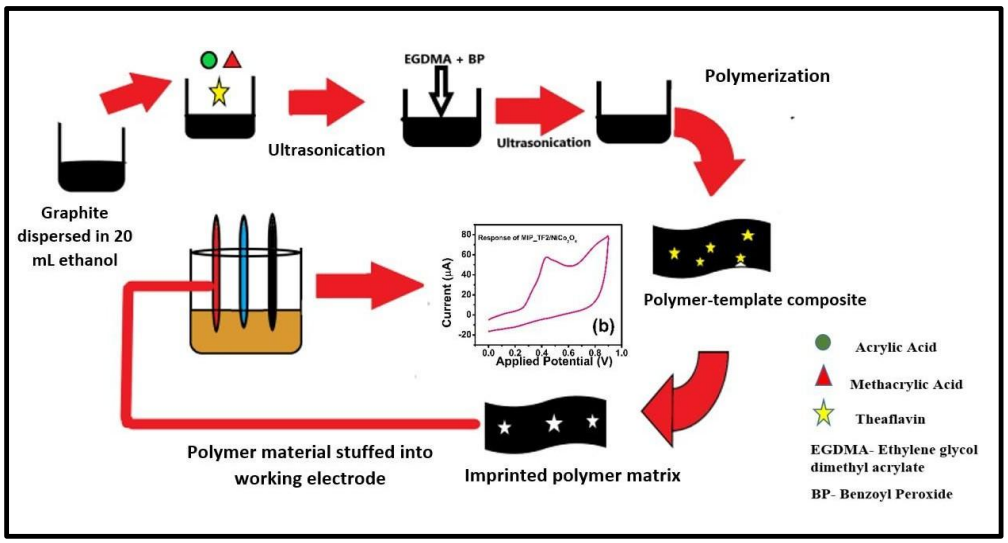

Fig. 4.8: *Schematic for the electrode preparation and overall experimentation*

4.3.1 Experimental study

4.3.1.1 Reagents and standards

The monomers, Acrylic Acid (AA) and methacrylic acid (MAA) were obtained from the companies Sigma Aldrich and E-Merck. Ethylene glycol dimethyl acrylate (EGDMA), a crosslinker, and benzoyl peroxide (initiator) were gathered from Sigma Aldrich. The graphite

powder was obtained from Loba Chemie Pvt. Ltd. Paraffin oil for binding the material and ethanol was purchased from Merk. Tannic acid was purchased from Nice Chemicals Pvt. Ltd., and caffeine (CAF) from Sigma Aldrich. Calcium chloride was obtained from Merck. TF extract was provided by the Tocklai Tea Research Institute, Assam. All the reagents used in the experimentation were analytical graded with a purity of 98%. The required testing solutions were all prepared using Millipore water (R= 18 Ω).

4.3.1.2 Extraction of TF

Black tea infusion was prepared to extract TFs using hot water. Caffeine is removed from the infusion prepared using dichloromethane (DCM). After decaffeination crude TFs were extracted with ethyl acetate. The crude TF is recovered from ethyl acetate using rotary evaporator at reduced pressure. The crude TF thus obtained was further purified by gel permeation column chromatography using Sephadex LH-20 as the column material. Water and acetone were used as eluents, starting with acetone: water ratio of 1:9 and gradually reaching 2:3 to get the purified TF fraction. TF from the eluted fractions was recovered, dried under vacuum, and kept in the dark under refrigeration until further use.

4.3.1.3 Apparatus and measurement

The surface morphology of the prepared electrode material and $NiCo_2O_4$ NPS was investigated using the instrument ZEISS EVO 18 at an acceleration voltage of 150000V of 15kV. The nanoparticles were analysed using an X-ray diffractometer (Philips PW1710) at 40 kV and 40 mA to obtain an XRD pattern. The electrochemical responses were carried out using the voltammetric techniques- cyclic voltammetry (CV) and differential pulse voltammetry (DPV), employing the three-electrode system employing the Autolab Potentiostat. The Ag/AgCl (reference electrode) and platinum electrode were used along with the working electrode (MIP_TF/NiCo2O4). The working potential range optimized was in the window 0.0 -1.0 V.

4.3.1.4 Production of Nickel-Cobalt Oxide Nanoparticles

The production the $NiCo_2O_4$ NPs was done in a similar way as given in literature [21]. These particles were used for electrode modification and characterization purposes.

4.3.1.5 Preparation of molecular imprinted polymer (MIP)

Graphite powder (99%) was dispersed in 20 mL ethanol by ultrasonication for 35 minutes. The 0.05 g of the monomers AA and MAA mixed in the ratio of 3:2 was added to

the solution along with the template TF (0.05g) and further sonicated for half an hour. Approximately 400 µL of crosslinker EGDMA and 1 mg of the initiator, benzoyl peroxide, were added in the mixture and was sonicated. The prepared material was then polymerized by continuous heating at 50ºC for 1 hour. After polymerization, the powdered sample was collected. The prepared material had to undergo the process of leaching to form the cavities for the adsorption of the template. Thus, the MIP material was prepared by repeatedly washing the material with distilled water, till no trace of TF was visible in the filtrate material. The non-imprinted polymer (NIP) was prepared similarly without TF during the synthesis procedure.

4.3.1.6 Preparation of the working electrode

MIP_TF2/$NiCo_2O_4$, the working electrode was prepared using 0.27g of MIP material mixed with 0.30g of the synthesized $NiCo_2O_4$ NPs, and a smooth paste was made using a couple of drops of binder, paraffin oil. The blended paste was inserted into a glass tube of inner diameter 2.5 mm and an electrical contact was established using a copper wire.

4.3.2 Material characterizations

4.3.2.1 X-ray Diffraction Characteristics of $NiCo_2O_4$ NPs

The XRD pattern in Fig 4.9 (a) confirms the cubic phase of $NiCo_2O_4$ NPs formed by comparing it with the JCPDS card No. 00-002-1074. It can be inferred the most intense peak was seen at 2 Θ= 28.7º (111). The obtained diffractogram showed pure crystalline formation. Using the Debye-Scherrer equation [22] the crystalline size was estimated at around 18.75 mm.

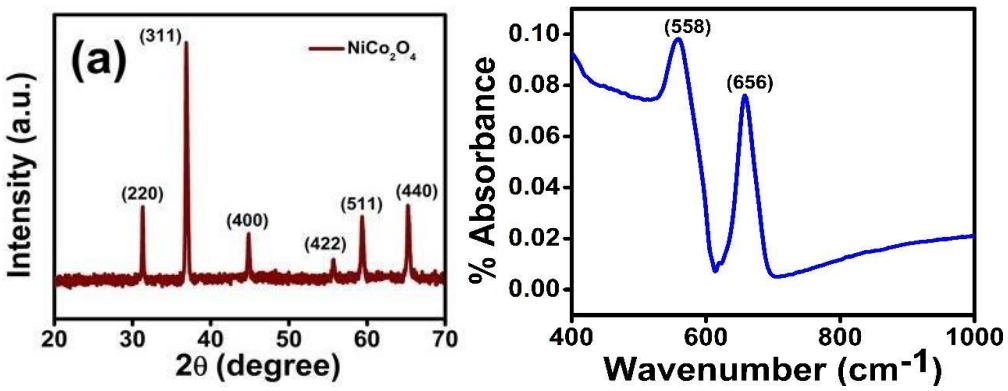

Fig. 4.9: *(a) XRD pattern for $NiCo_2O_4$ NPs (b) FTIR spectra of $NiCo_2O_4$ NPs*

4.3.2.2 FTIR analysis of $NiCo_2O_4$ NPs

Fig. 4.9 (b) shows the FTIR spectra of the synthesized $NiCo_2O_4$ NPs. It shows two prominent bands at 558 cm^{-1} and 656 cm^{-1}, respectively. As per literature [23] and [24], the two bands are characteristic features of metal-oxygen bonding between Ni^{2+} and O or Co^{2+} and O. The results are as per [21].

4.3.2.3. SEM Analysis

The morphological structures of $NiCo_2O_4$ nanoparticles and MIP_TF2/$NiCo_2O_4$ are portrayed in Fig.4.10 (a) and (b). It may be observed from Fig. 4.10 (a) that the $NiCo_2O_4$ nanoparticles are grain- like. Fig. 4.10 (b) illustrates that the surface of MIP_TF2/NiCo2O4 is wrinkled due to the formation of imprinted cavities for trapping the template molecule. Fig. 4.10 (c) depicts the almost smooth surface in the NIP material, which shows lack of cavities.

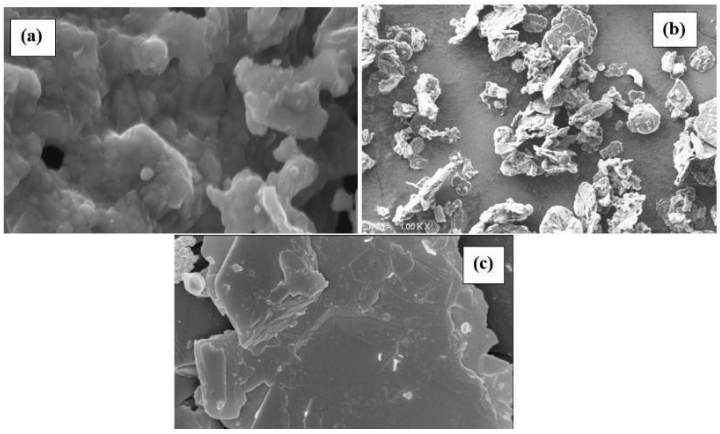

Fig. 4.10: *(a) SEM image of $NiCo_2O_4$ nanoparticles (b) MIP_TF2/$NiCo_2O_4$* ***(c)****NIP material*

4.3.3 Results and discussions

4.3.3.1 Monomer Optimization

An interesting feature was noted during experimentation, i.e. the choice of monomer was extremely important during electrode fabrication. In this regard, two electrodes were initially fabricated namely MIP_TF1/ $NiCo_2O_4$ (MIP electrode consisting of monomer AA and mixed with $NiCo_2O_4$ NPs) and MIP_TF2/$NiCo_2O_4$. From Fig. 4.11 (a) and (b) it is seen that the current response for MIP_TF1/ NiCo2Os was 9.62 µA, which was increased by nearly 5.92 times on mixing monomers AA and MAA in the ratio of 3:2. Therefore MIP_TF2/$NiCo_2O_4$ electrode was optimized for further experimentation.

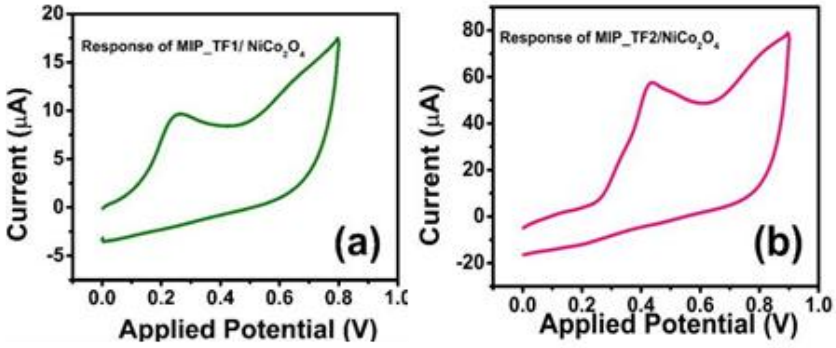

Fig. 4.11: *Comparative Current Response profile of (a) MIP_TF1/ $NiCo_2O_4$ (b) MIP_TF2/$NiCo_2O_4$.*

4.3.3.2 Effect of change of buffer and pH

MIP_TF2/$NiCo_2O_4$ was subjected to acetate buffer (ABS) having pH 5, 6, and 7. The response of electrodes is affected by a change in pH of the test solution. The resultant voltammogram is illustrated in Fig 4.12, depicts the oxidation peak current for MIP_TF2/$NiCo_2O_4$ is highest for ABS 5 compared to ABS 6 and 7. From Fig. 4.12, it is observed that the oxidation current intensity increases from pH 4 to 5, but tends to decrease when the pH is increased to 6. TF is inferred to be very stable in the pH window 4.5 to 5.5 [17]. With an increment in pH, there is a rise in phenolic anion production which increases the degree of solubility of TF in the liquid, restricting the adsorption on the electrode plane. Since the application of MIP_TF2/$NiCo_2O_4$ is essentially centered around the detection of TF in black tea samples, pH 4.9 to 5, the value of pH is fixed at 5 during the experiment.

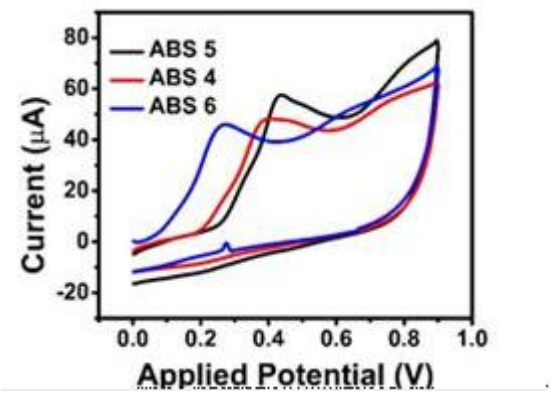

Fig. 4.12: *CV responses for 1 mM TF in ABS of pH 4 to 6*

4.3.3.3 Concentration Variation

The reliability of peak current on different TF concentrations was studied using MIP_TF2/$NiCo_2O_4$ using DPV over a concentration range of 40-1000 µM. The voltammograms in Fig. 4.13 (a) conclude that oxidation current is directly proportional to the

TF concentration and shows a linear rise with the increase in concentration from 80-1000 µM. The corresponding regression relationship (1) is given as

$$I_{TF} = 0.0706\, C_{TF} + 16.224\,;\, R^2 = 0.97 \quad (1)$$

The limit of detection (LOD) of the sensor is calculated as 13.89 µM from 3σ /m, here σ represents the standard deviation of the regression equation and m stands for slope of the calibration curve.

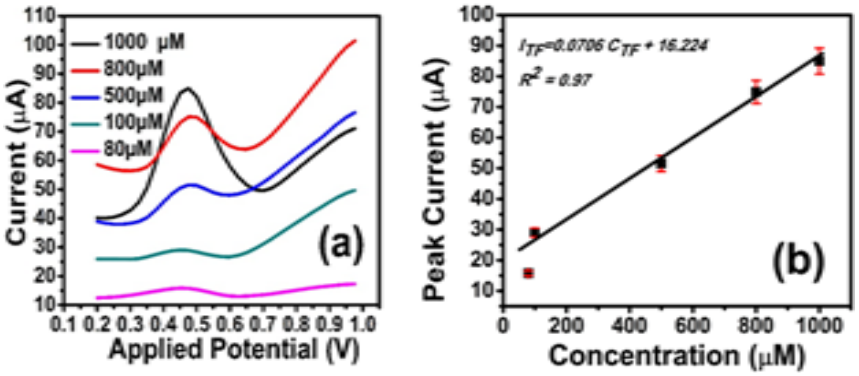

Fig. 4.13: *(a) DPV response of MIP_TF2/$NiCo_2O_4$ for different TF concentrations (b) Linear Variation of peak current with change in TF concentration*

4.3.3.4 Effect of scan rate on current response

CV analysis was carried out for scan rates ranging from the window 0.02 V/s to 0.2 V/s for analysing the electron transfer kinetics of the electrochemical system. Fig. 4.14 (a) shows the voltammograms for different scan rates. It is inferred that the oxidation peak current gradually increases linearly with the increase in scan rate as demonstrated in equation (2)

$$I_{TF} = 0.2667v + 6.0649\,;\, R^2 = 0.93 \quad (2)$$

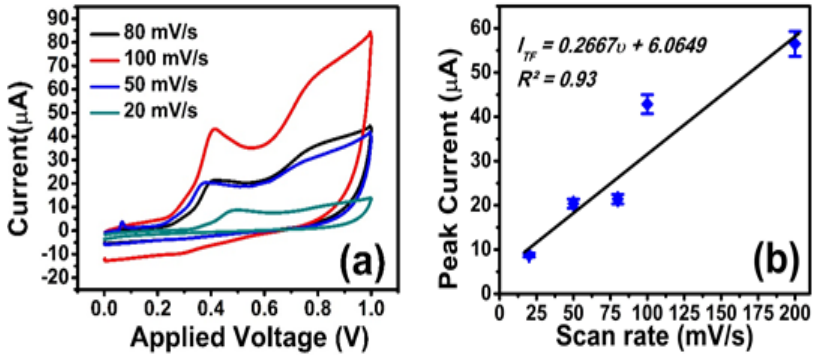

Fig. 4.14: *(a) Cyclic voltammograms showing the change of peak current with scan rate variation (b) Linear variation of peak current Vs scan rate.*

The no. of electrons transferred during the electrochemical process is calculated from the equation (3)

$$I_p = \frac{nFQv}{4RT} \quad (3)$$

'Ip' represents oxidation peak current, n = no. of electrons transferred during the electrochemical process, F represents Faraday's constant, R stands for the universal gas constant and 'T' represents the room temperature. The no. of electrons transferred was calculated to be 1.22 (approximately unity) which is attributed to the single electron adsorption protocol.

4.3.3.5 Repeatability, Reproducibility, and Stability Parameters

The repeatable nature of MIP_TF2/$NiCo_2O_4$ was concluded by subjecting it to 0.08 mM of TF solution under the same experimental setup. Due to repeated exposure of MIP_TF2/$NiCo_2O_4$ to the TF solution, the molecules get adsorbed on the electrode surface and show a slight decrease in the peak current. The relative standard deviation (RSD) is calculated as 0.71 % for three consecutive responses as shown in Fig 4.15 (a). Three different electrodes were prepared by the same protocol and subjected to 0.08 mM TF solution using ABS 5. The results in Fig 4.15 (b). show that MIP_TF2/$NiCo_2O_4$ is satisfactorily reproducible with an RSD value of 3.7%. All the measurements were taken at the room temperature. From Fig. 4.15 (c), it is seen that when the electrode was used for three consecutive readings at an interval of 20 days, the electrode showed an RSD value of 5.8%. Due to exposure to the TF solution, the molecules have been adsorbed on the surface of the electrode, and as a result, there has been a decrease in the oxidation peak current.

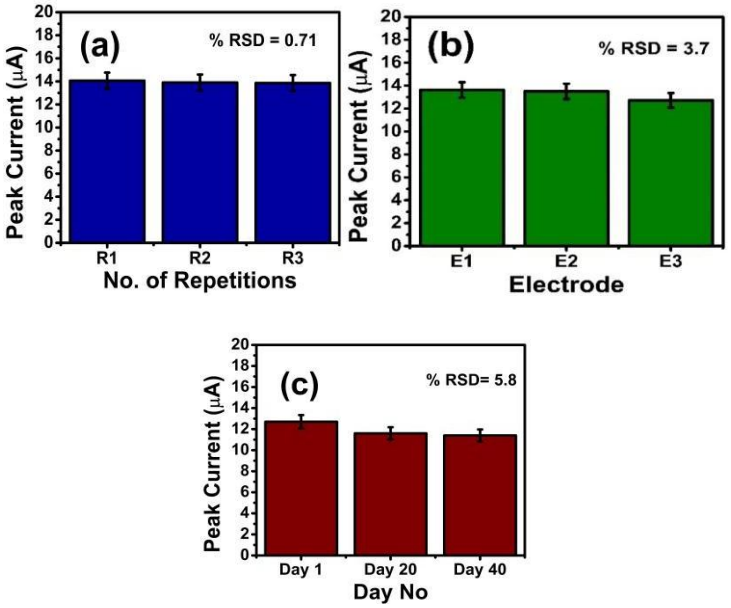

Fig. 4.15: *(a) Repeatability (b) Reproducibility, and (c) Stability of MIP_TF2/$NiCo_2O_4$*

4.3.3.6 Selectivity Profile

To conclude on the selectivity profile of MIP_TF2/$NiCo_2O_4$, the sensor was subjected to similar concentrations of CAF and TAN using CV. The responses are depicted in the form of a bar plot in Fig. 4.16. From the graphical analysis it is delineated that MIP_TF2/$NiCo_2O_4$ shows the maximum affinity towards TF. Thus, the selective nature of MIP_TF2/$NiCo_2O_4$ can be concluded.

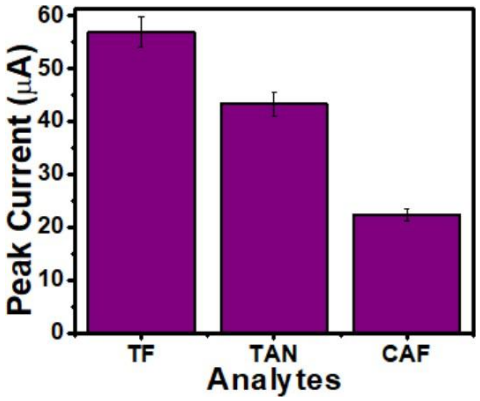

Fig. 4.16: *Selectivity Profile*

4.3.3.7 Comparative study of the fabricated electrode Vs. previous methods

The limited literature survey shows a wide linear range of operation of MIP_TF2/$NiCo_2O_4$ as compared to previously reported techniques. The LOD calculated using 3σ /m in this work is found to be significantly better with respect to literature [25] and [39]. As compared to the literature [17], MIP_TF2/$NiCo_2O_4$ shows improved repeatability as well as reproducibility. The aspect of stability was not previously addressed in the previous reports. Further, the use of $NiCo_2O_4$ Nps enhanced the current sensitivity of the electrode significantly. Table 4.2 below shows a comparative discussion of the different methodologies employed previously for the detection of TF.

Table 4.2: *Comparative literature study of different techniques used in the detection of TF*

Sl.	Methodology	Linear Range (µM)	LOD (µM)	Ref.
1.	CV	20-100	14	25
2.	HPLC	0.88-2.66	0.02	38
3..	S. phase extraction-HPLC	-	20-25	39
4.	**CV and DPV**	**80-1000**	**13.89**	**This work**

4.3.4 Analysis of black tea samples using Partial Least Square Regression (PLSR) Analysis

The tea liquor was prepared in a similar procedure as [17]. To study the practicability of the fabricated electrode, the PLSR model with a leave–one–out cross-validation (LOOCV) approach has been used. Ten repetitive DPV responses from six black tea samples were recorded. The generated matrix of the DPV responses as well as the HPLC values were divided into training and test subparts in the ratio of 80:20. It was observed that RMSEC value 0.088 was lowest for the two LVs. From Table 4.3, it is concluded that the average prediction accuracy was calculated to be 92.6%. A graphical comparison has been drawn in Fig. 4.17, which gives a clear impression of the predictability of the MIP_TF2/$NiCo_2O_4$.

Table 4.3: *Actual and predicted TF content from PLSR model*

Sample No	HPLC value (mg/ml)	Predicted TF (mg/ml) PLSR	Prediction Accuracy (%)
S1	0.90	1.00	90.00
S2	0.90	0.93	97.00
S3	1.10	1.06	96.00
S4	1.00	1.07	93.00
S5	1.20	1.14	94.00
S6	1.40	1.26	86.00
Average Prediction Accuracy			**92.6**

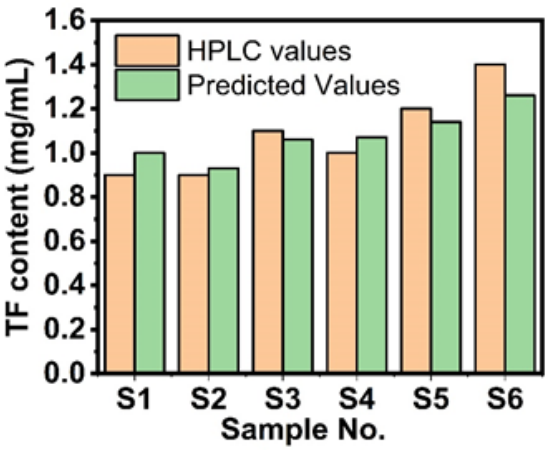

Fig. 4.17: *Bar plot showing TF content in black tea samples compared to standard HPLC values*

4.3.5 Summary

A MIP_TF2/$NiCo_2O_4$ electrode has been fabricated to comment on its practicability for the detection of TF in different black tea samples. Experimentally, it has been delineated that under optimized environmental conditions, MIP_TF2/$NiCo_2O_4$ exhibits a linear operating range of 80 to 1000 µM. This electrode shows satisfactory repeatability with an RSD value of 0.71%. To comment on the predictability of the fabricated electrode, it shows an average prediction accuracy of 92.6% when tested on the real tea samples. Thus, it can be established to be a novel, cost-effective, reusable, and highly selective MIP modified electrochemical sensor that can be instrumental in the quantification of TF in the tea industries.

4.4 Conclusion

In this treatise, two different sensors were fabricated for the quick and responsive detection of TF in black tea samples. In the first investigation, voltammetric responses in real black tea samples were thoroughly investigated. The fabricated electrode was successfully able to differentiate among the different samples based on their TF content using PCA. However, low molecular recognition ability led to the fabrication of a modified MIP based electrode. the MIP_TF2/NiCo2O4 electrode shows an LOD of 13.89 µM. This electrode shows satisfactory repeatability with an RSD value of 0.71%. To comment on the predictability of the fabricated electrode, it shows an average prediction accuracy of 92.6% when tested on the real tea samples. Thus, it can be established to be a novel, cost-effective, reusable, and highly selective MIP modified electrochemical sensor that can be instrumental in the quantification of TF in the tea industries.

This work has been published as cited *"Moulick M, Nag S, Das D, Hazarika AK, Sabhapondit S, Roy RB. A Molecular Imprinted Bi-polymer graphite electrode decorated with NiCo2O4 nano-cubes for rapid detection of theaflavin in black tea. IEEE Sensors Journal. 2025 Apr 3."*

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CHAPTER 5

Towards the development of an electronic tongue for the quality profiling of black tea

This chapter gives us an insight into developing an electronic tongue based on MIP technology for the overall quality profiling of different varieties of black tea. The various samples have been discriminated by the use of important biomarkers like theaflavin, theophylline and tannic acid.

Publication Outcomes

- Moulick M, Das D, Nag S, Paul A, Das DK, Roy RB. Simultaneous Detection of Epicatechin and Theophylline in Black Tea with Nano Cupric Oxide Infused Graphite Electrode. Nano LIFE. 2025 Feb 5;15(01) :2450009.
- Moulick M, Chowdhury S, Das M, Saha S, Bandyopadhyay D, Nag S, Roy RB. Development of an Electronic Tongue with MIP sensors for black tea quality evaluation. In 2024 IEEE Calcutta Conference (CALCON) 2024 Dec 14 (pp. 1-4). IEEE.
- Moulick M, Chowdhury S, Das M, Saha S, Naskar J, Nag S, Modak A, Roy RB. A customized electronic tongue by developing an array of molecular imprinted polymer based voltammetric electrodes for tea quality evaluation. IEEE Sensors Journal. 2025 Dec 8;26(2):1562-8.

CHAPTER 5

Towards the development of an electronic tongue for the quality profiling of black tea

5.1 Introduction

In the previous sections of this thesis, detailed discussions on the fabrication of dedicated sensors for the detection of the important biomarkers in black tea, like tannic acid, theophylline and total theaflavins have been presented. In this chapter, an approach towards the development of an e-tongue based on MIP sensors has been illustrated for the quality profiling of different black teas. Before taking an insight into the e-tongue development, an attempt was made to develop a single CuO modified graphite electrode that would be able to detect two different molecules i.e. epicatechin (EC) and theophylline (TH) from the same tea sample under the same operating conditions. EC ($C_{15}H_{14}O_6$) has strong anti-oxidant, anti-ageing, anti-allergic and anti-viral properties that contribute toward the quality of tea [1]-[2]; however, excess consumption of EC may lead to liver diseases [3]. TH, a methyl-xanthine derivative, is used in the treatment of chronic obstructive pulmonary disease caused by reversible airflow obstruction [4]. Quantitative detection of EC and TH present in black tea as taste-determining constituents is extremely important as a greater level of ingestion may result in human toxicity. Traditional methods for the individual detection of EC and TH include Raman spectroscopy [5], liquid chromatography (LC), chemiluminescence [6]-[7], electrophoresis [8] colorimetric aptasensor [9] and liquid chromatography-mass spectroscopy [10]. These methods require prior treatment of the sample, high-end instruments and a high degree of professional expertise. Electrochemical sensing of multiple molecules, dopamine and epinephrine using a single sensor was reported in [11]. In this chapter there are three sections. In the first section, a detailed description on the fabrication of a single electrode for the simultaneous detection of EC and TH from the same tea samples have been discussed. Secondly, an electronic tongue based on an array of MIP sensors was developed to record the signatures of the molecules TF, TAN and TH from a single tea sample. The third section of this chapter, deals with the employment of the aforesaid MIP based e-tongue in conjunction with a switching circuit, to detect the overall quality of five different samples of black tea.

5.2 Nano Cupric Oxide infused graphite electrode for the simultaneous detection of EC and TH in black tea

A novel cupric oxide nanoparticles (CuO NPs) infused graphite electrode (CuO@GP) was synthesized for simultaneous detection of epicatechin (EC) and theophylline (TH) in black tea. The CuO NPs were synthesized via the sol–gel method and characterized using X-ray diffraction and a scanning electron microscope. The electrochemical sensor performance was studied using cyclic voltammetry and differential pulse voltammetry revealing the presence of TH and EC simultaneously in black tea. The electrode demonstrated a linear range of 50.0–100.0 μM for both analytes, with a room temperature limit of detection (4.0 μM for EC, 27.0 μM for TH). Under the optimal conditions, it showed repeatability (with a relative standard deviation (RSD): 1.61% EC, 0.96% TH), reproducibility (RSD: 1.07% EC, 1.14% TH) and stability (RSD: 2.16% EC, 2.15% TH). CuO@GP exhibited notable selectivity and promising results in tea samples, achieving recovery rates of 102–107% for EC and 101.7–106% for TH.

5.2.1 Experimental details

5.2.1.1 Chemical standards and reagents

Both EC and TH were obtained from Tokyo Chemical Industry, Japan, and paraffin oil, and graphite powder was obtained from Sigma Aldrich, India Ethanol, cetyloyridinium chloride ($C_{21}H_{38}NCl$), copper chloride ($CuCl_2$), sodium hydroxide (NaOH) were purchased from Merck & Co. All the chemicals were of premium quality (99.0% purity) and hence required no refinement.

5.2.1.2 Synthesis of CuO NPs

The CuO NPs were synthesized in the laboratory using the sol–gel method. About 10.0 g of copper chloride $CuCl_2$ was rapidly stirred in 500.0 mL of ethanol. To this solution, surfactant cetylpyridinium chloride ($C_{21}H_{38}NCl$) was added and stirred continuously for half an hour in 0.1 M NaOH solution and was added dropwise to make the pH of the solution 8.9. On leaving the above solution undisturbed for some time, a green colour precipitate was obtained. The obtained precipitate was further filtered and dried in the oven for an hour at 50°C. After calcination at a temperature of 400°C for almost 3 h, the black-colored CuO NPs were obtained.

5.2.1.3 Characterization details and equipment specifications

The powder X-ray diffraction (PXRD) patterns of CuO samples are studied using a Philips PW 1710 X-ray diffractometer. The diffractometer is operated at 40 kV and 40 mA with CuKα radiation (λ =1.5406 Å) in a scan mode from 10° to 75°. Surface morphology of the polymer material were examined using a ZEISS EVO 18 SEM operated at an acceleration voltage (15 kV). Potentiostat (PGSTAT101), a three-electrode system comprising a CuO@GP, an Ag/AgCl reference electrode and a Pt counter electrode was used for testing. The Nova Electrochemistry software was used for graphical representations of CV.

5.2.1.4 Preparation of CuO@GP electrode

The CuO NPs and pure graphite particles were taken in three various weight ratios 1:9, 2:8 and 3:7. About 0.30 g by weight of each of the material was blended into a fine paste adding three drops of paraffin oil. The obtained paste was then inserted into a capillary tube of inner radius 1 mm. Platinum wires were inserted to establish electrical contact.

5.2.2 Results and discussions

5.2.2.1 Study of XRD characteristics of CuO NPs

The XRD pattern of CuO NPs is shown in Fig. 5.1. The formation of CuO was confirmed, where diffraction peaks were obtained at 32.47°, 35.49°, 38.68°, 38.89°, 46.19°, 48.65°, 53.40°, 61.45°, 66.1°, 68°, 72.32° and 75.12°, respectively. They can be assigned to the (110), (111), (202), (020), (113), (311) and (222) planes and the structure of CuO is monoclinic. The diffractogram indicates there is no contamination in the sample as there is no other intensified peak. The crystalline size of the nanoparticles is calculated as 41.6 ± 7.70 nm using the Debye–Scherrer method [12].

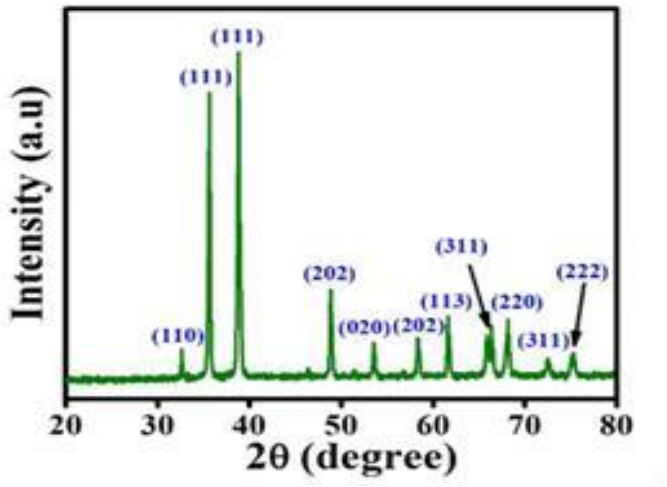

Fig. 5.1: *X-ray diffraction pattern of CuO nanoparticles*

5.2.2.2 SEM Analysis of CuO NPs and Pure Graphite

The morphological study of the surface of the prepared CuO nanoparticles and pure graphite was studied using SEM. The SEM image reveals that the prepared material contains nano-sized particles. CuO NPs and pure graphite are depicted in Figs. 5.2 (a) and 4(b). The particles size can be confirmed to be around 194 nm. [13].

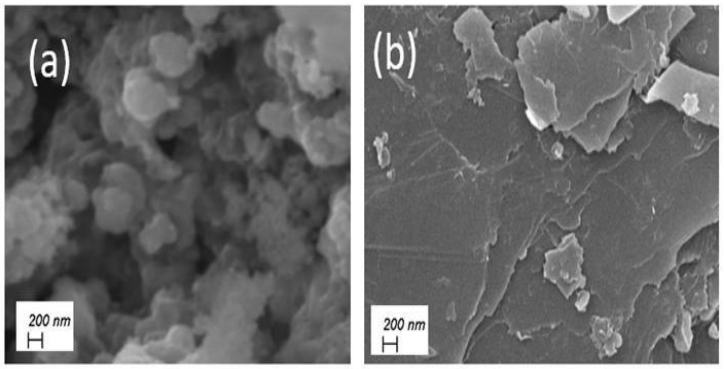

Fig. 5.2: *SEM image of (a) CuO nanoparticles and (b) pure graphite.*

5.2.2.3 FTIR analysis of CuO NPs

The FTIR spectra of the synthesized CuO NPs, shown in Fig.6.2, show several absorption bands approximately around 535 cm^{-1} and 584.3 cm^{-1}, which could be due to the characteristic stretching vibrations of Cu (II)–O bonds [14]. A broad absorption peak is observed at 3446.81 cm^{-1}, which is characteristic of OH bond as CuO NPs absorb moisture present because of the high surface-to-volume ratio [15].

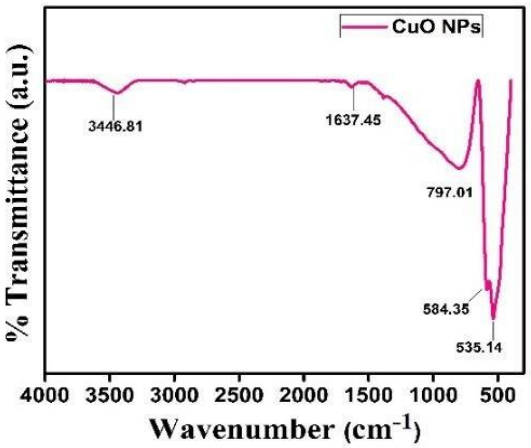

Fig. 5.3: *FTIR spectra of CuO NPs.*

5.2.2.4 Comparative Performance of CuO@GP with Bare GP Electrode

The electrochemical behaviour of the CuO@GP was thoroughly investigated and compared with the bare GP electrode. The CV responses were recorded and analysed. From Fig. 5.4 (a), it is revealed that the voltammograms recorded using the CuO@GP electrode show oxidation peaks at 0.4V and 1.2V for EC and TH respectively. Cupric oxide nanoparticles enable simultaneous detection of epicatechin and theophylline due to their specific attributes. The high surface area of these nanoparticles, coupled with their excellent conductivity and electrocatalytic activity, enhances the sensitivity of the detection process. This heightened sensitivity allows for the effective and concurrent identification of both EC and TH in a given sample. Essentially, the unique properties of cupric oxide nanoparticles make them well-suited for triggering simultaneous detection, offering precision in analytical applications. The oxidation peak currents recorded were 76.67 µA for EC and 92.03 µA for TH, which is almost 2.08 times for EC and 1.42 times for TH compared to the Bare GP electrode. This rise in the individual oxidation peaks for the two analytes can be due to the faster chemical kinetics occurring on the surface of CuO@GP due to the modification of the bare graphite electrode with CuO nps. The ratio of the modifier to graphite was experimentally decided. Initially, three such electrodes were prepared to bear modifier-to-graphite ratios as 1:9, 2:8 and 3:7. As inferred from Fig. 5.4 (b), the maximum peak currents for EC and TH were recorded when the modifier-to-graphite ratio was 1:9, hence considered as the optimum ratio throughout the experiment.

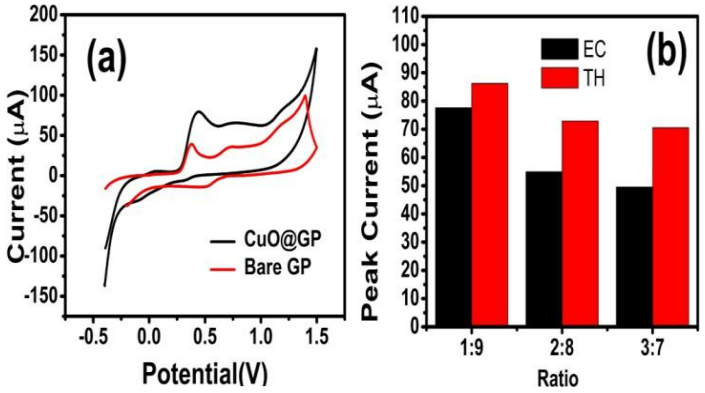

Fig. 5.4: *a) Comparative study of CuO@GP and Bare GP electrodes b) Double bar plot of modifier to graphite ratio*

From Fig. 5.5 (a), it is evident that the best observation is recorded for PBS. Further, we varied the pH of PBS and it was seen that in the case of PBS 2, we get a very sharp oxidation peak current for EC, however the oxidation peak for TH is completely suppressed, thus PBS 7 is optimized for further experimentation Fig. 5.5 (b).As shown in Fig. 5.5 (c), no oxidation peak current is observed at 0.4 V and 1.2 V in the absence of the analytes EC and TH. In addition to EC and TH in equal ratio, there can be seen a significant appearance of the oxidation peak currents for EC and TH respectively at 0.4V and 1.2V.

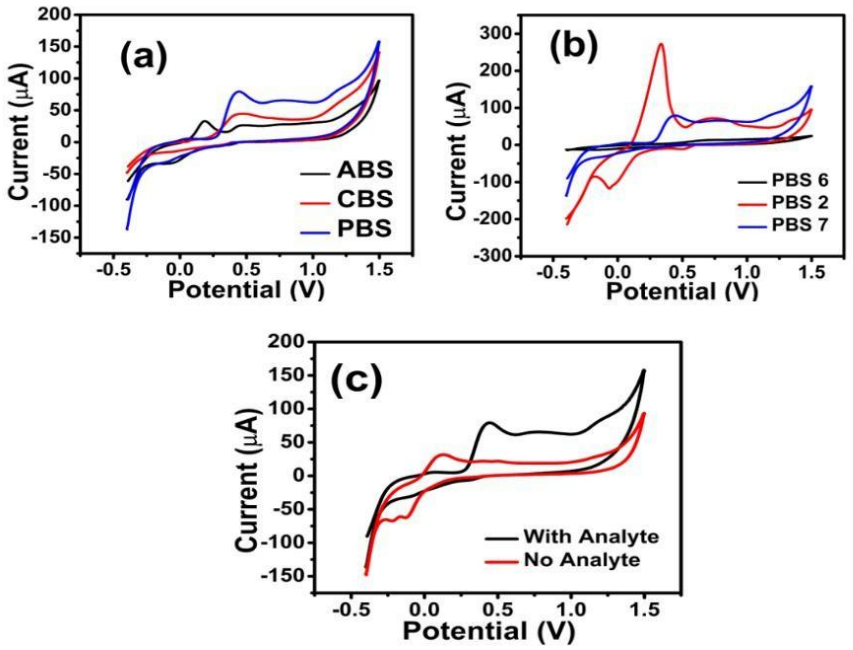

Fig. 5.5: *a) CV responses of CuO@GP in different buffers b) pH variation c) CV response of CuO@GP in PBS 7 with and without EC and TH respectively*

5.2.2.5 Effect of Scan Rate Variation

Applying the CV method, the influence of different scan rates on the electrochemical detection of EC and TH was studied across a potential window of -0.5 to 1.5V. The scan rate was linearly varied from 5 mV/s to 200 mV/s. The variation in scan rate and its influence on the peak current is illustrated in Fig. 5.6 a. It was inferred that on increasing the scan rate, the anodic peak current also increased. This observation suggests that the electrolytic oxidation of EC and TH on the surface of the CuO@GP electrode is a surface-controlled phenomenon. The plot between oxidation peak current vs. scan rate is shown in Fig. 5.6 b.

The linear regression equations of the individual analytes are as follows

$$I_{th} = 1.3295v + 2.982\ ;\quad R^2 = 0.97 \tag{1}$$

$$I_{EC} = 0.6038v + 16.297\ ; R^2 = 0.90 \tag{2}$$

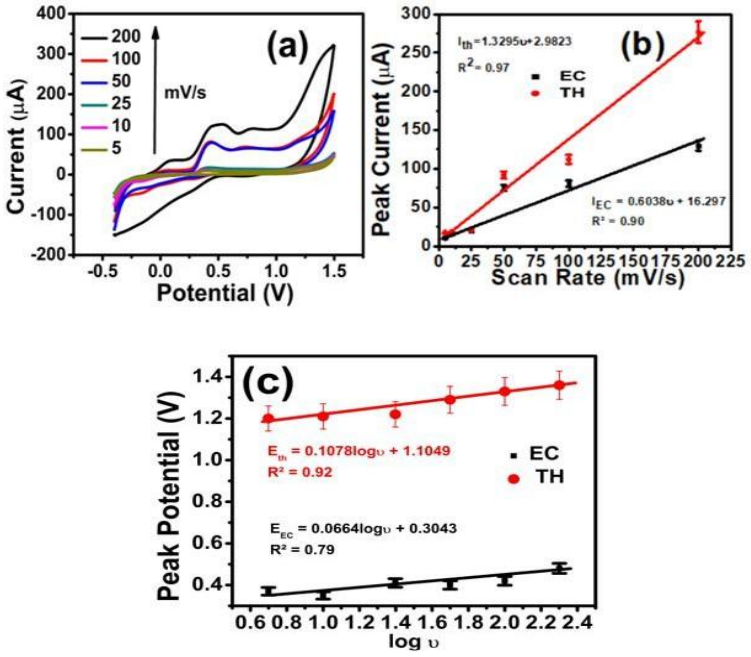

Fig. 5.6: *a) CV responses of variation of peak current with change in scan rate b) Linear plot of peak current vs scan rate*

The quantity of electrons transferred during this oxidation process is evaluated using the mathematical relationship (3).

$$I_p = \frac{nFQv}{4RT} \tag{3}$$

Where n, F, Q, υ, R and T denote the following quantities.

n=number of electrons transferred, F = Faraday's constant, Q=charge consumed v=scan rate, R =the universal gas constant and T= standard room temperature respectively. The value of n is calculated as 0.90 for EC and n= 1.04 for TH respectively, which are approximately unity. The charge transfer co-efficient for TH (α_{th}) and EC (α_{th}) is calculated using the slope of the calibration curve equal to 2.3RT/(1-α)nF and found to be 0.47 and 0.90 respectively.

5.2.2.6 Concentration Variation

DPV measurements (scan rate of 0.05 mV, modulation time of 0.05 s, modulation amplitude of 0.05 V, and a step value of 0.025 V) were recorded to study the effect of different concentrations of the testing solution on the oxidation peak current of EC and TH, we have varied the concentration range from 50.0 µM to 100.0 µM. It is observed that the oxidation peak current for both the analytes EC and TH increases linearly as shown in Fig. 5.7 a and b. Two linear regression equations are obtained as follows

$$I_{EC=}0.512C_{EC} - 13.17;\ R^2 = 0.94 \quad (4)$$

$$I_{th} = 0.321C_{th} + 15.49;\ R^2 = 0.92 \quad (5)$$

The limit of detection (LOD) is calculated using the formula $\mathbf{LOD = 3\sigma\ /m}$, where $\boldsymbol{\sigma}$ =standard deviation of the currents recorded at lowest obtained concentration and m= slope of the regression equation. Using the above formula, the value of LODs of EC and TH is obtained as 4.0 µM and 27.0 µM. respectively. The sensitivity of the electrode as calculated from the calibration curve, are 0.321 for TH and 0.512 for EC.

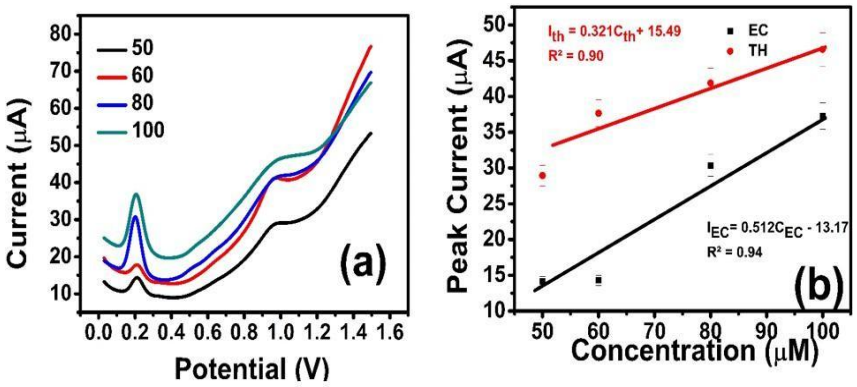

Fig. 5.7: *a) DPV plots showing the change in peak current with an increase in concentration b) linear plot of peak current vs. concentration change*

5.2.2.7. Study of Repeatability, Reproducibility and Stability

The analytical characteristics – repeatability, reproducibility and stability were studied using DPV responses. The CuO@GP showed RSD of 1.61% and RSD of 0.96% for EC and TH respectively for three consecutive repetitions as shown in Fig. 5.8 a. The sensor reported RSD 1.07% and 1.14% for EC and TH when voltammetric responses were recorded for three different electrodes as in Fig. 5.8 b. The same electrode was used at room temperature at an interval of 15 days for 45 days that gave RSD values of 2.16% and 2.15% respectively for EC and TH as reported in Fig. 5.8 c. It shows a very insignificant drop in the oxidation peak current on day 2 of the measurement, however, on the 3rd day there is a little fall of peak current which may be due to the ageing effect of the electrode.

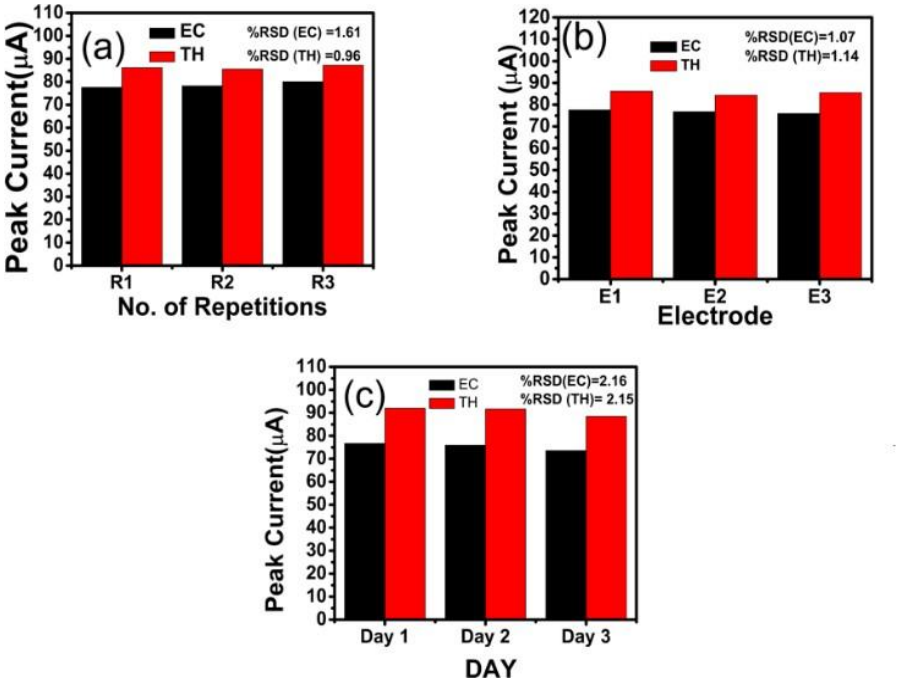

Fig. 5.8: *a) Study of Repeatability b) Reproducibility c) Stability*

5.2.2.8 Interference Study

The CuO@GP electrode was subjected to various interferents like Na+ and Cl- to study their effect on the electrochemical response of the sensor as shown in Fig. 5.9. The relative signal change was within a tolerance limit of ±5%. This behaviour shows the highly selective performance of the fabricated electrode.

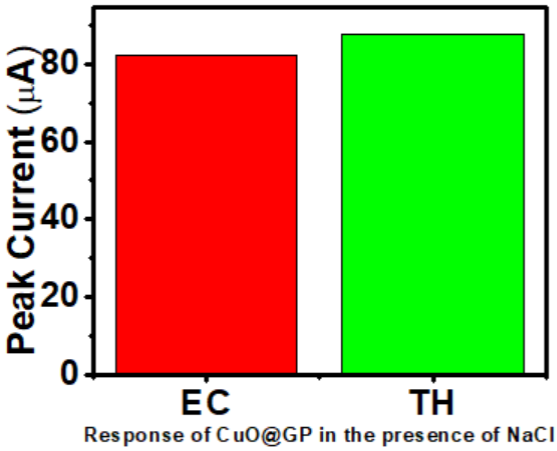

Fig. 5.9: *Response of CuO@GP in presence of NaCl.*

5.2.2.9. Real Sample Investigation

For the detection of EC and TH in the tea samples with the developed sensor, the electrode was dipped in a tea sample. The tea sample was procured from the local market. 200.0 mg of tea leaves were brewed in boiling water, and from it, 20.0 ml of the tea liquor was then measured by the standard addition technique. A DPV analysis was done on the tea sample by spiking it with various concentrations of EC and TH solutions simultaneously. The results are highlighted in the Table 1 below that indicate recovery rates of 102% and 106% for EC and TH respectively when spiked with 50.0 µM of the solutions, further, the DPV analysis shows a recovery rate of 107% and 101.7% when spiked with 80 µM of the solutions.

Table 5.1: *Determination of EC and TH*

Sample Name	Spiking (µM)	Detected (µM)		Recovery (%) [a]		± RSD (%) [b] N=3	
		TH	EC	EC	TH	EC	TH
Tea sample non-spiked	0	-	-	-	-	-	-
Tea Sample Spiked	50	51.07	53.14	102	106 .0	1.61	0.64

Table 5.2: *Comparative Study of Individual Sensors for the Detection of EC and TH with CuO@GP.*

Electrode	Linear Range(µM)	LOD(µM)	Technique	Analyte	Ref
MIP-Q@G	1 to 100µM 100 to 500 µM	0.33	CV and DPV	EC	[16]
Sm2O3@GP	10 to 1000µM	5.69	CV and DPV	TH	[17]
Pt	50 to 300 µM	1.8	DPV	EC	[18]
CuO@GP	50.0 µM to 100.0 µM.	4.0 for EC 27.0 for TH	CV and DPV	EC and TH	This Work

5.2.3 Summary

In this approach a quick and responsive detection of multiple molecules in black simultaneously using a single CuO@GP electrode has been discussed. Further, it has been observed that in comparison to individual sensors used to determine EC and TH, the developed sensor CuO@GP provides a higher anodic current for both analytes. This increase in the sensitivity of the electrode is due to the presence of the CuO nps which increases the surface area of adsorption of the analytes on the electrode surface. The developed CuO@GP electrode is repeatable in nature and also showed stability over a period of 45 days. The deployment of the electrode in real tea samples show excellent recovery rates. As an extension of this work, MIP based sensors for the detection of EC and TH were attempted, however, the complex and comparatively larger structure of EC in comparison to TH, led to the adsorption of all the analyte molecules in the sample into the cavities created for EC. Thus, it can be seen that during the experimentation with the MIP-based sensor, the size selectivity factor of EC suppressed that of TH.

This work has been published as cited *"Moulick M, Das D, Nag S, Paul A, Das DK, Roy RB. Simultaneous Detection of Epicatechin and Theophylline in Black Tea with Nano Cupric Oxide Infused Graphite Electrode. Nano LIFE. 2025 Feb 5;15(01):2450009."*

5.3 Fabrication of an e-tongue based on an array of MIP sensors directed towards black tea quality evaluation

An electronic tongue (E-Tongue), based on voltammetric principle, coupled with a switching circuit has been proposed to detect the overall quality of different samples of black tea. An E- Tongue with an array of three working electrodes has been designed where the electrodes are fabricated with the highly selective Molecular Imprinted Polymer (MIP) technology. The MIP sensors have been developed for the biochemical constituents tannic acid, theaflavin and theophylline. Developed E-Tongue shows the ability to measure the signatures of these three molecules simultaneously from the test tea sample. Hence can detect the tea quality more accurately based on chemical constituents present in the sample.

5.3.1 Experimental Section

5.3.1.1 Reagents and Apparatus

Pure graphite (98%) purity was obtained from Loba Chemie Pvt. Ltd. Ethylene glycol dimethyl acrylate (EGDMA), benzoyl peroxide and TH were obtained from Sigma Aldrich.The monomers Acrylic Acid (AA)Methyl methacrylic acid (MAA) were obtained from Sigma Aldrich and E-Merck respectively. Tannic acid (TAN) was purchased from Nice Chemicals Pvt. TF extract was obtained from Tocklai Tea Research Institute.

5.3.1.2 Synthesis of MIP material

For the synthesis of the MIP material for electrode fabrication, the following steps are followed as shown in Fig. 5.10. The same protocol was followed for preparation of the three working electrodes – MIP_TF, MIP_TAN and MIP_TH.

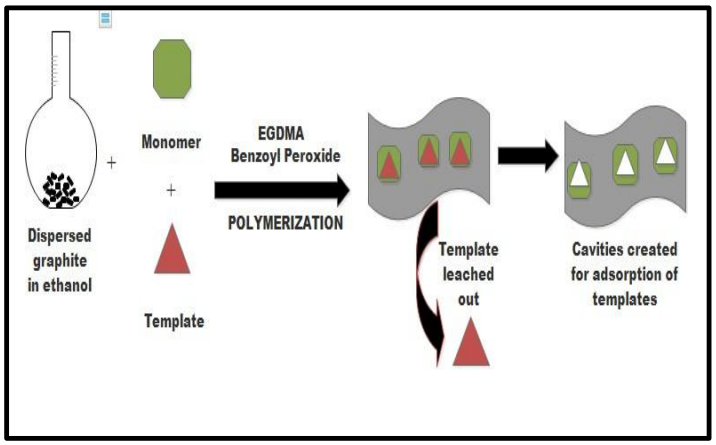

Fig. 5.10: *Flowchart for MIP material preparation*

5.3.1.3 E-Tongue Set Up

A setup featuring an electronic tongue has been developed, employing a configuration that includes three MIP electrodes, an Ag/AgCl electrode used as a reference and a Pt counter electrode to analyze three analytes in a tea sample, as shown in Fig. 5.11a. The functional block diagram of the E-tongue has been illustrated in Fig. 5.11b. The system comprises five main modules: 1) Power Supply, 2) Switching Circuit, 3) Sensor Array Cell, 4) Autolab potentiostat, and 5) Recorder. The components used in the switching circuit are 1) Arduino Board - The microcontroller board which has been used in the circuit to burn the code for switching circuit. The board also has a power supply which has been used as the power supply of the entire setup.2) 4 channel relay - The relay board has been used to observe the switching process to transfer the signal to the working electrode which is connected to the relay output through connecting wires. 3) Jumper wires - The Arduino and the relay are connected to each other via jumper wires.

After completing one set of measurement with one MIP working electrode, the switching circuit enables to select next MIP electrode of the array automatically keeping counter and reference electrodes unaltered. Keeping the scan rate fixed for all three sensor responses, a uniform data size has been obtained for all three sensors which can be used for further analysis. For the entire experimentation, the sample condition kept constant. The responses are obtained with the help of the data acquisition unit of NOVA Autolab.

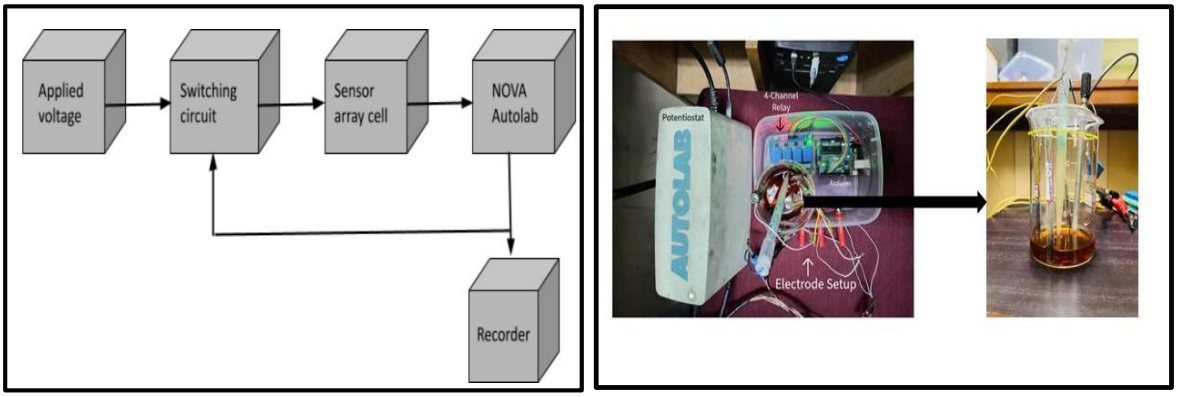

Fig. 5.11: *(a) Functional Block Diagram of E-Tongue Setup developed for simultaneous detection of taste affecting analytes in black tea (b) Top & Side View of the E-tongue system used in experimentation*

5.3.1.4 Data collection using E-tongue

To prepare the tea liquor, around 200 mg of tea sample was weighted and brewed in a flask containing approximately 50 mL of boiling water. Five minutes later, the liquor was by filtered. The tea liquor was allowed to cool to room temperature, thereafter 20 mL of the liquor was used for testing. The E-tongue, consisting of an array of three working electrodes were subjected to this sample for the detection of the analytes-TAN, TF and TH simultaneously The DPV response of the E-tongue for a particular tea sample is illustrated in Fig. 5.12 (a). Ten repetitive electrochemical responses were recorded from the tea sample at a constant scan rate of 50mV/s. The time duration of each cycle of response is 180 seconds.

5.3.2 Results and Discussions

Data Analysis using PCA

Ten consecutive DPV responses were recorded from the tea sample. For each repetition, 60 data points were obtained. The obtained data matrix [60x10x3] was subjected to PCA clustering which shows a satisfactory segregation among the three different analytes in a black tea sample as shown in Fig. 5.12(b). The PCA score plot shows 97.105% and 2.14 % of total variances. From the PCA analysis, the separability index (SI) is obtained to be 32.4305. This SI is a quantitative measurement of the three different analytes present in the tea sample among others.

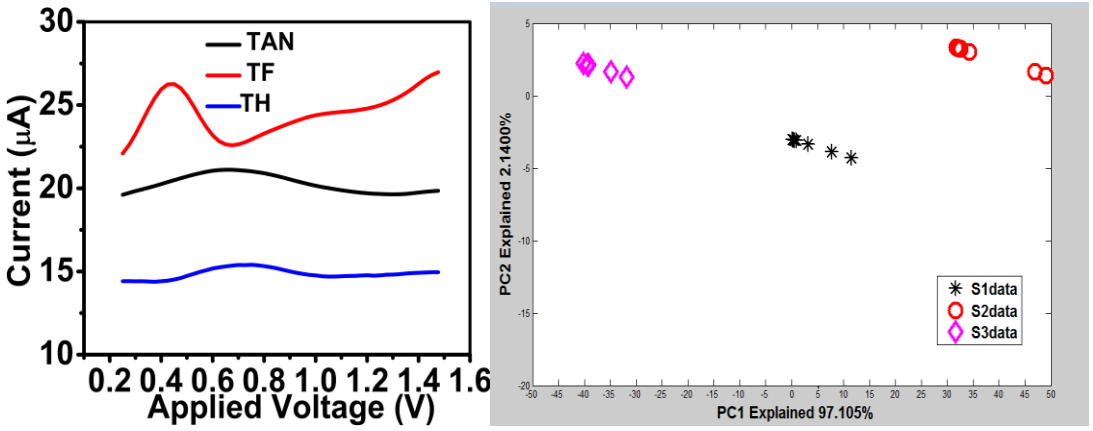

Fig. 5.12: *(a) DPV responses of the analytes- TAN, TF and TH from a single black tea sample (b) PCA Plot showing segregation among three analytes in the tea sample*

5.3.3 Summary

A simple E-tongue with an array of highly selective MIP sensors along with a switching circuit has been developed in this work, which enables the detection of different taste-affecting analytes from a real sample of black tea, thus enabling the overall quality determination of black tea and it has the potential to be used as a taste-assessment device in the tea-industry. The results from the PCA plot show a satisfactory segregation of the three different analytes in black tea.

This work has been published as cited *"Moulick M, Chowdhury S, Das M, Saha S, Bandyopadhyay D, Nag S, Roy RB. Development of an Electronic Tongue with MIP sensors for black tea quality evaluation. In2024 IEEE Calcutta Conference (CALCON) 2024 Dec 14 (pp. 1-4). IEEE."*

5.4 A customized electronic tongue by developing an array of molecular imprinted polymer-based voltammetric electrodes for tea quality evaluation

A well-developed voltammetric electronic tongue (e-tongue) consisting of an array of molecular imprinted polymer (MIP) electrodes has been designed for the detection of theophylline (TH), tannic acid (TAN) and total theaflavins (TF) in real samples of black tea. These compounds are responsible for having a high impact on the taste of tea in terms of astringency, thus attributing to the taste of tea infusion. The developed e-tongue has been found to detect the signatures of these analytes from the different tea samples under the same state of conditions. Thus, this e-tongue in conjunction with a switching circuit, has been proposed to detect the overall qualitative nature of black tea. The differential pulse voltammograms (DPV) responses were obtained to identify the performance on imbibing the MIP electrodes in the black tea samples. The obtained dataset was subject to the gradient boosting method. The prediction model was designed based on the responses of the e-tongue and yielded a mean- squared error (MSE) of 0.00003 and a coefficient of determination (R^2) of 0.967 for the fused data responses. From the explained variance score and R-square value, it is clear that the overall quality of tea has been predicted better for the fused dataset than considering the individual dataset from each sensor. On application of t-SNE (t-Distributed Stochastic Neighbour Embedding) on the fused data set, successful discrimination among the different black tea samples was obtained. The results point towards the possibility of an e-tongue based on an array of MIP electrodes to detect the quality of black tea liquor.

5.4.1 Materials and methodology

5.4.1.1 Chemical reagents and standards

Pure graphite (98%) purity was obtained from Loba Chemie Pvt. Ltd. Ethylene glycol dimethyl acrylate (EGDMA), benzoyl peroxide and TH were obtained from Sigma Aldrich. The monomers Acrylic Acid (AA), Methyl methacrylic acid (MAA) were obtained from Sigma Aldrich and E-Merck, respectively. TAN was purchased from Nice Chemicals Pvt.TF extract was obtained from Tocklai Tea Research Institute, Jorhat, Assam. In the process of developing and testing the electrodes, ethanol and paraffin oil from Merck & Co. were employed as binders and solvents, respectively. During experiments, distilled water (resistance(R) approximately 18 MΩ) of the Millipore water purification system was used for washing electrode materials.

5.4.1.2 Equipment specifications

PGSTAT101, a potentiostat system, employs a three-electrode configuration. This setup includes a platinum counter electrode (Pt), MIP_TAN, MIP_TF and MIP_TH as the working electrodes in the sensor array and an Ag/AgCl reference electrode. HPLC was used for precise quantitative measurement of TAN, TF and TH in real tea samples.

5.4.1.3 Synthesis of MIP electrodes

The synthesis of the MIP materials is similar to that followed in [19], [20] and [21].

5.4.1.4 E-tongue setup

An e-tongue set up has been designed using an array of three MIP electrodes selective to TAN, TH and TF analytes, an Ag/AgCl counter electrode and a Pt reference electrode. The description of the e-tongue set up has already been elaborated in section 5.3.1.3.

The complete circuit consists of two components (i) relay module, and (ii) Timing signal generator.

- ***Relay module***

  Each relay in the relay module has its isolated driver made of an opto coupler (PC817D) to provide isolation between controller and driver, a pull-down NPN transistor (2N3904) to give a ground path for the coil of relay, depending on the timing signal, and a PN junction diode (1N4148) connected in reverse bias with the coil. The diode helps to minimize the knocking current generated by the coil which may damage the transistor. The common terminal of the three relays is connected to the three MIP electrodes, respectively as shown in Fig.2. All the NO terminals of the relay are shorted together and connected to the working electrode port of the potentiostat device, and all NC terminals of relays are kept floating.

- ***Timing signal generator***

  An ATmega328p-based microcontroller development board (Arduino UNO) has been utilized as a timing signal generator. The microcontroller was programmed to trigger and hold each relay repetitively, with a 180-second time cycle. Relay1, Relay2 and Relay3 are connected with the D3, D4, and D5 pins of Arduino UNO, respectively.

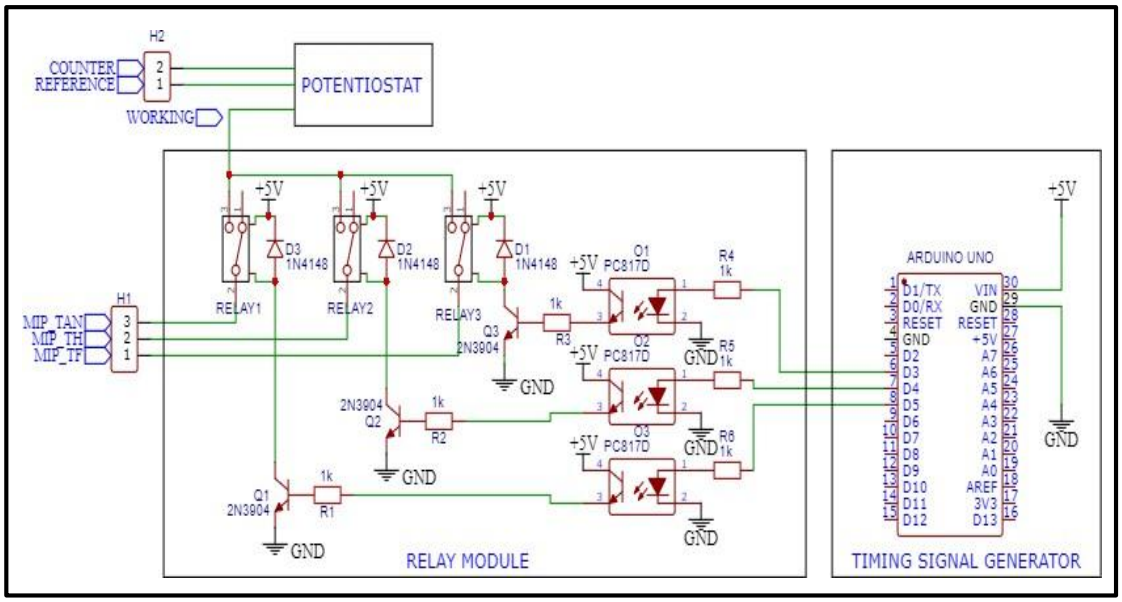

Fig. 5.13: *Circuit diagram*

5.4.1.5. Response recordings from the e-tongue

The tea liquor was prepared as in [21] and was allowed to cool to room temperature. Typically, 20 mL of the tea infusion was used for testing. The E-tongue was subjected to this sample for the detection of the analytes-TAN, TF and TH. After completing one set of measurements with the first MIP electrode, the switching circuit automatically shifts control to the next WE, keeping the RE and CE constant. after a period of 180 seconds. Ten repetitive electrochemical responses were recorded from each of the tea sample at a constant scan rate of 50mV/s. The duration of each cycle of DPV response is 180 seconds. As the scan rate fixed for the entire experimentation, a uniform data size has been obtained for all three sensors for each of the tea samples and used for further analysis.

5.4.1.6 Correlation of DPV responses with standard HPLC values

Experiments were conducted on five different samples of black tea. The standard values of TAN, TF and TH of each of the samples were obtained using HPLC method. The individual DPV responses obtained from the MIP electrodes were co-related with the HPLC data to estimate the sensors' accuracy. Further, it was observed that the co-relation of the HPLC data with the fused data responses yielded higher accuracy. Table 5.3 shows the HPLC values of the different analytes in the different samples of tea.

Table 5.3: *HPLC values of different analytes in the samples*

Sample	TAN (mg/mL)	TF (mg/mL)	TH (mg/mL)
S1	0.638	0.90	0.0027
S2	0.583	0.90	0.0011
S3	0.541	1.10	0.0010
S4	0.714	1.00	0.0004
S5	0.642	1.20	0.0026

5.4.2 Results and discussion

5.4.2.1 DPV responses obtained from the working electrodes

The fabricated e-tongue based on dedicated MIP sensors, was employed in a tea sample. It was able to distinctly detect the presence of the three analytes in the tea sample under the same experimental conditions. This e-tongue in conjunction with an effective switching circuit was able to transfer control from one WE to the next, keeping the counter and reference electrodes unaltered. The DPV responses for each working electrode from a single tea sample for the first cycle is shown below in Fig.5.14. To comment on the repetitive nature of the three working electrodes, ten repetitive DPV responses from each of the working electrode were recorded from a single tea sample at a constant scan rate of 50mV/s. Fig. 5.15 below shows a bar plot highlighting the ten repetitive cycles performed by each WE on the tea sample. All the three electrodes show satisfactory repeatability with RSD values 6.007%, 7.73 % and 3.86% for MIP_TAN, MIP_TF and MIP_TH respectively. Five different samples of tea were chosen for experimental purpose.

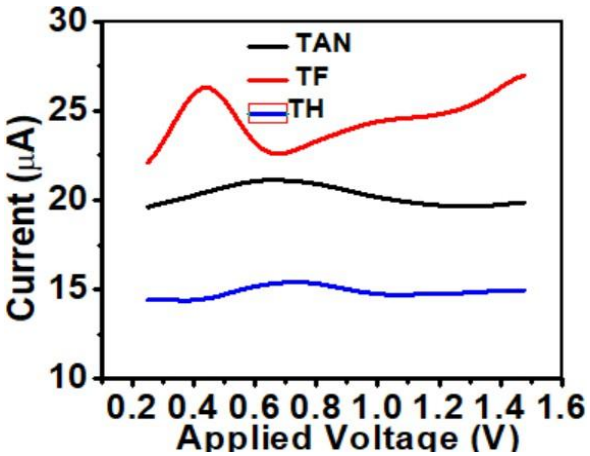

Fig. 5.14: *DPV responses of the three working electrodes obtained from the sample S1* [22]

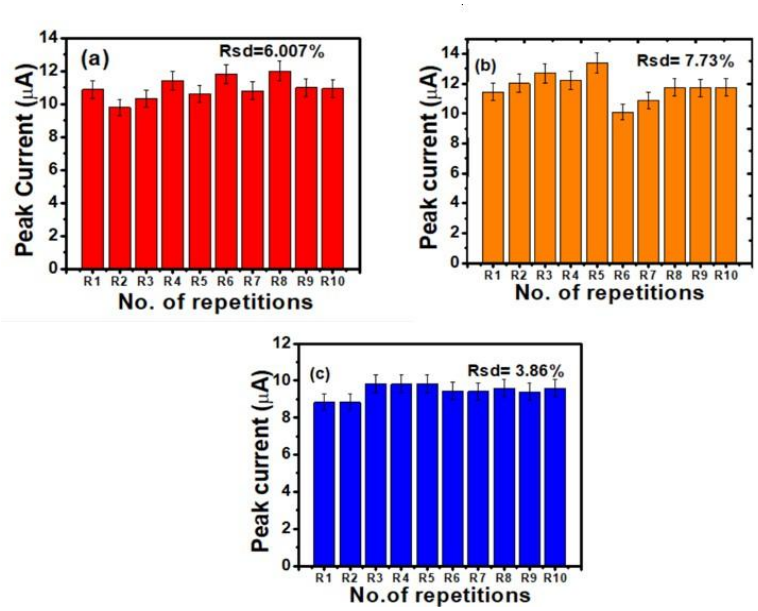

Fig. 5.15: *Bar plot showing the responses of each WE for ten consecutive DPV excitation.*

5.4.2.2 Clustering using t-Distributed Stochastic Neighbour Embedding (t-SNE)

t-SNE is a popular machine learning algorithm used to visualize high-dimensional data, introduced by Laurens van der Maaten and Geoffrey Hinton in 2008. Its main goal is to convert similarities between data points into probabilities and then create a low-dimensional map that represents these probabilities as accurately as possible. One of the most common applications of t-SNE is in the visualization of complex data sets. It is particularly useful for identifying patterns, clusters, and outliers in high-dimensional data such as those found in genomics, image recognition, and natural language processing. In this approach, the t-SNE algorithm was applied on the fused data set and it was successfully able to discriminate among the five different samples of black tea as shown in Fig. 5.16.

The input parameters for the successful clustering are:

Fused Data – 50x180

Total of Samples – 50 (5 Samples x Runs: 10)

No. of Features – 180 (TAN: 60+TF: 60+TH: 60) (Combination of TAN [TAN_0, TAN_1,….TAN_59], TF [TF_0, TF_1 … TF_59] and THEO [TH_0, TH_1, … TH_59])

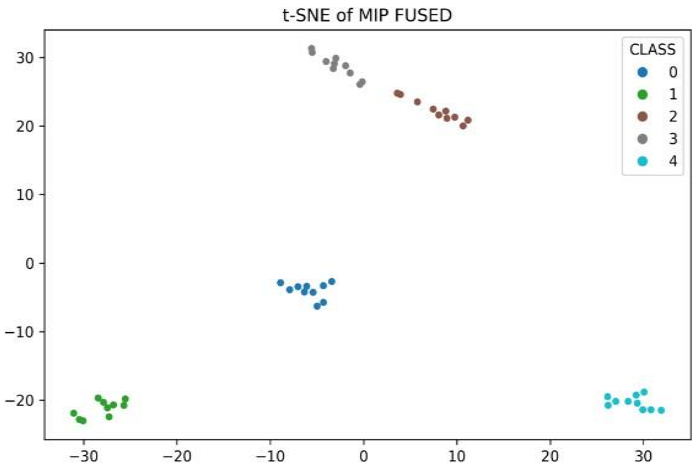

Fig. 5.16: *Discrimination among different black tea samples using t-SNE*

5.4.2.3 Gradient boost model

The Gradient Boost Regressor [23] is an ensemble boosting method that incrementally trains numerous models in a sequential and additive manner. To implement Gradient Boosting, the residual is identified and subsequently fit a new model to it. The new model is then integrated with the preceding model to initiate the next iteration. The approach of converging on the residues at each stage may be interpreted as a progression towards the negative gradient of the loss function. The generalization of the boosting process is facilitated by gradient descent. The default hyper parameters of gradient boost regressor function from the ensemble module of the ScikitLearn package has been considered for model training.

Procedure for individual and multiple input/output fusion process:

$X_{TAN}^{1}, X_{TAN}^{2}, X_{TAN}^{3}, \ldots, X_{TAN}^{64}$ are the input feature sets for TAN (X_{TAN})

$X_{TF}^{1}, X_{TF}^{2}, X_{TF}^{13}, \ldots, X_{TF}^{64}$ are the input feature sets for TF (X_{TF})

$X_{TH}^{1}, X_{TH}^{2}, X_{TH}^{3}, \ldots, X_{TH}^{64}$are the input feature sets for TH (X_{TH})

$$X_{TAN} \in \{X_{TAN}^{1}, X_{TAN}^{2}, X_{TAN}^{3}, \ldots, X_{TAN}^{64}\}$$

$$X_{TF} \in \{X_{TF}^{1}, X_{TF}^{2}, X_{TF}^{3}, \ldots, X_{TF}^{64}\}$$

$$X_{TH} \in \{X_{TH}^{1}, X_{TH}^{2}, X_{TH}^{3}, \ldots, X_{TH}^{64}\}$$

$y_{TAN}^{HPLC}, y_{TF}^{HPLC}, y_{TH}^{HPLC}$ are the HPLC values of TAN, TF and TH respectively.

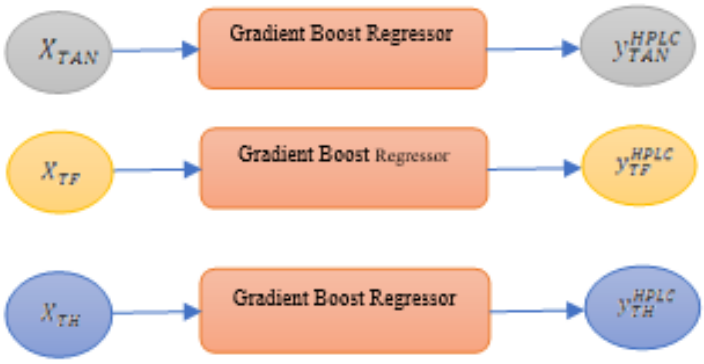

Fig. 5.17: *(a) Model for individual TAN, TF and TH responses*

The model for individual TAN, TF and TH in Fig. 5.17a can be represented as:

$$y_{TAN}^{HPLC} = f_{TAN}(X_{TAN})$$
$$y_{TF}^{HPLC} = f_{TF}(X_{TF})$$
$$y_{TH}^{HPLC} = f_{TH}(X_{TH})$$

here, f_{TAN}, f_{TF}, and f_{TH} are the regressor function for individual TAN, TF and TH respectively.

There are three different models created individually to predict the content of TAN, TF and TH present in the tea samples. As the data generated from different chemical sensors are for the same sample, therefore, the features from different chemical sensors are also inter-related. Hence, it will be more efficient to have a joint prediction so that the overall quality of tea sample can be established in a better way.

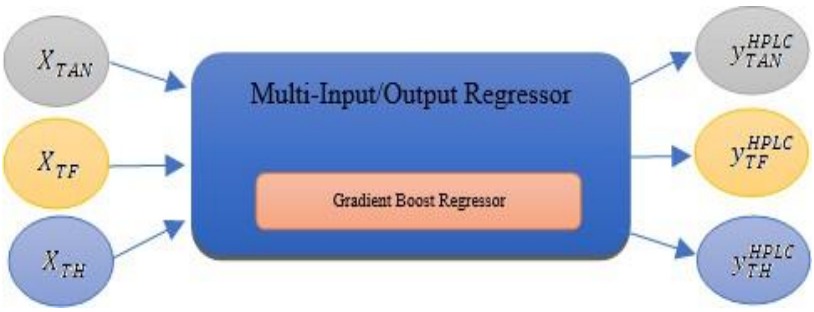

Fig. 5.17 b*. Model for fused data response*

The model for the fused dataset of TAN, TF and TH in Fig. 7b can be represented as:

$$y_{TAN}^{HPLC}, y_{TF}^{HPLC}, y_{TH}^{HPLC} = f_{fused}(X_{TAN}, X_{TF}, X_{TH})$$

Where f_{fused} is the , regressor function for the multi-input/output regressor.

The average values of performance metrics obtained from the individual and fused model are further considered to identify the overall quality of the tea sample is tabulated below in Table 5.4.

Table 5.4: *Individual sensor Vs Array of sensors performance*

Chemical	Individual						Fused					
	Mean Absolute Error (MAE) *10-3	Median Absolute Error (MedAE) *10-3	Mean Squared Error (MSE) *10-3	Mean Squared Log Error (MSLE) *10-3	Explained Variance Score *10-3	Score (R2) *10-3	Mean Absolute Error (MAE) *10-3	Median Absolute Error (MedAE) *10-3	Mean Squared Error (MSE) *10-3	Mean Squared Log Error (MSLE) *10-3	Explained Variance Score *10-3	Score (R2) *10-3
TAN	3.93	0.00144	0.25100	0.09840	925.0	899	4.41	0.0019	0.188	0.0728	941	917
TF	10.90	3.68000	1.76000	0.43800	850.0	827	3.46	0.472	0.137	0.0342	988	987
THEO	0.07	0.01310	0.00003	0.00003	968.0	960	0.000644	0.000032	0.00000000391	0.00000000390	1000	1000
Overall	**4.97**	**1.23**	**0.671**	**0.179**	**915**	**895**	**2.62**	**0.158**	**0.108**	**0.0356**	**976**	**968**

From the explained variance score and R-square value, it is clear that the overall quality of tea has been predicted better for the fused dataset rather than considering the individual dataset from each sensor. Also, the error metrics are also identified quite less for the fused dataset with respect to the individual dataset.

5.4.3 Summary

The current approach focuses on the development of an e-tongue comprising an array of highly specific MIP electrodes for the evaluation of tea quality with an efficient switching circuit. The switching circuit enables the selection one particular WE at a time, resulting in a higher chance of trapping that bio marker instead of others as the MIP sensors are highly selective and sensitive towards that particular molecule. Five different samples of black tea were used for the experimentation. Ten repetitive DPV signatures from each working electrode were recorded at a time and the control was then transferred to the next working electrode using the automatic switching circuit. Gradient boost algorithm has been used to develop a robust cognitive model. As can be seen from the results section the fusion of the three sensors performed better over the individual sensors as indicated by the R2 value of 0.967. Moreover, by using the t-SNE algorithm on the fused data, successful discrimination

was obtained among the five different samples of black tea. Addition of a few more specific sensors, targeting other major tea bio-markers to this array may be considered for further study. The developed system has the potential to be used as a taste profiling instrument on a frequent basis in the tea industry.

5.5 Conclusion

This chapter of the thesis deals with three very significant approach towards the qualitative measurement of tea biomarkers. In the first section of the chapter an attempt was made to simultaneously detect EC and TH in black tea samples using a CuO@GP electrode. The electrode demonstrated a linear range of 50.0–100.0 μM for both analytes, with alimit of detection 4.0 μM for EC, 27.0 μM for TH. After the successful simultaneous detection of two analytes, an e-tongue based on specific MIP electrodes were fabricated. This array of MIP sensors was able to successfully detect the traces of TAN, TF and TH in a single tea sample. DPV responses from the tea sample. were obtained and subjected to PCA clustering which shows a satisfactory segregation among the three different analytes in a black tea sample with a separability index (SI) of 32.4305. After successfully determining the traces of three different molecules in a single tea sample, an approach was extended to increase the number of tea samples and the same MIP array/e-tongue was used to discriminate among the tea samples using the t-SNE algorithm. Ten repetitive DPV signatures from each working electrode were recorded at a time and the control was then transferred to the next working electrode using the automatic switching circuit. Gradient boost algorithm was used to develop a robust cognitive model which signifies that the fusion of the three sensors performed better over the individual sensors as indicated by the R^2 value of 0.967.

This work has been published as cited *"*Moulick M, Chowdhury S, Das M, Saha S, Naskar J, Nag S, Modak A, Roy RB. A customized electronic tongue by developing an array of molecular imprinted polymer based voltammetric electrodes for tea quality evaluation. *IEEE Sensors Journal. 2025 Dec 8;26(2):1562-8.*

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CHAPTER 6

Conclusion and Future Aspects

This chapter highlights the research ideas conducted so far and also addresses the final comments. Some suggestions and a few proposals have also been discussed as the future scope of work in this chapter

CHAPTER 6

Conclusion and future aspects

6.1 Introduction

To start, the thesis exemplifies a novel technology for qualitative profiling of black tea in terms of taste and astringency. Currently, the tea industry engages a group of tea-tasters who evaluate the different tea samples on a scale between 1 and 10 based on a range of characteristics. The installation of analytical tools, which could estimate the different biochemical factors present in tea, is the second way. However, the high cost of this equipment prevents it from being installed in the tea industry. The E-tongue is an improvised variation of the previous one in which an electronic circuitry array of sensors is used to mimic the human taste sensation. Thus, this technique can eliminate human subjectivity and inconsistencies, making response patterns repeatable. However, the use of non-specific sensors is the major drawback of the E-tongue. It collects the general pattern of response from the different noble metal electrodes and using the right recognition algorithm, delivers an overall taste index of tea. Another disadvantage of the E-tongue is the use of noble metals as the sensing electrodes.

Electrochemical sensors were fabricated as a part of this thesis and were used in various experiments for quality profiling of tea. Sensor materials were developed incorporating metal as well as rare-earth metal oxide nano-cubes and the MIP approach. The MIP technology is a polymer-based approach that involves engraving pockets on the polymer bed similar to the target molecules in terms of their size. The following reasons [1]-[3] make the MIP-based sensors more effective in the tea industry.

Unlike noble metals or other electrodes, all the precursors utilized in MIP synthesis are readily available and inexpensive. The procedure for electrode fabrication is straightforward and easily reproducible. Due to their quick responsiveness, the electrodes are practical for real-time applications.

6.2 Summary of the various research works undertaken

6.2.1 Crafting of electrochemical sensors for the detection of tannic acid in black tea

Tannic acid is a polyphenol with antioxidant, anticarcinogenic, and anti-inflammatory properties. Black tea, a globally consumed beverage has the highest concentration of TAN among all types of tea. It is reported that the acceptable dose of tannic acid as an additive is 10–400 μg. In this thesis, two different methods have been discussed for the electrochemical detection of black tea.

(i) Nd_2O_3 modified graphite electrode for detection of tannic acid

A simple and affordable neodymium (III) oxide nanoparticles embedded carbon electrode (Nd_2O_3@GP) has been developed for the detection of tannic acid.A three-electrode setup has been used to assess the performance of the Nd_2O_3@GP electrode using cyclic voltammetry (CV). The CV responses reveal that the developed electrode is successfully able to trace the presence of tannic acid. The linear working range of the electrode is found to be 5 to 200μM. The limit of detection (LOD) was found to be 0.7μM. This electrochemical procedure can thus be used in the detection of tannic acid in various food and beverages consumed in our daily life.

(ii) Fabrication of a MIP-based electrode for tannic acid detection in black tea

The MIP technology is employed in this approach for the detection of TAN in black tea. The sensor has a wide linear range of operation from 0.1 to 500μM with an LOD 37nM under an optimized experimental setup. The MIP_TAN represents good repeatability and reproducibility. The electrode shows good stability over a period of 40 days. With real samples, a comparative finding between the HPLC analysis and the predicted TAN using MIP_TAN is presented as well. The average prediction accuracy using PLSR was obtained as 96.315 %. Thus, an easy-to-use, simple and cheap sensor for the determination of TAN is devised, which can be employed for the quantitative estimation of TAN in black tea.

6.2.2 Fabrication of electrochemical sensors for the detection of taste-affecting analytes in black tea: Theophylline

In this work, two different electrodes were developed for the rapid detection of TH in real samples of black tea. In the first investigation, analysis of the voltammetric responses in real samples of black tea while modifying the bare graphite electrode with samarium oxide nanoparticles to increase the electrode's efficacy, is highlighted. In the second approach, the excellent electrocatalytic properties of Gd_2O_3 nanocrystals have been thoroughly explored.

(iii) Detection of theophylline using Sm_2O_3 nanoparticles ingrained graphite electrode

This section illustrates the electrochemical detection of TH using Sm_2O_3 nanoparticles ingrained over graphite paste. An effective electrochemical sensor was thus developed. The fabricated electrode showed reliable sensory features in terms of repeatability, reproducibility and stability. This electrode showed a linear operational range of 10 to 1000 µM with a LOD of 5.69 µM.

(iv) Gadolinium oxide ingrained molecular imprinted polymer electrode for the electrochemical detection of theophylline in black tea

A molecular imprinted polymer electrode modified with nano-composites of gadolinium oxide (Gd_2O_3@TH-MIP-CPE) to detect theophylline in variants of black tea samples has been explored. The sensor shows a low limit of detection having value 2.004 µM and high selectivity towards the specific analyte, simplifying its usage in the detection of TH in real tea samples.The PLSR model was adopted to predict the quantity of TH in the unknown samples which showed an accuracy of 91.3 %.

6.2.3 Development of dedicated electrochemical sensors for the detection of total theaflavins in black tea

Theaflavin is a significant constituent in tea and is responsible for its quality profiling in terms of its astringency. Two different sensors were fabricated for the quick and responsive detection of TF in black tea samples. In the first investigation, voltammetric responses in real black tea samples were thoroughly investigated. The fabricated electrode was successfully able to differentiate among the different samples based on their TF content using PCA. However, low molecular recognition ability led to the fabrication of a modified MIP-based electrode, the MIP_TF2/NiCo2O4 electrode shows a LOD of 13.89 µM.

(v) Detection of theaflavin in black tea using TiO_2 nano-cubes modified graphite electrode

A reusable graphite electrode impregnated with TiO2 nps, to detect the total TF content in different black tea samples has been explored in this section. The electrode showed satisfactory linear range of operation and an LOD of 20.27 µM.

(vi) A Molecular Imprinted Bi-polymer graphite electrode impregnated with NiCo2O4 nano- cubes for rapid detection of theaflavin in black tea

A MIP_TF2/$NiCo_2O_4$ electrode has been fabricated to comment on its practicability for the detection of TF in different black tea samples. MIP_TF2/$NiCo_2O_4$ exhibits a linear operating range of 80 to 1000 µM. This electrode shows satisfactory repeatability with an RSD value of 0.71%. To comment on the predictability of the fabricated electrode, it shows an average prediction accuracy of 92.6% when tested on the real tea samples.

6.2.4 An attempt towards crafting a single CuO modified graphite electrode for simultaneous detection of epicatechin and theophylline

A novel cupric oxide nanoparticles (CuO NPs) infused graphite electrode (CuO@GP) was synthesised for simultaneous detection of epicatechin (EC) and theophylline (TH) in black tea. The electrochemical sensor performance was studied using cyclic voltammetry and differential pulse voltammetry, revealing the presence of TH and EC simultaneously in black tea. The electrode demonstrated a linear range of 50.0–100.0 µM for both analytes, with a room temperature limit of detection (4.0 µM for EC, 27.0 µM for TH). CuO@GP exhibited notable selectivity and promising results in tea samples, achieving recovery rates of 102–107% for EC and 101.7–106% for TH

6.2.5 Towards the development of an electronic tongue by coupling an array of MIP sensors for the quality evaluation of black tea

This segment of the thesis leads towards the development of an e-tongue based on an array of MIP- based sensors. This chapter is further dissected into two parts as below

(vii) Development of an Electronic Tongue with MIP sensors for black tea quality evaluation

An electronic tongue (E-Tongue) based on voltammetric principle, coupled with a switching circuit has been proposed to detect the overall quality of different samples of black tea. The MIP sensors have been developed for the biochemical constituents- tannic acid, theaflavin and theophylline. Developed E-Tongue shows the ability to measure the signatures of these three molecules simultaneously from the test tea

sample. Hence can detect the tea quality more accurately based on chemical constituents present in the sample. Principal component analysis was employed to distinguish among three different molecules in the black tea sample which shows a satisfactory separability index of 32.4305.

(viii) A customized electronic tongue based on molecular imprinted polymer technology for tea quality discrimination

A well-developed voltammetric electronic tongue (e-tongue) consisting of an array of molecular imprinted polymer (MIP) electrodes has been designed to detect theophylline, tannic acid and total theaflavins in real samples of black tea. this e-tongue, in conjunction with a switching circuit, has been proposed to detect the overall qualitative nature of black tea. The obtained dataset was subject to the gradient boosting method. The prediction model was designed from the responses of the e-tongue and hinted at the mean-squared error (MSE) of 0.00003 and the coefficient of determination (R^2) being 0.967. On applying t- SNE to the fused data set, successful discrimination among the different black tea samples was obtained. The results direct towards the possibility of an e-tongue based on an array of MIP electrodes to detect the quality of black tea liquor.

Table 6.1: *Major outcomes of this thesis*

Sl. No	Target analyte	Electrode	Precursors	Performance parameters (µM)		Ref
				Linear range	LOD	
1.	Tannic acid	Nd_2O_3@GP	Neodymium oxide nanoparticles	5 to 20	0.7	[4]
		MIP_TAN	Monomer: Acrylic acid Crosslinker: EGDMA	0.1-500	0.037	[3]
2.	Theophylline	Sm_2O_3@GP	Samarium oxide nano-cubes	10-1000	5.69	[5]
		Gd_2O_3@TH-MIP-CPE	Monomer: Acrylic acid Crosslinker: EGDMA Modifier: Samarium oxide nano-cubes	50-900	2.004	[6]
3.	Theaflavin	TiO_2@GP	Titanium oxide nanocrystals	20-500	20.27	[7]
		MIP_TF2/$NiCo_2O_4$	Monomer: AA and MAA Crosslinker: EGDMA Modifier: Nickel cobalt oxide nanoparticles	40-1000	13.89	[8]

6.3 Recommendations

The following suggestions can be proposed in the tea industry based on the findings of this thesis as discussed above.

(i) The MIP_TAN electrode fabricated for the detection of TAN in black tea could be used by the tea specialists to keep a check on the safe TAN content in different varieties of black tea. Excessive intake of tannic acid can lead to health complications like nausea and intestinal irritation [9].

(ii) The actual content of TH, TAN and TF in the different samples of black tea could be measured in situ, thus controlling the safety limit of consumption in every cup of tea.

(iii) These sensors could prove to be profitable in the tea industrial sector due to their easy maintenance and reproducibility. The cost-effectiveness of these electrodes could be beneficial too. Keeping a quick check on the various constituents of tea, could help in overall quality profiling of tea and could potentially increase their export yielding greater revenue.

6.4 Future scope

There are several areas in which the proposed methodology discussed in the previous sections can undergo improvement.

1. The sensors should be made more commercially viable for implementation in the tea industries. By employing disposable sensors and screen-printed electrodes, the hindrance of regenerating the adsorption surface every time when exposed to target analyte could be overcome.

2. The sensors fabricated in this thesis were tested using limited tea samples acquired from Asia school of tea and Tocklai Tea Research Institute. To prepare an electrode that can be implemented in the industry level it must be trained with datasets corresponding to different types of tea samples from different tea gardens.

3. In this thesis, the MIP-based sensors have been designed to replace the noble metal-based E-tongue for more objective quality profiling of tea. Thus, an important future scope lies on the fabrication of a portable e-tongue that can be used explicitly in the tea industry.

6.5 Conclusion

Though this research work is in its budding stage, it has the potential to pave the pathway for efficient quality profiling of tea. The electrodes fabricated are inexpensive, reusable and easily reproducible. These electrodes could be beneficial for both industry experts as well as consumers to assess the overall quality of the commodity. In addition to the health benefits of the discussed constituents of black tea, sustained perseverance towards the development of an e-tongue could be an instrumental breakthrough, not only for the tea sector but also for pharmaceutical organizations.

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