KUVEMPU UNIVERSITY

VOLTAMMETRIC STUDIES OF SOME BIOLOGICALLY IMPORTANT ORGANIC COMPOUNDS AT DIFFERENT MODIFIED ELECTRODES

Thesis to be submitted to the Faculty of Science
Kuvempu University

For the Award of the Degree of

Doctor of Philosophy

In

Industrial Chemistry

By

Mrs. SUKANYA M.Sc.,

Guide

Dr. B.E. KUMARA SWAMY M.Sc., Ph.D.,
Professor
Department of Post Graduate Studies and Research
in Industrial Chemistry
Kuvempu University,
Jnana Sahyadri, Shankaraghatta - 577451,
Shivamogga, Karnataka, India

2023

KUVEMPU UNIVERSITY

VOLTAMMETRIC STUDIES OF SOME BIOLOGICALLY IMPORTANT ORGANIC COMPOUNDS AT DIFFERENT MODIFIED ELECTRODES

Thesis to be submitted to the Faculty of Science
Kuvempu University

For the Award of the Degree of

Doctor of Philosophy

In

Industrial Chemistry

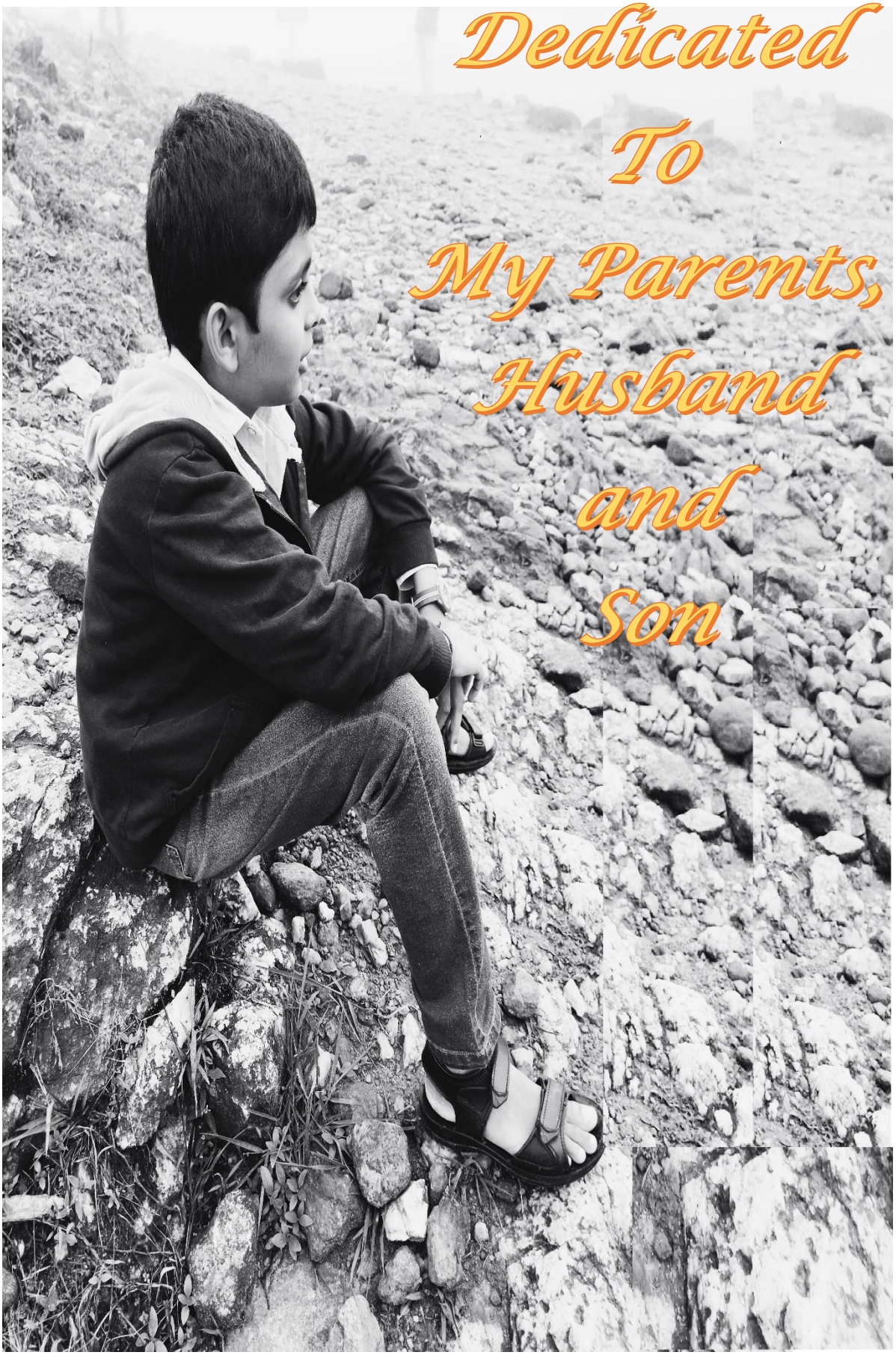
By

Mrs. SUKANYA M.Sc.,

Guide

Dr. B.E. KUMARA SWAMY M.Sc., Ph.D.,
Professor
Department of Post Graduate Studies and Research
in Industrial Chemistry
Kuvempu University,
Jnana Sahyadri, Shankaraghatta - 577451,
Shivamogga, Karnataka, India

2023


Dedicated
To
My Parents,
Husband
and
Son

KUVEMPU UNIVERSITY

Mrs. SUKANYA M.Sc.,
Research Scholar
Department of Industrial Chemistry
Jnana Sahyadri
Kuvempu University
Shankaraghatta
Email: sukanyavenur@gmail.com

Declaration

I hereby declare that the Ph.D., thesis entitled **"VOLTAMMETRIC STUDIES OF SOME BIOLOGICALLY IMPORTANT ORGANIC COMPOUNDS AT DIFFERENT MODIFIED ELECTRODES"** embodies the results of my investigation and this has been composed by me under the Supervision of **Dr. B. E. Kumara Swamy,** Professor, Department of PG Studies and Research in Industrial Chemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shimoga and the same has not been previously formed the basis for the award of any degree, diploma, associateship, fellowship etc., of any other University or Institution.

Place: Shankaraghatta

Date: 30/01/2023

(SUKANYA)

KUVEMPU UNIVERSITY

Dr. B.E. Kumara Swamy M.Sc., Ph.D.
Professor
Department of PG Studies and
Research in Industrial Chemistry
Jnana Sahyadri
Shankaraghatta – 577 451
Shimoga, Karnataka, INDIA
Email: kumaraswamy21@yahoo.com
Cell: +919900513796

Certificate

This is to certify that the work reported in this thesis entitled "**VOLTAMMETRIC STUDIES OF SOME BIOLOGICALLY IMPORTANT ORGANIC COMPOUNDS AT DIFFERENT MODIFIED ELECTRODES"** submitted by **Mrs. Sukanya,** to the Faculty of Science, Kuvempu University, for the award of **Doctor of Philosophy in Industrial Chemistry** is a record of the bonafide and original research work carried out by her under my guidance.

I further certify that this or part thereof has not been previously formed the basis of the award of any degree, associateship, fellowship, etc., of any other University or Institution.

Date: 30/1/23
Place: Shankaraghatta

(Dr. B.E. KUMARA SWAMY)
Prof. B.E. Kumara Swamy
Dept. of PG Studies and
Research in Industrial Chemistry
Kuvempu University
SHANKARAGHATTA-577451

Acknowledgment

Before I savour the wonderful moments of completion of my thesis work, to be sure, at this juncture, I would like to record my humble gratitude to all the people who have eased my burden by assisting me in all aspects to complete this work with their blessings and moral support.

At the very outset I thank and owe my gratefulness to the ***Almighty God*** for his blessings on my life.

I am delighted to offer my heartfelt gratitude and appreciation to my supervisor, ***Dr. B. E. Kumara Swamy***, Professor, Department of Post Graduate Studies and Research in Industrial Chemistry, Kuvempu University, Shankaraghatta-577451, Shimoga, Karnataka, India, for his mentoring during all stages of my research work. It is really a privilege to work with a person who has a high spirit of life, an outstanding teacher, and a great scientist. His inspiration, motivation, simplicity, and creation of a courageous environment will be cherished in all walks of my research work. The knowledge gained by working with him will definitely be useful in my academic evolution. I am forever indebted to you.

I am very grateful to ***Prof. Vasantha Kumar Pai***, ***Prof. H. S. Bhojya Naik, Prof. Yadav Bodke,*** and ***Mrs. Itte Pushpavathi,*** for their good wishes and constant support behind this research work at every stage since inception.

I am grateful to ***Prof. B. P. Veerabhadrappa***, Vice-Chancellor, Kuvempu University, Shankaragatta, Shimoga, for his constant support for providing the necessary facilities throughout my research work.

I express my sincere thanks to ***Prof. B. S. Sherigara***, Former Vice-Chancellor of Kuvempu University, Shankaragatta, Shimoga, who has been my forever inspiration and sole reason to pursue my doctoral degree. He has been my driving force and a constant well-wisher.

Special thanks go to our beloved guide family members ***Mrs. Deepa Kumara Swamy, Pratham Swamy*** and ***Pradvi Swamy*** for their constant encouragement and support during my research stay at university.

I wholeheartedly treasure the constant support of my research mate ***Dr. Shashikumara J. K.*** for his meticulous support, co-operation and friendly advice received during the programme of my work.

I also place on record my appreciation to my senior researchers ***Dr. P. S. Ganesh, Dr. Chethan Kuskur, Dr. Mohan, Dr. K. R. Mahanthesha, Dr. K. V. Harish,*** and in addition to my magnificent bunch of co-researchers ***Dr. Chetan Kumar Naik, Dr. Madhu Chandra, Dr. Deepa S, Ms. Rukaya Bhanu, Mrs. Shruthi, Mr. Manjunath K. G and all Research Scholars*** for their active support. They always helped me out in solving any difficulties or queries regarding my research work and for making the working environment very lively.

I thank the ***non-teaching staff members*** of the Department of Industrial Chemistry, for their timely help.

A special gratitude for ***Dr. M. Mohan Alva,*** Chairman, Alva's Education Foundation Moodubidire and ***Prof. Kurian***, Principal, Alva's college, Moodubidire for being so supportive throughout my academic career. I thank my colleagues at Post Graduation Department of Analytical Chemistry, Moodubidire, namely, ***Prof. Rajkumar Bhat, Ms. Ranjitha R Acharya, Mr. U Rajesh Kumar***, and ***Mr. Arun Kumar Dandavathi*** for their help in many ways. I express my sincere thanks to ***Prof. Sundar Moily***, Retd. Professor of Chemistry, SVS college, Bantwal, for his valuable suggestions and support.

I remember with gratitude for the support and help made by good friends ***Dr. Sandya Rani, Dr. Reshma Kayarmar, Mrs. Manjula S,*** and ***Dr. Poornima Nayak.***

I have no words to express my deep sense of gratitude to my parents ***Smt. Kumudini*** and ***Sri. Lingappa Devadiga*** for their love and blessings and giving me a strength to chase my dreams. I owe my gratitude to my husband ***Mr. Santhosh Devadiga*** and my son ***Sudhanshu S Devadiga*** for their affection, encouragement, and sacrifice. Sincere thanks to my brother ***Mr. Suvarna Devadiga*** and uncle ***Mr. Prabhakar Devadiga*** for their support and encouragement.

Finally, I express my immense gratitude to ***all my teachers, friends, relatives,*** and ***well-wishers*** who have helped me in various ways in my career.

Sukanya

LIST OF ABBREVIATIONS

AA	=	Ascorbic acid
ABS	=	Acetate buffer solution
ADR	=	Adrenaline
AE	=	Auxiliary electrode
ASV	=	Anodic stripping voltammetry
BCPE	=	Bare carbon paste electrode
CC	=	Catechol
CE	=	Counter electrode
CPE	=	Carbon paste electrode
CV	=	Cyclic voltammetry
DA	=	Dopamine
DNA	=	Deoxyribonucleic acid
D_0	=	Diffusion coefficient
DPV	=	Differential pulse voltammetry
E_f	=	Final potential
E_i	=	Initial applied potential
E_{pa}	=	Anodic peak potential
E_{pc}	=	Cathodic peak potential
E_v	=	Vertex potential
E^0	=	Formal potential
ERY	=	Erythrosine
FA	=	Folic Acid
GCE	=	Glassy carbon electrode
GE	=	Graphite electrode
GPE	=	Graphite pencil electrode
HQ	=	Hydroquinone
5-HT	=	5-hydroxytryptamine (Serotonin)
I_{pa}	=	Anodic peak current

I_{pc}	=	Cathodic peak current
K_0	=	Heterogeneous rate constant
LOD	=	Limit of Detection
LOQ	=	Limit of Quantification
LSV	=	Linear sweep voltammetry
MCPE	=	Modified carbon paste electrode
MGCE	=	Modified glassy carbon electrode
MPGE	=	Modified pencil graphite electrode
mM	=	Millimolar
mV	=	Millivolt
mVs^{-1}	=	Millivolt per second
PBS	=	Phosphate buffer solution
PA/PAR/PC	=	Paracetamol
PGE	=	Pencil graphite electrode
RE	=	Reference electrode
SCE	=	Saturated calomel electrode
SEM	=	Scanning electron microscopy
SHE	=	Standard hydrogen electrode
SR	=	Scan rate
SR	=	Scan speed
SWV	=	Square wave voltammetry
UA/UAC/URI	=	Uric acid
WE	=	Working electrode

LIST OF PAPER PUBLICATIONS / COMMUNICATED

1) **Sukanya,** B. E. K Swamy, J. K. Shashikumara, Poly (benzydamine) sensor for electrochemical resolution of catechol and hydroquinone, ***Materials Science for Energy Technologies*** 3 (2020) 640
2) **Sukanya,** B. E. K Swamy, J. K Shashikumara, Voltammetric investigation of uric acid in existence of dopamine at Poly(benzydamine) modified carbon paste electrode, ***Sensors International*** 1 (2020) 100045
3) **S. D. Sukanya,** B. E. K Swamy, J. K. Shashikumara, S. C. Sharma & S. A. Hariprasad, Poly (Orange CD) sensor for paracetamol in presence of folic acid and dopamine, ***Scientific Reports*** 11 (2021) 22332
4) **Sukanya,** B. E. K Swamy, J. K. Shashikumara, S. C. Sharma, Poly (yellow PX4R) carbon paste electrode sensor for paracetamol: A voltammetric study, ***Inorganic Chemistry Communications*** 140 (2022) 109394
5) **S. D. Sukanya,** B. E. K. Swamy, J. K. Shashikumara, Electroanalytical detection of Uric acid on Blue HEGN modified glassy carbon electrode by voltammetry, ***Analytical and Bioanalytical Electrochemistry*** 14 (2022) 1114
6) **S. D. Sukanya,** B. E. K Swamy, J. K. Shashikumara, S. C. Sharma & S. A. Hariprasad, A novel, extreme low-cost poly (Erythrosine) modified pencil graphite electrode for determination of Adrenaline (***Scientific Reports***)
7) **Sukanya,** B. E. K. Swamy, J. K. Shashikumara, An Affordable Yellow DS5R polymeric film modified glassy carbon electrode for voltametric assay of Uric acid **(*Inorganic Chemistry Communications*)**

List of National / International Seminars / Conferences / Workshops

1) **Sukanya,** B. E. K Swamy, Electrochemical Sensor for Uric acid in presence of Dopamine using poly (benzydamine) modified carbon paste electrode: A voltammetric study at National Level Conference on *"Recent Advances Materials science"* on 5th February 2019 at Field Marshal K. M. Cariappa College, Madikeri
2) **Sukanya,** B. E. K Swamy, Poly (Benzydamine) modified sensor for Catechol in presence of hydroquinone: A voltammetric study at International Conference on *"Nanotechnology"* on 18th & 19th October 2019 at Srinivas University, Mukka, Mangalore
3) **Sukanya,** B. E. K Swamy, Voltammetric resolution of Paracetamol in presence of Folic acid at poly (Yellow PX$R) Modified Carbon Paste Electrode at National Level Conference on *"Innovations in Chemical Sciences (NCICS-2020)"* on 30th and 31st January 2020 at University of Mysore
4) **Sukanya,** B. E. K Swamy, Electrochemical Sensor for Uric acid in presence of Dopamine using poly (benzydamine) modified carbon paste electrode: A voltammetric study at National Level Conference on *"Recent Advances Materials science"* on 12th February 2020 at Field Marshal K. M. Cariappa college,Madikeri
5) **Sukanya,** B. E. K Swamy, Electrochemical Investigation of Folic acid in presence of Paracetamol at poly (benzydamine) modified carbon paste electrode: A voltammetric study at National Level Conference on *"Frontiers of Chemical and Agricultural Sciences Development: Challenges and Opportunities (FCASDCO-2020)"* on 3rd March 2020 at Govinda Dasa College, Surathkal. (**Best poster presentation Award**)
6) **Sukanya,** B. E. K Swamy, Electrochemical Determination of Catechol in presence of Hydroquinone at Poly (Orange CD) modified carbon paste electrode: A Voltammetric study at National Level Conference on *"Climate change and Agriculture: Impacts, Adaptation and Mitigation (NCCCAIAM-2021)"* on 26th March 2021 at Govinda Dasa College, Surathkal
7) **Sukanya,** B. E. K Swamy, Electrochemical Determination of Uric acid in presence of Dopamine at Poly (yellow DS5R) modified glassy carbon electrode: A Voltammetric study at National Level Conference on *"Impact of Chemistry and Biology to the Society and Industry (ICBSI)"* on 20th and 21st May 2022 at Kuvempu University, Shankaraghatta, Shivamogga, Karnataka

WEBINARS:

1) Webinar on "Natural Nano materials chemistry exemplified by cellulosic Fibers and products" on 14th June 2020 at Department of Chemistry, Reva University, Bengaluru
2) National Webinar on "Recent Advances in Modern Analytical Techniques" held on 22-24 June 2020 at A. U college of Science and Technology, Andhra University & PerkinElmer India Pvt. Ltd.
3) International Webinar on "Diabetes and its Management" on 9th July 2020 at Department of Chemistry, Sri Bhuvanendra College, Karkala
4) Webinar on "Applications of Cyclic Voltammetry in Research" on 12th July 2020 at Department of Chemistry, Reva University, Bengaluru
5) Webinar on "Trends in Future Research in Chemistry" on 13th July 2020 at Department of Chemistry, St, Aloysius College, Mangaluru
6) National webinar on "Industrial Applications of Electrochemistry" on 13-14 July 2020 at Department of Chemistry, PES Institute of Technology and management, Shivamogga
7) Webinar on "Hypervalent Iodine oxidants and their synthetic applications" on 17th July 2020 at Department of Chemistry, Reva University, Bengaluru
8) National webinar on "Current Trends, opportunities and Future in Clinical Research" held on 1st August 2020 at Ramaiah College of Arts, Science and Commerce, Bengaluru
9) Webinar on "Drug Design-is it really that easy?" on 7th August 2020 at Department of Chemistry, St, Aloysius College, Mangaluru
10) International Webinar on "Frontier Research in Chemical Sciences 2020" held on 10-12 September 2020, Department of Chemistry, Mangalore University, Mangalagangothri

Summary of the Thesis

The goal of the thesis is to design an electrochemical sensor by altering the electrode surface (carbon paste electrode, glassy carbon electrode, and pencil electrode) to examine the electrochemical behaviour of some biologically important organic compounds employing cyclic voltammetric techniques. The biologically important compounds like paracetamol, uric acid, adrenaline, catechol, and hydroquinone were chosen for electrochemical investigations. The following aspects like number of electrons involved in the electrochemical reaction, impact of sweep rate, the surface area of the electrode, concentration, pH condition of electroactive species, simultaneous analysis and real sample were observed.

The work carried out in this thesis is divided and described into seven chapters.

Chapter–1

Introduction and Overview of Cyclic Voltammetry

This chapter involves the introduction about voltammetry and its techniques, fundamental principles, theoretical aspects, and applications of cyclic voltammetry. Moreover, the solvents, supporting electrolytes and electrode interaction can be seen in this section. A brief review of cyclic voltametric investigations of some biologically important compounds has been presented. Also, objective and scope of the present thesis were discussed in this chapter.

Chapter-2

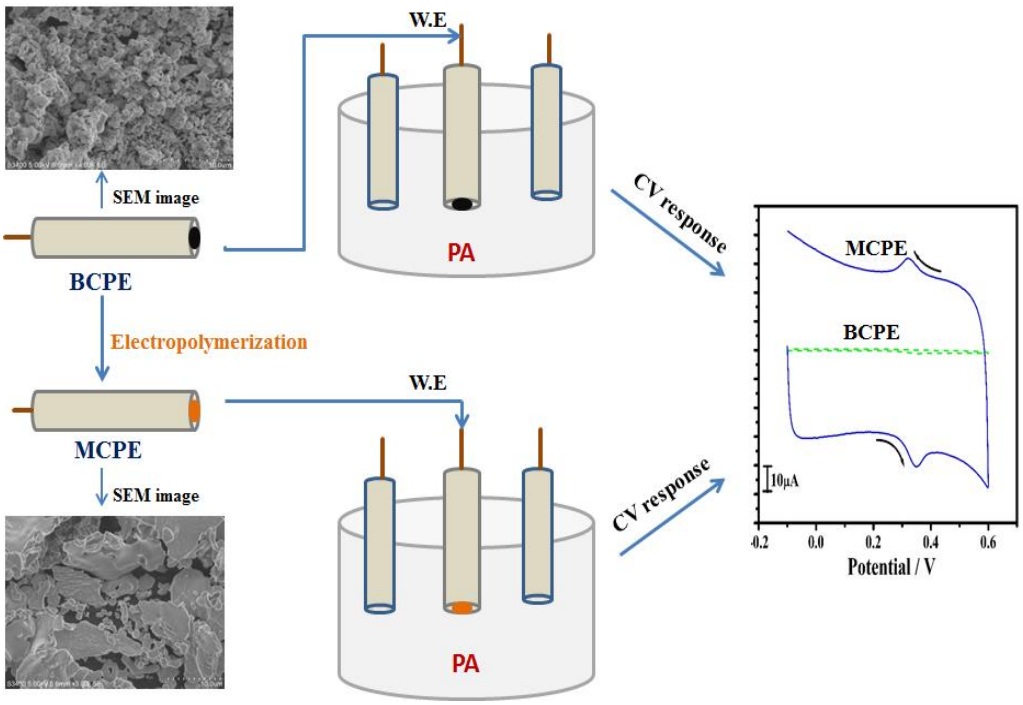
Experimental

This chapter describes the basic experimental setup which is very much essential for voltammetric techniques. Also, procedure for the preparation of bare carbon paste electrode and its modification was explained in detail.

Chapter-3

Poly (Orange CD) sensor for paracetamol in presence of folic acid and dopamine

In the present work, Orange CD was chosen as an intriguing modifier for the electropolymerization on the surface of CPE by the CV technique. A novel, sensitive, and cost-effective poly (Orange CD) MCPE (PoOCD/MCPE) sensor was utilized for the selective detection of paracetamol (PA) in 0.2 M phosphate buffer solution (PBS) of pH 7.4. The oxidation peak current of PA was vastly enhanced at the sensor. The scan rate study is suggested that electro-oxidation of PA was adsorption-controlled. The pH study testifies the redox pathways transport with the same quantity of electrons and protons. The detection limit of PA is found to be 2.64 µM. DPV results show that substantial peak separation between PA, folic acid (FA), and dopamine (DA) could be facilitating their individual and simultaneous determination on the sensor. The decorated sensor demonstrates high sensitivity, stability, reproducibility, repeatability and has been successfully exploited for the detection of PA in a tablet with promising results.

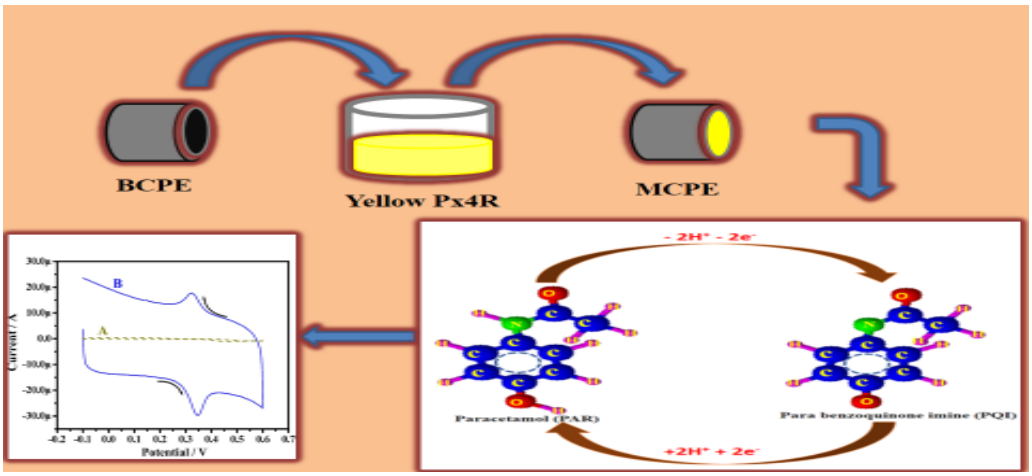


Published in Scientific Reports (2021) 11:22332

Chapter-4

Poly (yellow PX4R) carbon paste electrode sensor for paracetamol: A voltammetric study

A simple, novel and less cost yellow (PX4R) modified carbon paste electrode (Po-YPX4R-MCPE) was successfully fabricated for the detection of paracetamol (PAR) in PBS (0.2 M, 7.4 pH). This reported senor showed excellent electrocatalytic activity, increased sensitivity, and fast electron transfer rate towards the oxidation of PAR in comparison to BCPE. The surface morphologies of BCPE and Po-YPX4R-MCPE were analyzed using scanning electron microscopy (SEM). The influence of pH, scan speed, and impact of concentration was studied at the sensor. The pH study reveals that the same amount of electrons and protons are involved in the redox reaction of PAR, and the scan speed study depicts that the electro-oxidation was adsorption controlled. In an optimal operating condition, Po-YPX4R-MCPE exhibits a fine linear increase for PAR oxidation in the concentration range of working dynamic range 10 to 60 µM with an estimated LOD of 0.084 µM. The DPV data indicate that there is a significant peak divergence of 0.097 V for PAR and 0.292 V for dopamine (DA) which could make it easier to determine them alone and simultaneously on the sensor. The established sensor also contributed to good stability and reproducibility. The described method has been employed for the determination of PAR in pharmaceutical tablets. Good recovery values indicate the efficacy and applicability of the sensor in detecting PAR.



***Published in Inorganic Chemistry Communications* 140 (2022) 109394**

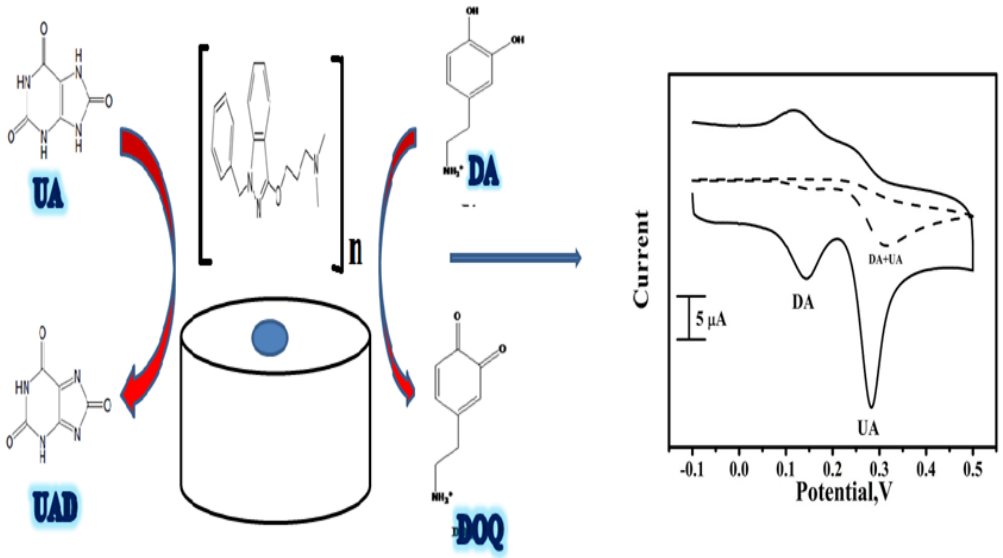
Chapter-5

This chapter is divided into two parts such as Part-A and Part-B

Part-A

Voltammetric investigation of uric acid in existence of dopamine at Poly(benzydamine) modified carbon paste electrode

Electropolymerization of Benzydamine was achieved on carbon paste electrode surface by cyclic voltammetric technique. The poly (benzydamine) MCPE exhibited an excellent electrocatalytic behavior of uric acid (UA). The critical examination was conducted on the electrochemical measurements at poly (benzydamine) MCPE for analysis of UA and dopamine (DA) by CV and DPV techniques. From the scan rate study, it was found that the oxidation of UA was diffusion controlled. Uric acid exhibits good detection limit (11.33 nM) at modified electrode in DPV technique. Hence, the investigated sensor poly (benzydamine) MCPE shows excellent selectivity and sensitivity towards resolution of UA in existence of DA.

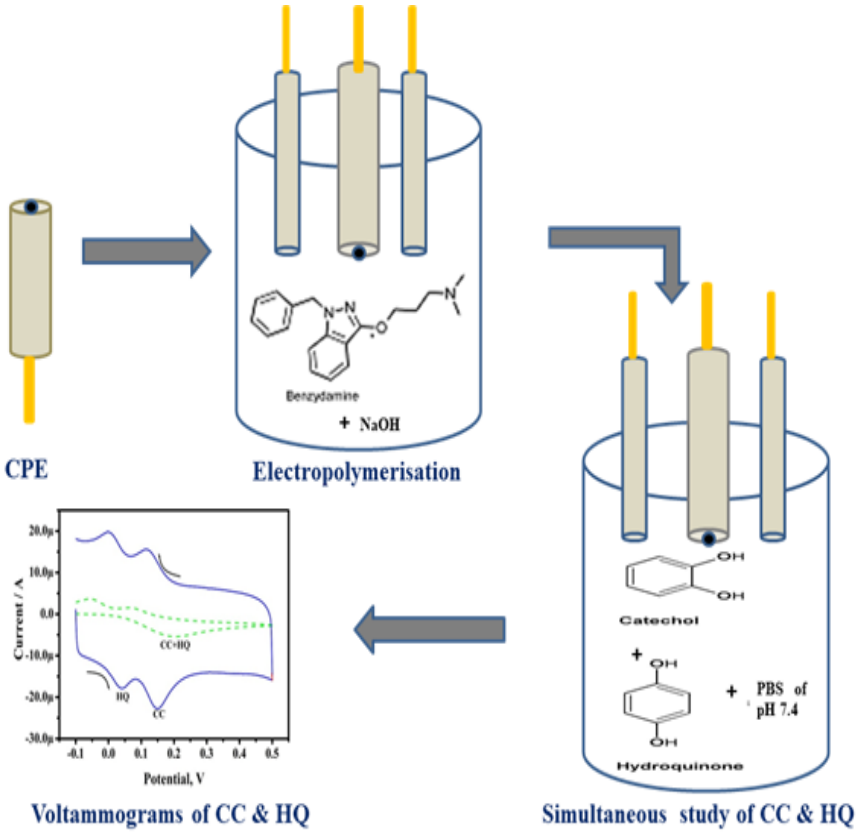


Published in Sensors International 1 (2020) 100045

Part-B

Poly (benzydamine) sensor for electrochemical resolution of catechol and hydroquinone

The carbon paste electrode was modified with poly (benzydamine) by cyclic voltammetric technique (CV) and its electrocatalytic activity towards the oxidation of catechol (CC) and hydroquinone (HQ) was studied. The detailed investigation on the various parameters influencing the activity was also carried out. From the obtained result, it was concluded that the process was adsorption - controlled and the involvement of same number of electron and proton. The simulations resolution of CC and HQ along with excellent selectivity and sensitivity were also exhibited by the studied MCPE. The detection limits 5.79 and 3.78 l µM were found for CC and HQ, respectively.

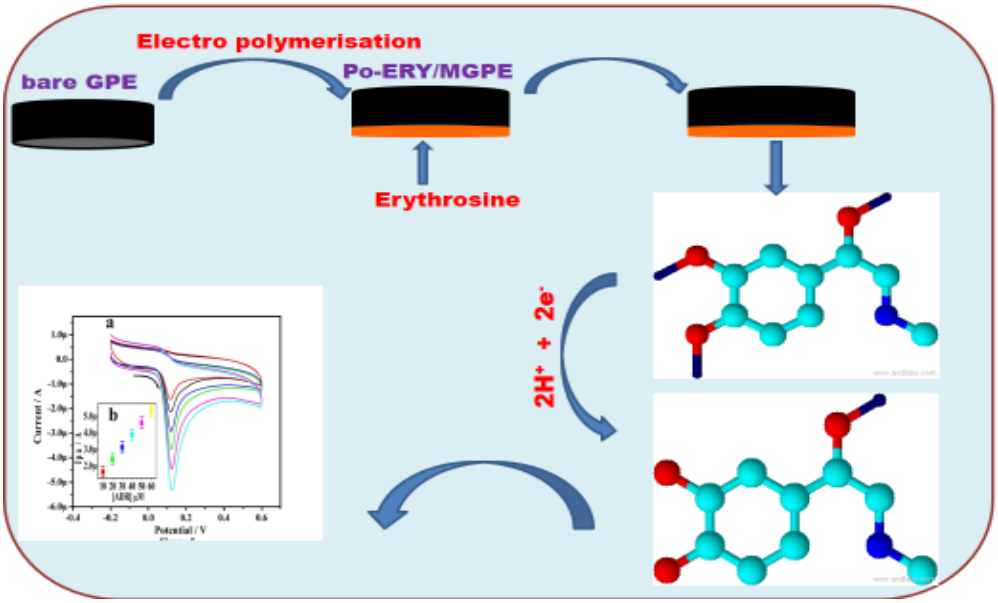


Published in Materials Science for Energy Technologies 3 (2020) 640–647

Chapter-6

A novel, extreme low-cost poly (Erythrosine) modified pencil graphite electrode for determination of Adrenaline

A simple, novel, and less cost yellow (Erythrosine) modified pencil graphite electrode (Po-ERY/MGPE) was successfully fabricated via electropolymerization method using cyclic voltammetric (CV) techniques. The fabricated Po-ERY/MGPE opted as a sensor for the detection Adrenaline (ADR) in 0.2M PBS (7.4 pH). This reported senor displayed excellent electrocatalytic activity, increased sensitivity, high stability, superior electron transfer kinetics in the oxidation of ADR once relative to BGPE. The significance of pH, sweep speed, and impact of concentration was assessed at the sensor. As per the pH and sweep speed study, redox routes carry the same number of electrons and protons, and electro-oxidation of ADR was adsorption controlled, respectively. The LOD of ADR was found to be 0.499 µM. The DPV data indicate that there is a significant peak divergence among ADR and Uric acid (UA) which could make it easier to determine them alone and simultaneously on the sensor. The described method has been employed for the determination of ADR in injection sample. Good recovery values indicate the efficacy and applicability of the sensor in detecting ADR.



Communicated to Scientific Reports (Revised manuscript submitted)

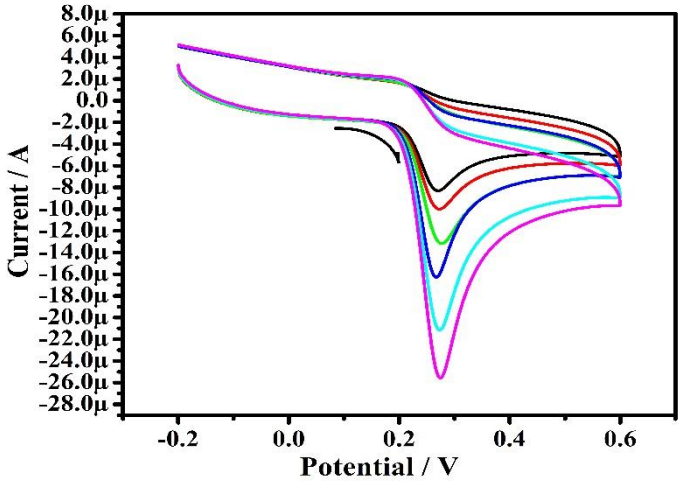
Chapter-7

This chapter is divided into two parts such as Part-A and Part-B

Part-A

An Affordable Yellow DS5R polymeric film modified glassy carbon electrode for voltametric assay of Uric acid

This work focuses on the development of a novel and affordable yellow DS5R polymeric film on the glassy carbon electrode surface (Po-YDS5R/GCE) for the detection of Uric acid (UAC). Po-YDS5R/GCE was effectively developed using cyclic voltammetry techniques (CV). The sweep velocity study revealed that the overall electrode reaction was diffusion controlled. The static linear range of 10 - 60 µM of UAC was used to compute the LOQ and LOD, and the results yield 3.12 and 0.87µM, respectively. The interference of dopamine on the electrooxidation of UAC was tested at the sensor. It was discovered that the modified sensor allowed for the selective detection of UAC. These impressive outcomes showed that a simple and less-cost Po-YDS5R/GCE sensor has the potential to be a significant improvement over current electrochemical sensor technology.



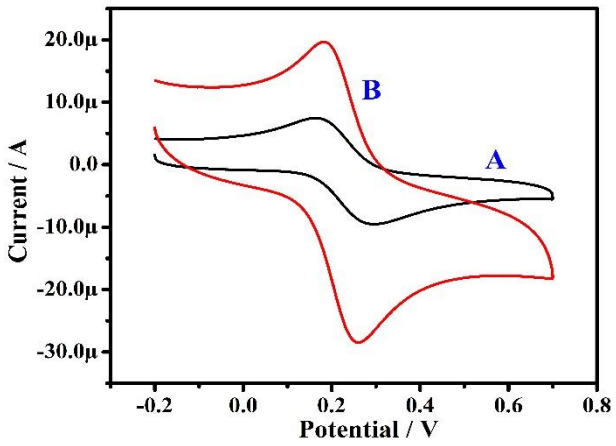
CVs of UAC in 0.2M PBS (pH 7.4) at Po-YDS5R/GCE at a sweep velocity of 50 mV/s with varied concentrations (10µM - 60µM)

Communicated to Inorganic Chemistry Communications

Part-B

Electroanalytical detection of Uric acid on Blue HEGN modified glassy carbon electrode by voltammetry

An electroanalytical technique was advanced for the detection of uric acid (URI) relying on its oxidation behaviour. Using cyclic voltammetry (CV) techniques, the electrochemical performance and detection of URI were easily accomplished on poly (Blue HEGN) modified glassy carbon electrode (Po-BHEGN/GCE). The role of pH on anodic peak current and potential was examined. Phosphate buffer of pH 7.4 was selected for subsequent data analysis. Sweep rate studies were carried out and showed that electrode reaction was a diffusion-controlled process. A linear calibration curve was established in the URI concentration levels from 10- 70 µM. The LOD and LOQ were determined to be 0.94 and 3.15µM, respectively. A simultaneous study of URI and dopamine (DA) revealed that well-separated peak at Po-BHEGN/GCE compared to GCE. To sum up, a straightforward and inexpensive sensor Po-BHEGN/GCE is built for the sensitive and focused detection of URI in samples.



Cyclic voltammogram recorded for URI at bare GCE (A) and Po-BHEGN/GCE (B) in presence of 0.2M PBS (pH 7.4) at the scan rate of 50mVs^{-1}.

Published in Analytical and Bioanalytical Electrochemistry 14 (2022)1114-1125

CONTENTS

Chapter -2

Experimental

Chapter-3

Poly (Orange CD) sensor for paracetamol in presence of folic acid and dopamine

Chapter-4

Poly (yellow PX4R) carbon paste electrode sensor for paracetamol: A voltammetric study

Chapter-5A

Voltammetric investigation of uric acid in existence of dopamine at Poly(benzydamine) modified carbon paste electrode

Chapter-5B

Poly (benzydamine) sensor for electrochemical resolution of catechol and hydroquinone

Chapter-6

A novel, extreme low-cost poly (Erythrosine) modified pencil graphite electrode for determination of Adrenaline

Chapter-7A

An Affordable Yellow DS5R polymeric film modified glassy carbon electrode for voltametric assay of Uric acid

Chapter-7B

Electroanalytical detection of Uric acid on Blue HEGN modified glassy carbon electrode by voltammetry

PUBLICATIONS

CONFERENCE CERTIFICATES

Chapter -1

Introduction And Overview of Cyclic Voltammetry

1.1 Introduction

Electrochemistry is a distinct area of current research. This includes electrochemical cells, photovoltaic cells, energy conversion devices etc. It is an area of chemistry that deals with the study of the movement of electrons from one substance to another. This generates a current, and the amplitude of the current reveal's information about the substance. The basic principle of electrochemistry is used in analytical chemistry as well [1-7].

Analytical chemistry is extremely important to all the areas of science like medicine, forensics, environmental science, engineering, and the food industry, etc. It uses conventional, wet chemical, and sophisticated instrumental techniques to obtain data regarding the sample materials and their structure. A quantitative study establishes the absolute or relative quantity of the analytes. The qualitative study allows us to determine the chemical composition of the analyte. In this regard analytical chemistry is helpful. Instrumental technique is the backbone of mainstream analytical chemistry. This gives a significant level of accuracy as well as precision in the result. Spectrometry, chromatography, electroanalytical and thermal analysis are the most frequently employed effective methods [8-10]. Electroanalytical chemistry is an effective and sensitive method for qualitative and quantitative examinations across a wide range of concentrations. This relies on the electrochemical characteristics of the sample solution which forms the constituent of an electrochemical cell. The investigation of chemical characteristics like analyte's concentration is connected to electrical behaviors like potential, charge, current, and resistance. This behavior can be detected by the sensors.

A sensor is a device composed of an active sensing component and a signal transducer. It is categorized into electrochemical sensors and biosensors. The electrochemical sensors respond to the behavior of the analyte by producing an electrical output corresponding to it. A new category of sensor known as electrochemical biosensor, where the electrochemical methods are applied for the fabrication and operation of a biosensor has been created by the combination of electrochemical sensor and biosensor [11-13]. The electrochemical approach has gained attention in detecting biomolecules because of its swift reaction, ease of operation, excellent sensitivity, simplicity, reproducibility, and durability. Due to their distinctive characteristics, researchers are competing to develop innovative sensors. The utilization

of these in electrochemical analysis has substantial influence in the disciplines of environmental monitoring, clinical analysis, industrial quality control, etc. These techniques are beneficial as they enable us to separate ionic species along with their detection. Besides this, they are flexible, convenient, affordable, efficient, and sensitive. Among many of the existing electrochemical methods, voltammetry is the most prominent and extensively employed technique for measuring electroactive analytes [14-18].

1.2 Voltammetry

Jaroslav Heyrovsky, a chemist from Czechoslovakia discovered polarography in 1922. In 1959, he was awarded the Nobel prize. Voltammetry was developed from his work on polarography. Currently, it is one of the subfields of electroanalysis. Voltammetry measures the current flowing at an electrode in response to the applied potential. It involves the three electrodes system to record both voltage and current in contrast to potentiometric measurements which only use two electrodes. The potentiostat device and utilization of three electrodes- working, reference, and counter electrodes- enable the precise application of potential functions and determination of the resulting current.

A current versus potential technique called voltammetry switches the applied potential at the electrode solution interface and estimates the developed current. This technique is frequently used by researchers for diverse purposes such as basic studies on the redox process, mechanism, and kinetics of transfer of electrons, and thermodynamic properties of solvated molecules, etc. Thus, some electroactive biomolecules such as neurotransmitters, vitamins, hormones, antibiotics, analgesics, etc., are best studied by this technique.

The importance of the technique is currently shifting from its invention or modification to its application to specific systems. Such advancements are also accelerated by recent findings like conductive polymers modified electrodes. In connection with this, several advancements in the experimental approach are noted with each experimental report [19-22]. This has led to constantly innovative concepts, views, and perspectives in experimental facets.

1.3 Theoretical aspects of voltammetric technique

The potential applied to the working electrode to cause the electrochemical reaction and the material chosen as the working electrode was adopted to differentiate the voltammetric procedure from one another. Different voltage waveforms or excitation signals are applied to trigger redox reactions at an electrode to produce various kinds of voltammetric approaches. Considering this finding, the following categories of voltammetric methods are developed [23-25].

- Linear sweep voltammetry
- Staircase voltammetry
- Square wave voltammetry
- Anodic stripping voltammetry
- Cathodic stripping voltammetry
- Differential pulse voltammetry
- Normal pulse voltammetry
- Fast scan cyclic voltammetry
- Cyclic voltammetry

A contemporary electrochemical approach called cyclic voltammetry (CV) allows for the thorough analysis of the nature and kinetics of electrode reactions. The current monitored in opposition to the applied voltage generates a cyclic voltammogram. The scan rate, electrolyte concentration, and electrode material all influence the width and height of the cyclic voltammogram.

1.4 Cyclic Voltammetry

Cyclic voltammetry (CV) is employed to examine the electrochemical characteristics of the analyte solution. In 1938 it was first reported and described by Randles [26]. It is more often used to get qualitative data on electrochemical reactions. The advantage of CV is that it provides significant information quickly on the thermodynamics of redox processes. It also gives information on the kinetics of heterogeneous electron transfer processes and couple chemical reactions or adsorption processes. Since it enables quick identification of the redox potentials of the electroactive species and is convenient in assessing the impact of media upon the redox

process [27-28]. CV is more often the first experimental technique used in electroanalytical research.

Application of linear potential sweep to the working electrode results in a cyclic voltammogram. A current pass through the electrode that either oxidizes or decreases the analyte as the potential is swept back and forth past the formal potential, $E_{0,}$ of an analyte. CV finds application in the analytical determination of the concentration of analyte because the current is proportional to its concentration in the solution.

1.4.1 Fundamentals of cyclic Voltammetry

1.4.1.1 Circuit

The two circuits used in voltammetric analysis are a polarizing circuit that imparts voltage to the cell and a measuring circuit that records cell constant. The working electrode is controlled by the potentiostatic method. A voltammogram is a current vs potential graph that results from systematic varying of the potential.

1.4.1.2 Scan rate

The linear waveform, which continually changes the potential as a linear function of time, is frequently employed in electrochemical studies. Scan rate is the rate at which potential changes with time.

1.4.1.3 Switching potential

In CV, an electrode's potential is cycled between two predetermined values known as the switching potentials in an unstirred solution, and the resulting current is recorded. The excitation signal, which is a linear potential scan with a triangle waveform and is applied across the working electrode (WE) and reference electrode (RE), is the controlling potential (Fig.1.1). The excitation signal produces a negative scan of the potential from +0.8V to -0.2V relative SCE, after which the scan direction is reversed, creating a positive scan back to the initial potential of + 0.8V. Single cycle or multiple cycles can be made use of.

1.4.1.4 Potential control

Utilizing a potentiostat and a three-electrode system, potential management of

the external point is accomplished. This can be carried out by adjusting the WE's potential concerning the RE, saturated calomel electrode (SCE), or silver-silver chloride (Ag/AgCl) electrode. The current flow between WE and the counter electrodes. CV has received much interest in the electrochemical studies of novel systems owing to its increased experimental simplicity and has been established as a sensitive instrument for acquiring information about complex electrode reactions.

The CV technique uses a reverse (i.e., anodic) scan to reoxidize a species that undergoes a reduction during a cathodic polarization of the WE in an unstirred solution. For many electrochemical reactions, the relationship between the cathodic and anodic peak currents and the variations between their potentials with the potential scan rates has been investigated mathematically [29-30]. The scan rates of CV and single sweep voltammetry can be the same.

1.4.1.5 Characteristic parameters of a cyclic voltammogram

Peak potential and peak current are key cyclic voltammogram variables. The redox reaction has two peaks namely, anodic peak potential (Epa) and cathodic peak potential (Epc). The related currents are anodic peak current (Ipa) and cathodic peak current (Ipc). A typical voltammogram for a reversible process is depicted in Fig. 1.2 as current versus potential. The horizontal axis can also be considered as a time axis as the potential changes linearly over time. The oxidation and reductions occur at a faster rate by increasing the positive and negative potentials, respectively.

The insight into the kinetics and thermodynamics of the redox system can be obtained from the results of the parameters of the cyclic voltammogram [31]. From Fig. 1.3, the electrode becomes a better reductant when the potential is scanned in the negative direction and a better oxidant when the potential is scanned in the positive direction.

1.5 Theory and Principle

The potential sweep strategy in CV incorporates sweeping the electrode potential between fixed potential limits E_1 and E_2 at a predefined sweep/ scan rate. The sweep is inverted to E_1 to achieve a cyclic scan once it attains the limit E_2. The CV scan displays the potential at which the redox reaction occurs by plotting current versus

potential. The time axis is the potential axis which is related to the scan rate [32]. The excitation signal for CV is a linear potential scan with a triangle waveform [33]. The triangle potential excitation signal shifts the potential of an electrode between two values. This is known as the switching potential. In cyclic voltammetry, a cathodic polarization of the working electrode in an unstirred solution is re-oxidized. This results in a reduction of the species. To investigate the various electrochemical reactions, a mathematical analysis of the relations between the cathodic and anodic peak currents and variations between their respective peak potentials with the rates of voltage sweeps has been performed. The sweep rate used in a single sweep and cyclic voltammetry is identical. The current recorded during this operation is generally normalized to the electrode surface area termed as the current density. The plot of current density versus the applied voltage gives the cyclic voltammogram. A potential corresponding to the measured peak current is reflective of the characteristic electrode reaction. For a specific process, the peak width and height are influenced by the electrolyte concentration, sweep rate, and electrode material [34]. An oxidation process is performed using a positive potential gradient. An anodic peak current is observed when the electroactive species loses an electron resulting in an oxidation peak at the given potential. Similarly, when a negative potential is applied, the cathodic current is observed indicating a reduction peak. It is possible to detect cathodic currents, which often produce a reduction peak at a specific potential. The CV is performed at a potential if the species are typically electroactive.

1.5.1 Faradaic current in CV

The faradaic process abides by Faraday's law and represents charge/electron transport across the electrode solution interface [35]. Hence, the amount of electricity passed (charge) is proportional to the number of moles of reactant transformed. The current resulting from the analyte's redox reaction is called a faradaic current. It is useful to calculate the kinetic, transport, and thermodynamic parameters of redox systems [36].

1.5.2 Non-Faradaic current in CV

It involves charging the interface, like a capacitor, but without an electron transfer. The junction between the working electrode and the electrolyte behaves as the

capacitor. Therefore, the current is essential to modify the applied voltage to the working electrode. This current is known as non-faradaic or residual current. Charging current, the current generated by contaminants in the solvent, electrolyte, or electrode, and dissolved oxygen are the primary sources of residual current [9, 10]. A CV experiment's oscillating potential results in a fairly constant charging current that serves as the prime source of the background current. The faradaic current varies linearly with the square root of the scan rate, while the charging current varies linearly with the sweep rate [37].

1.6 Solvent

The choice of solvent for electrochemical operations depends on various physicochemical parameters [35, 38]. The significant features of solvent in this regard are,

- The solvent should be pure with a convenient liquid range
- It should dissolve the analyte completely
- It should possess strong solvent power
- It should not lead to an undesired reaction with the analyte or product
- It should have the appropriate acid-base characteristics
- It should have a sufficiently high dielectric constant

Universal solvent water is extensively used and is the least expensive. It dissolves numerous compounds of electrochemical interest effectively. Meanwhile, it is efficiently reduced or oxidized to H_2 or O_2. The outcome of this is, it has only 2.0V areas for the various studies and the formation of an oxide layer on the solid electrode surface also influences reproducibility and reactivity.

1.7 Supporting electrolyte

Supporting electrolytes are any ionic salts present in the solvent. They influence the electrochemical processes in various ways. The functions of the supporting electrolyte are:

- It should remain electroactive within the desired applied potential range
- They impart conductivity to the solvent and hence maintain constant current flow in the solution

- Higher supporting electrolyte concentration minimizes the analyte migration
- It should not be adsorbed on the electrode
- It should not involve in any undesirable reactions with the reactants or the products
- The supporting electrolyte primarily governs the acidity of the ionic solution
- They must avoid becoming adsorbed on the surface, where they could catalyze or hinder further processes.

Supporting electrolytes must be of high purity. Otherwise, it interferes with further studies. H_2SO_4, $HClO_4$, and HCl are used in acidic media while NaOH or KOH are employed in alkaline media. In neutral media, buffers like phosphate, acetate, citrate, and pyrophosphate can be made use of. Any electrolyte can be used if the redox reaction does not involve acid-base reactions. Any electrolyte and no need of any buffer in case the redox reaction does not involve acid/ bases in it.

1.8 Electrodes

The CV utilizes an electrochemical cell of the three-electrode system such as

- Reference electrode (RE)
- Auxiliary (AE) / counter electrode (CE)
- Working electrode (WE)

A current will flow when a potential difference is applied across the WE-solution interface. It is measured as a function of the applied potential. The function of AE is to allow the same current to flow through WE. In CV, the potential is applied between WE and RE, and current is recorded between WE and AE.

1.8.1 Reference electrode

RE is the standard electrode used for regulating the potential of WE. It is a potential device [39-43]. It provides a constant potential value, expressed as a potential difference, to which other potentials can be compared. The commonly used REs in the voltammetric technique is,

- Saturated calomel electrode (SCE, Hg / Hg_2Cl_2 / $KCl_{(saturated)}$, E^0= 0.244V vs. NHE at 25°C)
- Silver-silver chloride electrode (Ag /AgCl/ $KCl_{(saturated)}$, E^0= 0.222V vs. NHE at 25°C)
- Mercury-Mercury oxide electrode (Hg / HgO /H_2O, E^0= 0.244V vs. NHE at 25°C)

The efficiency of a potentiostat can be greatly influenced by RE, as they have negligible impedance. The other important features of RE include a reversible reference redox pair and a constant liquid junction potential.

1.8.2 Auxiliary or counter electrode

Only a minimal quantity of current passes through the reference electrode as the counter electrode furnishes an alternative path for current flow. The choice of the electrode depends on the chemical inertness in the specific analyte solution and the surface area provided. Generally, a coil of platinum wire is utilized. In non-corrosive conditions and where metal cation interference is not an issue even stainless steel, copper or aluminium wire may also be made use of. The current passing through the RE is kept near zero by using AE to drop off the current in the electrochemical cell mutually with large input resistance.

1.8.3 Working electrode

The WE are an electron conductor where essential electrochemical modifications take place. The operations of voltammetric procedures are greatly influenced by WE. Glassy carbon, graphite, carbon paste, various carbon, diamond, Pb, Au, Pt, Ag, etc., are some of the most typical solid inert WEs. Its shape can be a tiny wire or disc or sphere. It can also be a single crystal of metal or semiconductor, an evaporated thin film, or pressed to discs/pellets.

Several electrodes are constructed using a variety of carbon-based compounds as electrode substrates. The sensor surfaces can be conveniently renewed for electron exchange owing to the fragile properties of carbon. Large potential windows, minimal background current and greater surface activity describe carbon materials. The carbon material is incredibly inexpensive. As a result, carbon electrode materials are

extensively used in research works. CPE, PGE, and GCE are the three most often utilized carbon electrodes.

Smoothing the electrode surface is essential to make sure that there are no physical imperfections that could cause erratic responses. The desired properties of it are,

- ❖ It should have an extremely clean metal surface with a well characterized geometry
- ❖ It should have long-term stability and be inert to both electrochemical and chemical reactions
- ❖ It should be highly pure and have a reproducible surface
- ❖ It must have a wide potential window for both cathodic and anodic operations
- ❖ The WE should have the minimum geometrical area possible to have the maximum current density

1.9 The pathway of the mass transfer process

At the electrode surface, an electrochemical charge transfer process occurs. This results in the depletion of the electroactive substrate and the creation of a concentration slope at the electrode. When exploring electrode reactions at WE are using the electroanalytical approach, faradic processes are generally of interest. The movement of electroactive species arising by variations in electrical or chemical potential at the two places is depicted by mass transfer in electrochemistry. Diffusion, migration, and convection are the three types of mass transfer that have affects on the electrolysis reaction shown in Fig. 1.4

1.9.1 Diffusion

It refers to the natural migration of a species due to the concentration gradient. The concentration of the analyte at the WE surface will drop significantly when the analyte undergoes electrolysis. Consequently, more analytes diffuse from the bulk of the solution and flow toward the WE. It is commonly seen in quiescent solution voltammetry.

1.9.2 Migration

It refers to the movement of a charged species due to the potential gradient. By this method, the movement of charged species in the electrolytic solution occurs. The driving force behind this is purely electrostatic. In voltammetry, it is not a significant form of mass transfer. By introducing an excessive amount of a supportive electrolyte, the migration of the analyte is prevented but not the electrolyte.

1.9.3 Convection

It refers to the movement of a species occurring by mechanical forces. External mechanical energy from stirring, rotating, flowing solutions, or vibrating the electrode is the primary driving factor behind this. Additionally, it occurs because of temperature and density gradients. In typical voltammetric methods, it is prevented by maintaining a still, stable environment within the cell.

1.10 The electron transfer process

The important aspect of an electrode reaction is the exchange of electrons at the interface between the electrode and electrolyte. The formation of an electrical double layer at the electrode surface is due to the movement of electroactive species from the bulk of the solution through convention or diffusion is influenced by the electrode.

The species entering the double layer experiences structural orientation by gaining or losing the electron from or to the electrode surface when an appropriate voltage is applied giving the current. This state is referred to as the “transition state” of the reactant species. Due to its instability, the species transforms into a product by releasing activation energy and becoming reduced or oxidized. After proper reorientation, the product either deposits on the electrode surface or moves away from the electrode surface into the bulk solution. The movement of electrons to or from the substrate is a process that has been activated.

The electron transfer process can be

- Reversible process
- Irreversible process
- Quasi-reversible process

1.10.1 Reversible electron transfer process

If the electron transfer is faster compared to the mass transfer, the reaction is said to be electrochemically reversible. Both forward and reverse peaks are seen in reversible. Both forward and reverse peaks are seen in reversible systems, as illustrated in Fig. 1.5. It considers that both oxidized and reduced species are in equilibrium and reflects Nernst's behavior. In CV, a peak current recorded for the electrochemically reversible process can be calculated by Randles-Sevick's equation is given below

$$I_p = \left(2.69 \times 10^5\right)\ n^{3/2}\ A\ D_0^{1/2}\ C_0\ \upsilon^{1/2}$$

Where A is the electrode's area in cm^2, D_0 is the diffusion coefficient of the oxidized species in $cm^2\ s^{-1}$, C_0 is the oxidized species concentration in mol/cm^3. υ is the scan rate in Vs^{-1}, and n is the number of electrons involved in the stoichiometric reaction. The following are the diagnostic standard for electrochemically reversible systems at 25 ˚C.

- $\triangle E_p = E_{pa} - E_{pc} = 59.1/n$
- $E_0 = [\ E_{pa} + E_{pc}\ ] / 2$
- $I_{pa} / I_{pc} = 1$
- $I_p \propto \upsilon^{1/2}$
- E_p is independent of υ

1.10.2 Irreversible electron transfer process

If the electron transfer is slower than the mass transport process, the reaction is said to be electrochemically irreversible. Irreversible systems can only have one of two peaks: an oxidation peak or reduction peak as illustrated in Fig. 1.6. But sometimes, when there is a potential difference of more than 59.1/n mV, both peaks are seen. This system does not show Nernst behavior. The following equation is used for peak current measurement in CV for electrochemically irreversible systems.

$$I_p = \left(2.69 \times 10^5\right)\ n\ (\alpha n)^{1/2}\ A\ D_0^{1/2}\ C_0\ \upsilon^{1/2}$$

$$(\alpha n)^{1/2} = 47.7 / E_P - E_{p/2}$$

Where, α is the charge transfer coefficient.

The following are the diagnostic standard for electrochemically irreversible systems at 25˚C.

- No reverse peak
- $I_p \propto \upsilon^{1/2}$
- E_p shifts = 30/ αn mV
- $E_P - E_{p/2}$ = 47.7/ αn mV

1.10.3 Quasi- reversible electron transfer process

If the rates of mass and electron transfer are similar or competitive, the reaction is said to be electrochemically quasi-reversible. It is the intermediate case of reversible and irreversible systems. For quasi-reversible systems, both forward and reverse peaks are detected, as shown in Fig. 1.7. It does not show Nernst behavior. The following are the diagnostic standard for electrochemically quasi-reversible systems at 25˚C.

- I_p increase with υ, but not proportional to it
- I_{pa}/ I_{pc} = 1, provided α = 0.5
- $\triangle E_p$ is greater than 59.1/n mV and it increase with increasing υ
- E_p shifts with increasing υ

1.11 Applications of Cyclic voltammetry

The development of CV approaches is intended for both qualitative and quantitative applications. The CV technique can be utilized extensively for the estimation of various electroactive substances since the peak current response is directly associated to the change in concentration. In analytical chemistry, organic chemistry, physical chemistry, inorganic chemistry, biochemistry fields, etc., CV strategies are being used.

- It is used with high accuracy in analytical applications
- It is used for the measurement of E^0
- It finds application in the study of electrode mechanism
- In the qualitative and quantitative diagnosis of the electrode reactions it is made use of
- It also finds application in structural and functional group characterization

- ❖ It is used in the detection of an eluted analyte in high-performance liquid chromatography (HPLC) and flow injection analysis
- ❖ These methods are useful in both oxidation reduction process and to study the number of electrons involved in it
- ❖ In-vivo analysis in the rat brain, bacteria, and plants
- ❖ It is extensively used in the evaluation of thermodynamics and kinetic parameters (electron transfer, rate constant, diffusion coefficient of an analyte etc.
- ❖ It is also used in the development of sensors for the identification of some bioactive molecules

1.12 A brief literature survey of cyclic voltammetric investigation

Several researchers have reported several studies utilizing cyclic voltammetric techniques to explore the electrochemical properties of numerous analytes. The function of chemically modified electrodes in the field of electrochemical study of some biologically active compounds offers several advantages. The unique features of the working electrode can lower the overpotential, increase the reaction rate, and improve the selectivity and sensitivity in the analysis. Research preferences involve the modification of carbon paste, glassy carbon electrodes, and pencil graphite electrodes by modification methods like electropolymerization. Various biomolecules, including paracetamol, catechol, hydroquinone, uric acid, and adrenaline, have been studied using the fabricated sensor. The literature survey reveals the following prominent references.

Y. J. Chang *et al*, investigated a paper-based biosensor modified with graphene oxide and 5-amino-1,3,4-thiadiazole (ATT) by electropolymerization. It was used for the quantitative determination of uric acid in biological samples. The developed sensor showed good sensitivity and selectivity [44].

A. Kannan *et al.* reported selective and simultaneous determination of DA and PA using a glassy carbon electrode with electropolymerized AHMP thin film (Poly-AHMP/GCE). Poly-AHMP/GCE electrode showed good electrocatalytic activity and reduced over potential towards sensing of DA and compared to bare GCE [45].

S. Alpat *et al*, developed novel CPE modified with a multiwalled carbon nanotube (MWCNT), tyrosine, and Nafion membrane (CP/MWCNT/Tyr/Nafion) for the voltametric determination of epinephrine (EP) in the presence of AA and UA. The

results showed good sensitivity, selectivity, and stability [46].

W. Ren *et al.* reported simultaneous voltammetric measurement of ascorbic acid, epinephrine, and uric acid at a GCE modified with caffeic acid. The explored modified electrode exhibited potent and persistent electron mediating behavior followed by well-separated oxidation peaks towards AA, EP, and UA with activation over potential [47].

T. Tavana *et al.* have fabricated novel ionic liquid modified carbon nanotubes paste electrode (IL/CNTPE) by using hydrophilic ionic liquid 1-methyl-3-butylimidazolium bromide [MBIDZ]Br as a new binder. The formed sensor was successfully applied for the determination of EP and AC in human urine, pharmaceutical, and serum samples [48].

P. S. Ganesh *et al.* have investigated simple and simultaneous electrochemical sensor fabricated by electropolymerization of allura red on GCE for the interference - free detection of dihydroxy benzene isomers. They reported electrode kinetics as adsorption controlled. The LOD for CC and HQ was calculated to be 0.126 µM and 0.132 µM in the linear range of 0 to 80.0 µM and 0 to 110.0 µM, respectively [49].

S. A. Kumar *et al.* reported a new PAY/nano-TiO_2/GC hybrid film modified GC electrode fabrication, characterization, and its applications for the detection of DA and AP by CV and LSV. The results indicated that the hybrid film provided a good platform for the determination of DA and AP [50].

Rekha *et al.* carried out voltammetric study of poly (alcian blue) MCPE for the estimation of catechol in presence of hydroquinone. A good analytical performance in terms of sensitivity, selectivity, linearity, and detection limits have been observed [51].

P. S. Ganesh *et al.* investigated cyclic voltammetric electropolymerization and electrochemical characterization of celestine blue (CB) on GCE which was used for the electrolysis of HQ and CC. They reported the diffusion-controlled kinetics phenomenon at the MGCE [52].

N. Hareesha *et al,* investigated electrochemically polymerized DL-phenylalanine graphite electrode (poly (DLPA)-GE) for dopamine sensing. The developed electrode showed fine selectivity and sensitivity for DA analysis in the presence of interfering moieties such as AA, UA, and organic dyes [53].

S. S. Shankar *et al.* studied carbon quantum dot-MCPE based sensor for selective and sensitive determination of Adrenaline. The performance of the fabricated

electrode was highly reproducible and repeatable [54].

Y. Zao *et al.* reported the electrocatalytic oxidation of AA or UA on a Tb-doped Ta_2O_5 MGCE. In this, they report high catalytic properties at 0.1 M PBS pH=7.0 containing AA or UA [55].

B. B. Prasad *et al.* investigated one monomer doubly imprinted dendrimer nanofilm modified PGE for simultaneous estimation of norepinephrine and uric acid. It was used for the analysis of biological and pharmaceutical samples [56].

C. M. Kuskur *et al.* reported a voltammetric study of a poly (Eosin Y) film-based sensor for the determination of epinephrine in the presence of uric acid. The work provides a simple and easy approach to selectively determine EP in the presence of UA [57].

S. B. Tanuja *et al.* investigated nevirapine MCPE for the determination of paracetamol in presence of folic acid. The scan rate effect revealed that the electrode process was adsorption controlled [58]

R. Mangaiyarkarsi *et al.* reported the electrochemical performance of imidazolium ionic liquid crystal and carbon paste composite electrode for the sensitive detection of paracetamol. They observed excellent electrocatalytic activity, good stability, and the anti-interfering ability of the sensor [59].

S. M. Garcia *et al.* investigated biometric sensor selective to folic acid on carbon paste modified with graphene oxide and Fe_3O_4 nanoparticles coated with molecularly imprinted polymer in the coreq@shell format by polyol method. The advantages reported were the high robustness, selectivity, and low cost of the method [60].

Q. A. Moallem *et al.* developed a simple and sensitive electrochemical sensor (Cu- BTC MOF modified CPE and used for the detection of dopamine in presence of uric acid. During the electro-oxidation process of the analytes, two separate peaks were observed on the modified electrode [61].

H. Karimi-Maleh *et al.* investigated the simultaneous determination of cholesterol, ascorbic acid and uric acid at a CPE modified with copper oxide decorated reduced graphene with 1-methyl-3- octylimidazolium tetrafluoroborate. The work shows remarkable sensitivities towards the determination of the analytes and reports well-defined and clearly separated oxidation peaks [62].

M. Taei *et al.* fabricated AuNPs/PTAT/GCE by electrodeposition and electropolymerization. This sensor was effectively used for the detection of Cys, UA,

and Tyr in real samples [63].

J. X. Qiao *et al.* used pretreated GCE by electrochemical activation for the simultaneous investigation of epinephrine and uric acid. They have reported LOD under the optimum condition [64].

M. C. Rodriguez *et al.* reported the highly selective and sensitive uric acid quantification in the presence of ascorbic acid using GCE modified with multi-wall nanotubes dispersed in polylysine. The sensor made possible the clear definition of the oxidation processes of ascorbic acid and uric acid [65].

S. He *et al.* fabricated p-GLY/GO/GCE and used it for the simultaneous determination of DA, UA, GU, and AD. It exhibited prominent selectivity, reproducibility, and stability [66].

M. Buleandra *et al.* reported simultaneous determination of epinephrine and norepinephrine using an electrochemically activated PGE. It was sensitive, simple, reliable individual and/or simultaneous EP and NP determination [67].

1.13 Objectives of the thesis

The main goal of this thesis is to fabricate an electrochemical sensor by modifying the electrode surface (Carbon paste electrode, glassy carbon electrode, and pencil graphite electrode) to examine the electrochemical behavior of some biologically important organic compounds to meet the following objectives

- To select the modifier to modify the surface of the electrodes
- To establish the modified electrode for the electrochemical study of the biomolecules
- Determination of kinetics of modified electrode process
- Simultaneous and interference-free determination of analytes in a mixture by using CV and DPV techniques
- To achieve the lower detection limit

1.14 Scope of the present study

The aim of the thesis is to design electrochemical sensors by modifying the electrode surface (Carbon paste electrode, glassy carbon electrode, and pencil graphite electrode) by electropolymerization process. The modified electrode was used to

determine some biologically important organic compounds such as paracetamol, catechol, hydroquinone, uric acid, folic acid, dopamine, and adrenaline, both separately and simultaneously using cyclic voltammetric and differential pulse voltammetric methods.

Due to their unique electrode surface qualities, the fabrication of electrochemically modified electrodes offers several benefits. The aim of modification was to enhance the lower detection limit of the targeted analyte and make it free from the interferences by other molecules. These chemically altered electrodes can improve the selectivity of some biologically important organic compounds decrease over potential and speed up reactions.

In this work, a great deal of focus has been placed on investigating the electrode process near the electrode surface, including electro catalytic activities, the number of electrons involved, kinetics, and diffusion-controlled processes, the impact of the concentration of electroactive species, the pH effect, and low detection limit.

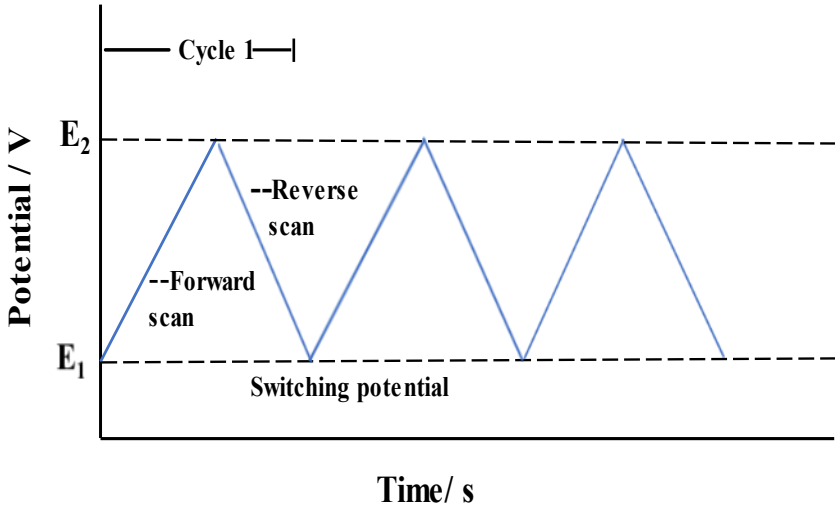


Fig. 1.1 Typical potential excitation signal in cyclic voltammetry

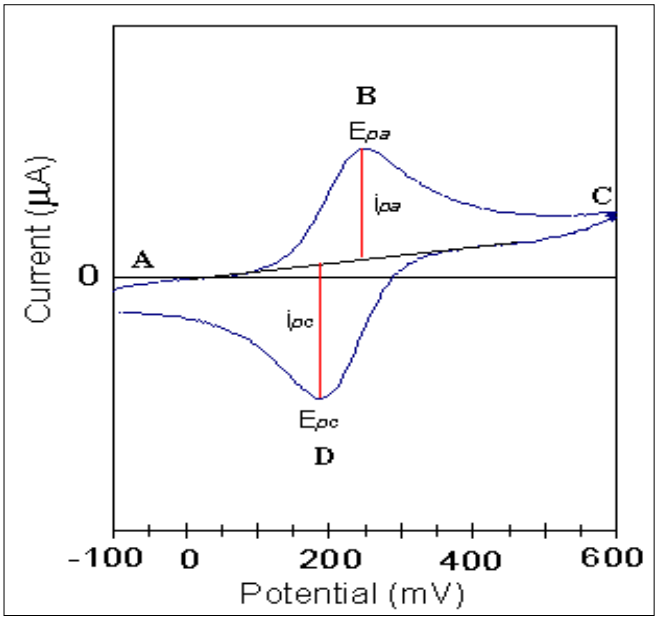


Fig. 1.2 Typical cyclic voltammogram for reversible process

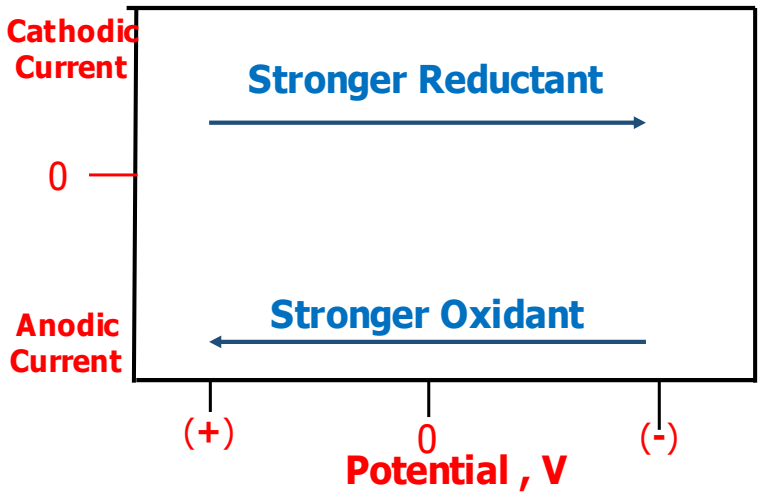


Fig. 1.3 Potential -current axes for cyclic voltammetry

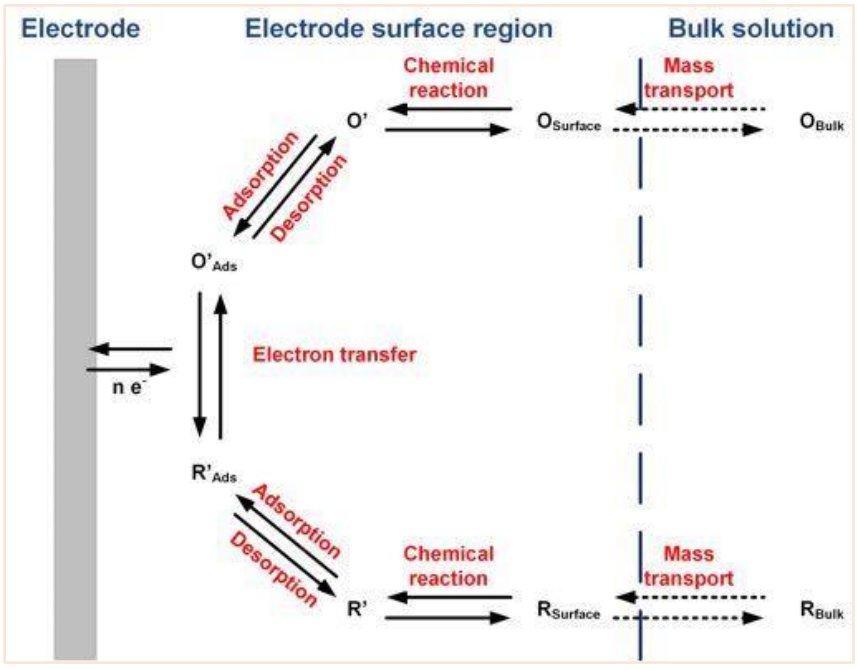


Fig. 1.4 Pathway of general electrode reaction

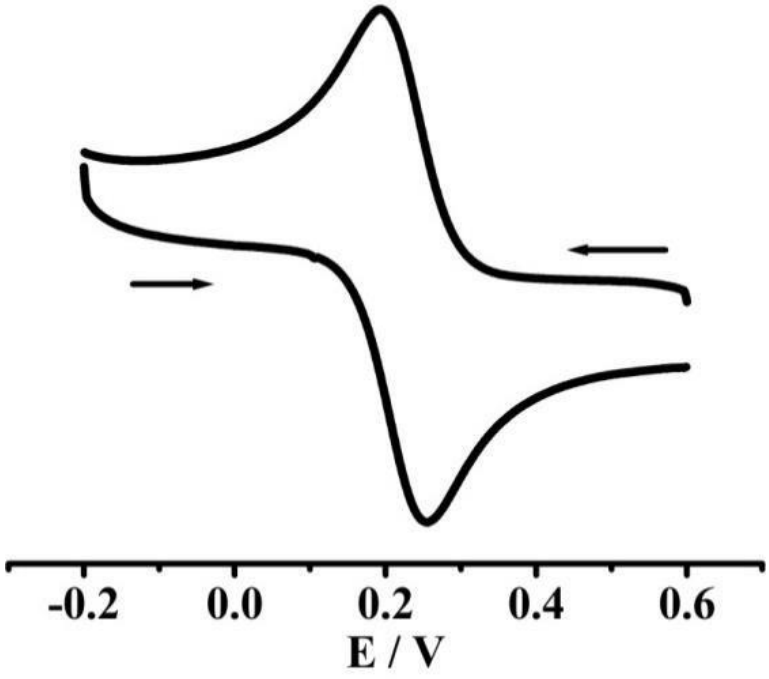


Fig. 1.5 Typical voltammogram for reversible process

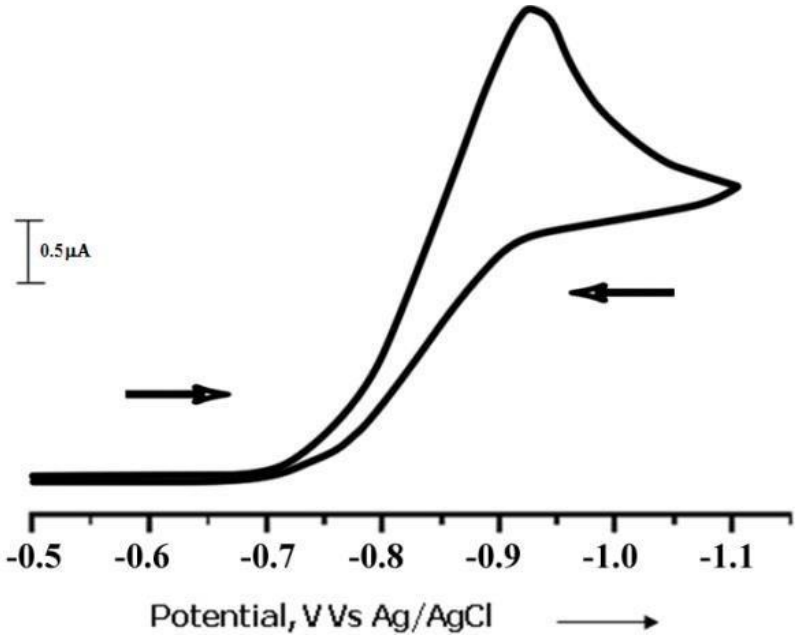


Fig. 1.6 Typical voltammogram for an irreversible process

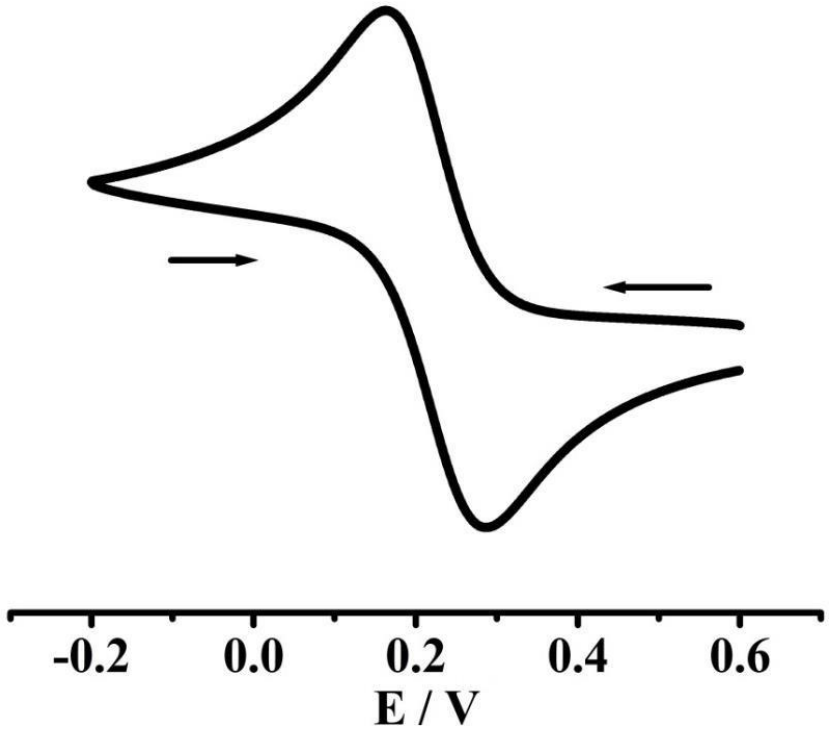


Fig. 1.7 Typical voltammogram for Quasi-reversible process

1.15 References

[1] S. Chia-Liang, S. Chun-Hao, W. Jhing-Jhou, *Biosens Bioelectron.,* 67 **(2015)** 327

[2] S. Mohammadi, H. Beitollahi, A. Mohadesi., *Sensor Lett.,* 11 **(2013)** 388

[3] T. Zahra, M. A. Mohammad, N. Hossein, H. Beitollahi, N. Maryam, Z. H. Reza. *Anal Sci.,* 24 **(2008)** 1039

[4] M. Mazloum-Ardakani, G. Bahram, H. Beitollahi, A. M. Kazem, M. Fakhradin, N. Hossein, *Electrochim. Acta.,* 56 **(2011)** 9113

[5] D. M. Fernandes, Costa Marta, P. Clara, B. Bachiller-Baeza, I. Rodriguez-Ramos, A. Guerrero- Ruiz, C. Freire, *J. Colloid Interface Sci.,* 432 **(2014)** 207

[6] H. Beitollahi, H. Karimi-Maleh, H. Khabazzadeh., Anal. Chem. 80 (2008) 9848

[7] T. I. Hudai. *J. Food Drug Anal.,* 22 **(2014)** 527

[8] O. Arias-Carrion, E. Poppel, Dopamine, learning and reward-seeking behavior, Act Neuro. Exp. 67 **(2007)** 481

[9] D. A. Skoog, D. M. West, F. J. Holler, S.R. Crouch, *Fundamentals of Analytical Chemistry*, 8th ed., Thomson Asia Pvt. Ltd., **2005**

[10] D. A. Skoog, F.J. Holler, S.R. Crouch, *Instrumental Analysis*, 6th ed., Cengage Learning India Pvt. Ltd., **2010**

[11] U. Yogeswaran, S. M. Chen, *Sensors.,* 8 **(2008)** 290

[12] G. S. Wilson, R. Gifford, *Biosens. Bioelectron.,* 20 (2005) 2388

[13] T. Sakaguchi, Y. Morioka, M. Yamasaki, J. Iwanaga, K. Beppu, H. Maeda, Y. Morita E. Tamiya, *Biosens. Bioelectron.,* 22 **(2007)** 1345

[14] O. Saglam, D. G. Dilgin, B. Ertek, Y. Dilgin, *Mater. Sci. Eng. C*. 60 **(2016)** 156

[15] G. A. Saleh, H. F. Askal, I. H. Refaat, A. H. Naggar, F. A. M. Abdelaal, *Arabian J. Chem.,* 9 **(2016)** 143

[16] S. C. Avendano, G. A. Angles, M. T. R. Silva, G. R. Pina, M. R. Romo, M. P. Pardave, *J. Electroanal. Chem.*, 609 **(2007)** 17

[17] Wei-Fang Hsu, Tzong-Ming Wu, *J. Mater. Sci: Mater. in Electron.,* 30 **(2019)** 8449

[18] Y. V. M. Reddy, B. Sravani, S. Agarwal, V. K. Gupta, G. Madhavi, *J. Electroanal. Chem.,* 820 **(2018)** 168

[19] E. Colın Orozco, S. Corona Avendano, M. Romero Romo, M. Palomar Pardave, M. T. Ramırez-Silva., *ECS Trans.* 36 **(2011)** 373

[20] J. G. Manjunatha, M. Deraman, N. H. Basri, I. A. Talib. *Adv. Mater. Res.,* 895 **(2014)** 447

[21] F. Pariente, E. Lorenzo, H. D. Abruna, *Anal. Chem.,* 66 **(1994)** 4337

[22] J. J. Sun, D. M. Zhou, H. Q. Fang, H. Y. Chen, *Talanta,* 45 **(1998)** 851

[23] J. Wang, D. B. Luo, P. A. M Farias, J. S. Mahmoud, *Anal. Chem.,* 57 **(1985)** 158

[24] J. G. Osteryoung, *Acc. Chem. Res.,* 26 **(1993)** 77

[25] J.G. Osteryoung, R.A. Osteryoung, *Anal. Chem.,* 57 **(1985)** 101A

[26] Randles, *Trans. Far. Soc.,* 44 **(1948)** 327

[27] A. E. Kaifer, M. G. Kaifer, *Supramolecular Electrochemistry* (Willey, VCH, New York) **1999**

[28] A. J. Bard, L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications,* (John Willey & Sons) **1996**

[29] R. S. Nicholson, *Anal. Chem.,* 37 **(1965)** 1351

[30] R. S. Nicholson, I. Shain, *Anal. Chem.,* 37 **(1965)** 190

[31] A. Salimi, H. Mam-Khezri, R. Hallaj, *Talanta,* 70 **(2006)** 823

[32] E. Katz, I. Willner, J. Wang, *Electroanal.,* 16 **(2004)** 19

[33] E. Gileadi, E. K. Eisner, J. Penciner, *Interfacial Chemistry: An Experimental Approach,* (Addison-Wesley, U.S.A), **1975**

[34] V. S. Bagotsky, *Fundamentals of Electrochemistry,* 2nd ed., Wiley Inter science, **2005**

[35] M. Noel, K. I. Vasu, *Cyclic Voltammetry and the Frontiers of Electrochemistry,* 1st ed., Oxford and IBH publishing Co. Pvt. Ltd., **(1990)** 104

[36] M. Faraday, Phil Trans. *Roy. So.c,* A 124 **(1834)** 77

[37] R. G. Compton, C. E. Banks, *Understanding Voltammetry,* 2nd ed., World Scientific Publishing Co. Pte. Ltd., **(2011)** 94

[38] B. E. Conway, H. Angerstein-Kozlowska, W.B.A. Sharp, E. Criddle, *Anal. Chem.,* 45 **(1973)** 1331

[39] P. Millet, J. *Chem. Edu.,* 73 (1996) 956

[40] C. Zhao, J. Xiao, T. Liu, H. Shi, Q. Li, Z. Ruan, *Int. J. Electrochem. Sci.,* 17 (2022) 22096

[41] I. Anshori, G. F. Mufiddin, I. F. Ramadhan, E. Ariasena, S. Harimurti, H. Yunkins, C. Kurniawan, *Sens. Bio-Sens. Re.,* 36 **(2022)** 100490

[42] R. Banu, B. E. K. Swamy, E. Ebenso, *Inorg. Chem. Commun.,* 145 **(2022)** 110013

[43] M. S. Vishwanath, B. E. K. Swamy, K. A. Vishnumurthy, *Mater. Chem. Phys.,* 289 **(2022)** 126443

[44] Y. J. Changa, M. C. Lee, Y. C. Chien, *SLAS Technology* 27 **(2022)** 54

[45] A. Kannan, R. Sevvel, *J. Electroanal. Chem.,* 791 **(2017)** 8

[46] F. Alpat, K. Özdemir, S. K. Alpat, *J. Sensors*, 5653975 **(2016)** 9

[47] W. Ren, H. Q. Luo, N. B. Li, *Biosen. Bioele.,*21 **(2006)** 1086

[48] T. Tavana, M. A. Khalilzadeh, H. Karimi-Maleh, A. A. Ensafi, H. Beitollahi, D. Zareyee, *J. Mol. Liq.,* 168 **(2012)** 69

[49] P. S. Ganesh, G. Shimoga, S. H. Lee, S. Y. Kim, E. E. Ebenso, *J. Anal. Sci. Technol.,* 12 **(2021)** 20

[50] S. A. Kumar, C. F. Tang, S. M. Chen, *Talanta* 76 **(2008)** 997

[51] Rekha, B. E. K. Swamy, P. S. Ganesh, *J Biosens Bioelectron* 7 **(2016)** 217

[52] P. S. Ganesh, G. Shimoga, S. H. Lee, S. Y. Kim, E. E. Ebenso, *ChemistrySelect* 6 **(2021)** 2379

[53] N. Hareesha, J. G. Manjunatha, *J. Electroanal. Chem.,* 878 **(2020)** 114533

[54] S. S. Shankar, R. M. Shereema, V. Ramachandran, T. V. Sruthi, V. B. S. Kumar, R. B. Rakhi, *ACS Omega*, 4 **(2019)** 7903

[55] Y. Zhao, C. Yan, P. Tian, L. Wu, Y. Song, P. Wang, X. Wang, *Mater. Chem. Phys.,* 250 **(2020)** 122958

[56] B. B. Prasad, S. Fatma, *Electrochim. Acta.,* 232 **(2017)** 474

[57] C. M. Kuskur, B. E. K. Swamy, H. Jayadevappa, K. Shivakumar, *Anal. Bioanal. Electrochem.,* 10 **(2018)** 1120

[58] S. B. Tanuja, B. E. K. Swamy, K. V. Pai, *J. Electroanal. Chem.,* 798 **(2017)** 17

[59] R. Mangaiyarkarasi, S. Premlatha, R. Khan, R. Pratibha, S. Umadevi, *J. Mol. Liq.,* 319 **(2020)** 114255

[60] S. M. Garcia, A. Wong, S. Khan, M. D.P.T. Sotomayor, *Talanta*, 229 **(2021)** 71

[61] Q. A. Moallem, H. Beitollahi, *Microchem. J.,* 177 **(2022)** 107261

[62] H. Karimi-Maleh, O. A. Arotiba, J. *Colloid Interface Sci.,* 560 **(2020)** 208

[63] M. Taei, F. Hasanpour, H. Salavati, S. H. Banitaba, F. Kazemi, *Mater. Sci. Eng: C,* 59 **(2016)**120

[64] J. X. Qiao, H. Q. Luo, N. B. Li, *Colloids Surf. B.,* 62 **(2008)** 31

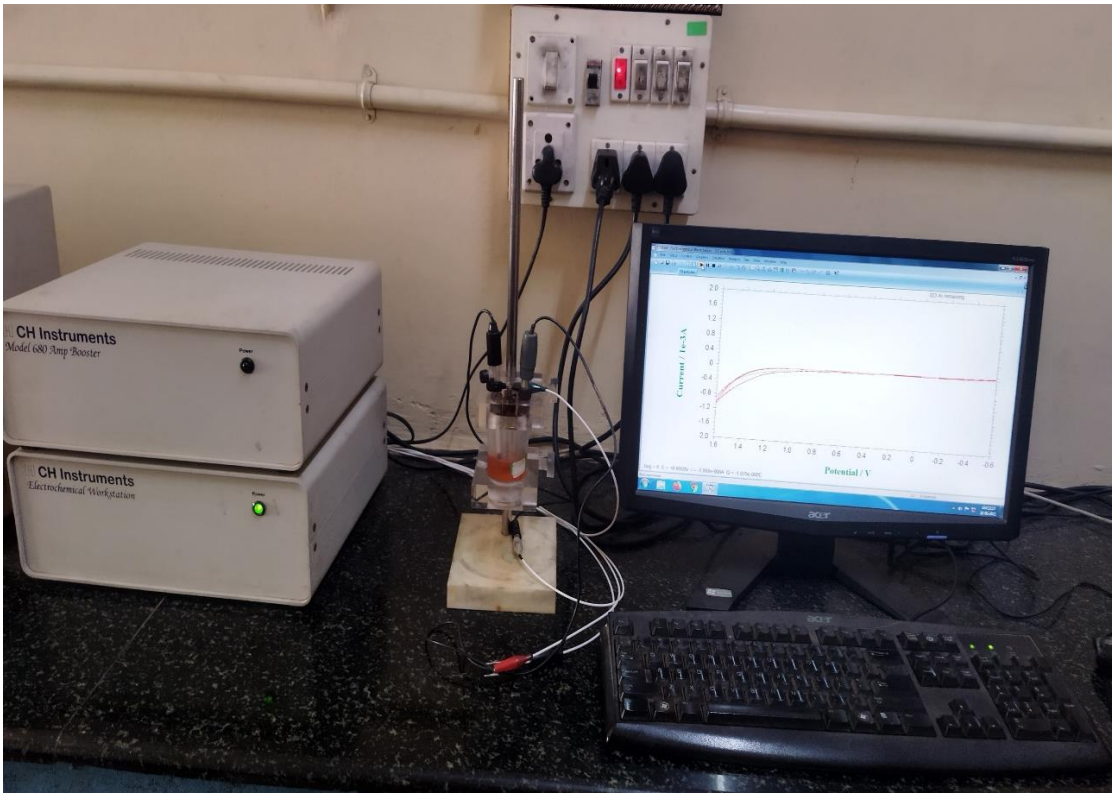
[65] M. C. Rodríguez, J. Sandoval, L. Galicia, S. Gutiérrez, G. A. Rivas, *Sen. Actuators B: Chem.*, 134 **(2008)** 559

[66] S. He, P. He, X. Zhang, X. Zhang, K. Liu, L. Jia, F. Dong, *Anal. Chim. Acta,* 1031 **(2018)** 75

[67] M. Buleandră, D. E. Popa, I. G. David, A. A. Ciucu, *Microchem. J.*, 160 **(2021)** 105621

Chapter -2

Experimental



2.1 Introduction

This chapter discusses the experimental procedures, instruments, and fundamental electrochemistry equipment such as a potentiostat, a recording device, and an electrochemical cell. The electrode systems employed in this study are described in detail, with a focus on carbon paste electrodes, glassy carbon electrodes, and pencil graphite electrodes. The fabrication and characterization of carbon paste electrodes, as well as their modification using the electropolymerization method and the procedures used in this work, are described in depth. Furthermore, an overview of the theories, characterization methodologies, and relevant equations are provided.

2.2 Experimental Techniques

Cyclic voltammetry and differential pulse voltammetry were the electroanalytical techniques used throughout this work. Each method is outlined briefly below.

2.2.1 Cyclic Voltammetry (CV)

Cyclic voltammetry is a dynamic electroanalytical approach in which the applied potential at the working electrode is swept between two predetermined potential limits while the current is measured. The scan rate is constant when this operation is carried out. The sweep is reversed and returned to the final potential (E_f), while the initially applied potential (E_i) is swept to a vertex potential (E_v), which is generally as same as the original potential (E_i). When this process is performed several times, it produces a cyclic effect. Therefore, CV is the most significant and frequently used technique in electroanalytical chemistry. It is typically used to obtain data on both simple and complex electrode reactions. CV was initially utilized to investigate the electrochemical properties of biologically electroactive compounds at modified and unmodified carbon paste electrodes, either individually or in the existence of potential interferences.

2.2.2 Differential Pulse Voltammetry (DPV)

Pulse voltammetry was designed to improve the sensitivity of voltammetric studies. If the double layer capacitance is reduced to zero, the recorded current will be

completely faradaic. There are different kinds of pulse voltammetry, including normal, differential, and square wave. The base potential is increased and incremented at a constant rate in DPV. At each stage, the current is measured both before and after the pulse is applied, while the magnitude of the pulse applied remains static. The difference between these two values as a function of the applied potential must then be recorded.

2.3 Instrumentation

2.3.1 Potentiostat

The potentiostat is connected to working, reference, and auxiliary (counter) electrodes immersed in an analyte placed in the electrolytic cell. It controls the potential of the working electrode (WE) concerning the reference electrode (RE) and measures the current flowing between WE and the auxiliary electrode (AE). The potentiostat serves three purposes:

- Controls the applied potential, which is the potential difference between the WE and RE (the applied potential determines which half-reactions occur at the WE)
- Allows current to flow between the WE and AE without passing through the RE (which would change its potential if current passes through it)
- Converts the cell current to a voltage for recording devices

The potential of the WE (relative to the RE) must be brought to the desired level in a short interval of time by the potentiostat. The time taken for controlling the WE potential by the potentiostat is referred to as a rise time. Except for the transfer of a small amount of current between the WE and RE, the internal feedback circuits of the potentiostat inhibit all types of current. Because the control of the electrode potential is a significant property of voltammetry, a function generator is required to give the potential sweep or pulse sequence to be applied to the WE. The potentiostat's input is the connection to the electrodes in the cell. The potentiostat's output are the signal lines that indicate the current and potential of the WE (Fig. 2.1)

The electrochemical experiments were performed using a potentiostat equipped with the Data Acquisition PC interface Card Model CHI-660c (CH instrument-660 electrochemical workstation, USA). The instrument has a high speed, high accuracy,

and an electrolysis mode that comprises a high-gain operational amplifier with controllable potential circuits. The voltammograms were all measured at the ambient temperature of 25 ± 0.5˚C. The WE current signal is monitored and handled uniquely. This signal line is also shown as a voltage signal; however, the voltage level is proportional to the current flowing through the WE. The potentiostat contains an internal 'current converter' circuit that performs the necessary current-to-voltage conversion automatically.

2.3.2 Recording Device

Computers were introduced in the electroanalytical instrument around 1967 [1]. There have been reports of computer applications in stationary electrode voltammetry and CV [2-6]. Through potentiostat, computers can be utilized to adapt potential programmes to the WE. The initial potential, final potential, can rate, pulse nature, current sensitivity, and other parameters can be digitally fed to the computer. Computers may be incredibly useful in data acquisition. An A/D converter may transform the applied values and consequent current values into digital information, which enhances the signal- to- noise ration of the experimental cyclic voltammograms. Each experiment may be replicated by a computer under identical conditions. Computers are used to analyze data. It accurately measures the peak current or peak potential [7, 8] by subtracting the background current [4]. Voltammetric curves can be distinguished to produce more precise peak potentials [9]. The information recorded, such as peak current, peak potential, and peak width at different concentrations, can then be related to the theoretical aspect of the nature of processes and to evaluate rate parameters.

2.3.3 Electrochemical Cell

In the current study, electroanalysis was performed using a standard three-electrode electrochemical cell. The electrolysis cell is a glassware that can hold a precise volume of an analyte solution containing one or more electroactive species. The cell is then kept oxygen-free by-passing nitrogen to the solution through the nitrogen inlet. The electrochemical cell is composed of three electrodes dipped in the solution and electrically connected to the potentiostat. Standard calomel electrode (SCE) was the RE used during the entire study. In all the studies, the platinum electrode (Pt) was served as the AE. The carbon paste electrode (CPE) or various modified carbon paste

electrodes (MCPE) were immersed in the solution (Fig. 2.2). Custom glassware designs include convenient fittings for mounting electrodes, as well as gas inlets and outlets for passing deoxygenating gas. The cell is thermostatically controlled to maintain the desired temperature. The cell is thermostated for the desired temperature because the limiting (peak) current in any sort of voltammetry is temperature dependent.

2.4 pH Meter

A Systronic Digital model 335 pH meter was used to measure and adjust the pH of the solutions using a combination of glass and SCE.

2.5 Scanning Electron Microscopy (SEM)

SEM is a technique for obtaining high-resolution image objects in which electrons, rather than light beams, are employed to generate an image. Such images offer information about the object's surface morphology and topography. The primary electrons are high-energy electron beams that hit the object's surface under study and eject the electrons from the surface of the object. Positive detectors capture these secondary electrons and convert them into signals. These signals are later amplified, analyzed, and translated into images. The sample used for SEM analysis must be conductive. Sample preparation for SEM analysis is quite easy.

2.6 Electrodes

The current work employs three electrode systems, namely RE, AE, and WEs. The RE taken was an SCE that was frequently isolated from the solution by a salt bridge to avoid contamination from RE leakage. The platinum (Pt) foil was used as AE. Carbon paste electrode (CPE)/modified carbon paste electrode (MCPE), glassy carbon electrode (GCE)/modified glassy carbon electrode (MGCE), and pencil graphite electrode (PGE)/modified pencil graphite electrode (MPGE) were used as WE's.

2.6.1 Working Electrodes

The working electrode material has a significant impact on the voltammetric procedure's performance. The WE should have a high signal-to-noise ratio and a repeatable response. As a result, the choice of WE are deduced by the redox behavior of the analyte under study as well as the background current over the potential region

required for the measurement. The other factors to be taken into account are potential windows, electrical conductivity, surface reproducibility, mechanical properties, cost, availability, and toxicity. WEs for electroanalytical applications are made from various types of materials. Those involving WEs are Pb, Au, Ag, Pt, glassy carbon, pencil graphite, carbon paste, diamond, indium, tin oxide etc., which are most commonly used.

In the current electroanalysis aspect, solid carbon electrodes are more appealing. They are widely used because of their broad potential window, low background current, affordability with ease of preparation, high chemical stability, and practicability for a diverse range of sensing and detection applications. Electron transfer rates observed on carbon surfaces, on the other hand, are slower than those observed on metal surfaces. The carbon surface morphology influences electron transfer activity. Carbon paste, graphite pencil, and glassy carbon are the most common carbon-electrode materials.

2.7 Development of Carbon Paste as Electrode Material

CPE and related sensors have been in rapid progress. Its inspiring history, displaying the potentialities of electrochemistry, reveals numerous connections with current electrochemistry trends. CPE was initially used mainly to examine the mechanisms of electrode reaction of various organic compounds [10]. The first modification, in which an organic compound was dissolved in binder [11], was considered a revolutionary step in the field of CPEs because it enabled us to study the electrode behavior of the substance itself. Modified CPE was first prepared in 1965 by rubbing a modifier into the paste [12].

CPEs have progressed considerably since their invention in 1958. The modifying agent is normally a single substance, but the paste can also be altered with two or more components. Modifiers can be dissolved directly in the binder [13-14] or mechanically mixed into the paste during homogenization [15]. It can also be achieved by soaking graphite in a modifier solution and then vaporizing the solvent [16]. Electropolymerization is the most efficient process for retaining a polymer layer on the electrode surface. Electropolymerization incorporates the repeated voltammetric scanning of a monomer-containing solution at the electrode surface with a specific potential window [17-18]. This can be oxidative or reductive voltammetric scanning, with monomer radicals joining to form a polymer on the electrode surface. The

multilayered polymer covering on the film thickness by adjusting the polymerization procedure parameters such as time, scan rate, potential range, and electrolyte type.

2.8 Carbon / Graphite Powder

The major constituent of carbon paste is finely grained carbon/graphite. This must operate effectively as an electrode material or sensor in electrochemical analysis. The utility of CPE is determined by the qualities of graphite powder and pasting liquid, as well as their ratio. Carbonaceous materials should fulfill the following aspects:

- The particles size of the micrometer range
- Uniform particle distribution over a specified dimension range
- High chemical purity
- Low adsorption capability

Generally, the typical properties of the carbon paste mixture depend on the type, quality, and quantity of graphite in the mixture. Even till date, the most commonly used carbon powder has been spectroscopic graphite with particles in the micrometric scale (typically 20- 50 µM).

2.9 Binders

The desirable qualities in binders are,

- Non-conducting
- Non-miscibility with aqueous solutions
- High chemically and electrochemically inertness
- High viscosity
- Non-volatility

The most commonly used binders are paraffin oil and various silicon oils. Pasting liquids also include organic esters such as tri cresyl phosphate [19-21], di-octyl phthalate, and di-nitrophenyloctyl. The electron transfer rate at the surfaces (slower kinetics) is reduced by using an excess of pasting liquid. This results in increased over potential compared to homogeneous electrodes. The high hydrophobicity of the liquid reduces the access of the analyte to the surface [22-26]. The degree of surface hydrophobicity of the surface can be reduced using pre-treatments.

2.10 Unmodified/Bare Carbon Paste Electrode

Bare carbon paste is a homogeneous mixture composed by combining an appropriate quantity of carbon powder and viscous liquid of non-electrolytic character [27]. In carbon pastes, the appropriate electroactive moiety is carbon powder with micrometric (20-50 µM) particles of high purity with uniform distribution. For this purpose, spectroscopic graphite available in the market is used. Non-electrolytic organic binders such as Nujol [28-30] and silicone oil [31] are used as pasting liquids. They satisfy all the important criteria such as chemically inertness, insulating behavior, non-volatility, water-immiscibility, and capability to form fine paste mixtures. Liquid organophosphate can also be made use of as binders. Even though they have high ion-pairing ability, they are less stable and exhibit typical signal-to-noise characteristics. This necessitates special pre-treatments.

2.11 Modified Carbon Paste Electrode

Generally, modified carbon paste is a combination of carbon powder, a non-electrolytic binder, and a modifier [23, 26, and 28]. The modifying agent is normally a single substance. However, the paste can also be modified with two or more components. The quantity of modifier in the paste usually ranges from 10 to 30% (w/w), depending on the modifying agent's nature and ability to form enough active sites in the modified paste, such as functional groups immobilized at the electrode surface [13] or molecules of an extractant in the bulk [19].

Modifying an electrode matrix enables us to develop a new sensor with desired, often pre-defined properties. Conductive substrates are modified with electroactive thin films, monolayers, or thick coatings in chemically modified carbon paste. The MCPEs are developed for a specific application which may not be possible with a bare CPE. This may lead to a significant rise in the electron transfer rate. Modifications can be brought about by mechanical grinding, self-assembled layers, covalently bonded electrolyzers, and surfactant immobilization, etc., surface modifications act as a catalytic agent, and minute changes in surface characteristics determine measurement sensitivity in electroanalytical applications.

Modification of the surfaces results in,

- Prevention of electrode surface fouling

- ❖ Transference of the modifier's physicochemical properties to the electrode
- ❖ Improved electrocatalytic activity owing to the use of materials with a large surface area. This result in improved current sensitivity
- ❖ Exhibition of improved selectivity and reproducibility towards the analyte due to immobilization of functional groups and dopants
- ❖ Increased diffusion rate in the case of some materials

2.12 Fabrication of Carbon Paste Electrode

CPE fabrication is based on a short Teflon rod (shaped like a robust plug) with a well drilled in it to refill with carbon paste. A graphite rod is placed at this same end of the Teflon tube, and the end of this graphite rod is connected to a copper wire that emerges from the other end of the Teflon tube to establish electrical contact with the external circuit. Various types of holders like glass, PVC tubes, and Teflon rods with end holes are used [32]. The actual diameter of the end-hole forming the proper carbon paste surface for common CPEs is chosen between 2 to 10 mm, which is suitable for the majority of electrochemical determinations [25].

2.13 Distinct properties and Advantages of CPE

- ❖ Low background currents less than 1µA resulting in higher signal-to-noise ratio in faradaic measurements
- ❖ Large potential window depending on the component quality and supporting electrolytes in it
- ❖ The composition of CPEs influences the specific reaction kinetics. As the hydrophobic nature of the CPE surface repels hydrophilic species, it influences the electrode reactions
- ❖ Simple fabrication procedure
- ❖ The surface is easily renewable
- ❖ Enhanced compatibility with various types of modifiers
- ❖ Inexpensive
- ❖ Possibilities for various modification procedures with detection limits as low as nanomolar
- ❖ Biocompatible with low toxicity

2.14 Storage and aging of CPE

To get reproducible results, the CPE is stored in a beaker filled with distilled water, with the tip filled with paste completely submerged in the water. This keeps carbon paste from drying out. The CPE stored in this manner is extremely stable. Carbon paste's irregular response was attributed to its aging effect. The response of carbon paste prepared with volatile binders such as organic esters change over time. CPEs achieved long-term stability for up to six months in our research study.

2.15 Preparation and Standardization procedures of CPE in present work

The reversibility of the electrode is influenced by an increase in the volume of the pasting liquid. The higher peak separations $\triangle E_p$ in the studied potassium ferrocyanide model system reflected this. The binder's lower volume and appropriate ratio offer stability to the electrode. The carbon powder to silicon oil ratio was optimized in this study to 0.24g: 0.04ml. The paste was homogenized by carefully mixing it with a pestle in an agate mortar. The freshly prepared carbon paste was inserted into the electrode's cavity. Fresh electrode surfaces were polished on weighing paper to get a smooth and shiny surface. the steps involved in the fabrication of the CPE are depicted schematically in Fig. 2.3. the electrode surface was mechanically regenerated after each analysis by removing some paste (3.0 mm) and polishing it on the weighing/tissue paper solution interface.

2.16 Glassy Carbon Electrode (GCE) as working electrode

Owing to its excellent mechanical and electrical properties, wide potential window, chemical inertness (solvent resistance), and relatively reproducible performance, GCE is widely known. Zittel and Miller [33,34] were the first to use glassy carbon as an electrode material. Yamada and Sato [27] explained the properties of glassy carbon.

Before conducting the electrochemical measurements, the GCE surface was polished to get a mirror-like finish with 0.05 mm gamma alumina slurry on a polishing pad. This was followed by extension rinsing with doubly distilled water between each polishing step.

2.17 Pencil Graphite Electrodes (PGE) as working electrode

PGE is a new type of carbon electrode that finds application in voltammetric methods [35-36]. When compared to other types of carbon electrodes, disposable PGE is affordable and provides an attractive alternative to "high-tech" carbon electrodes [37]. The experimental procedure for modifying carbon-based electrodes can be simplified by adding the modifying agent to the background electrolyte. This process is known as in situ modification.

A porous composite PGE is made up of graphite particles, a polymeric binder, and other ingredients such as clay. PGE has numerous applications in neurotransmitter analysis and metal ion detection. It is attributed to its high electrochemical reactivity, high electrical conductivity, good mechanical rigidity, low cost, ease of modification, renewal, low background current, and miniaturization [38- 41]. Because of its larger active surface area, it can detect low limits of concentrations [42-47].

The use of PGE has several advantages, including the avoidance of simple contamination, ease of use due to the lack of pre-treatment, consistent sensitivity, selectivity, and reproducibility.

2.18 Experimental procedure to record the Voltammograms

To prevent contamination, the electrochemical cell was extensively washed and dried. The desired concentration of analyte solutions was prepared and placed in the electrochemical cell along with the supporting electrolytes. The RE, AE and WE were dipped into the cell so that the electrode tip comes in contact with the analyte solution. In absence of an analyte, the blank voltammogram was recorded using the supporting electrolyte. Before each voltammetric measurement, the test solution was deoxygenated for 20 minutes with pure (99.9%) nitrogen gas. The potentiostat is set up to take measurements, the voltammogram was recorded using a known test solution in supporting electrolyte. Before performing any analysis, the electrode surface was renewed by removing some paste and filling the new one and then polishing the electrode on a transparent polishing paper. The experiments were carried out in an undisturbed solution, and all voltammograms were recorded at temperature of 25 ± 0.5 ˚C.

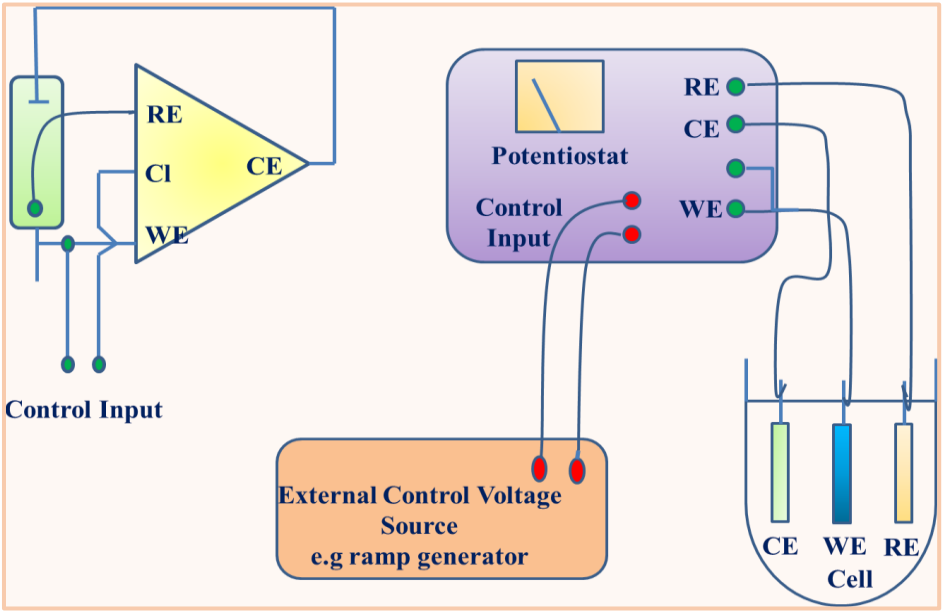


Fig. 2.1 Experimental set up of potentiostat for cyclic voltammetry

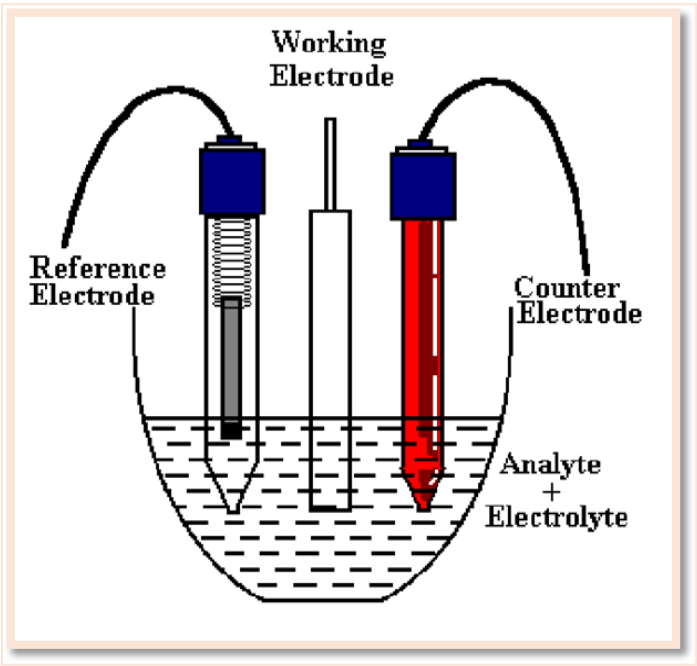


Fig. 2.2 Schematic representation of electrochemical cell

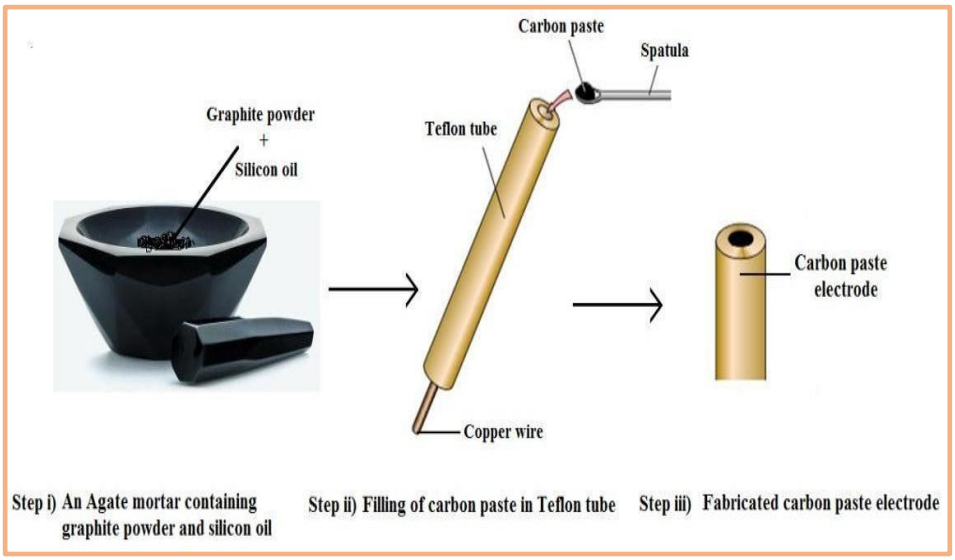


Fig. 2.3 Steps involved in the fabrication of carbon paste electrode

2.19 References

[1] G. Lauer, R. Abel, F. C. Anson, *Anal. Chem.,* 48 **(1976)** 1616

[2] S. P. Perone, J. E. Harrar, F. B. Stephens, R. E. Anderson, *Anal. Chem.,* 40 **(1968)** 899

[3] S. P. Perone, D. O. Jones, W. F. Gutknecht, *Anal. Chem.,* 41 **(1969)** 1154

[4] S. P. Perone, J. W. Frazer, A. Kray, *Anal. Chem.,* 43 **(1971)** 1485

[5] S. C. Creason, R. J. Loyd, D. E. Smith, *Anal. Chem.,* 44 **(1972)** 1159

[6] P. E. Whiston, H. W. V. Born, D. H. Evans, *Anal. Chem.,* 45 **(1973)** 1298

[7] B. Aalstad, V. D. Parker, *J. Electroanal. Chem.,* 112 **(1980)** 163

[8] R. Eliason, V. D. Parker, *J. Electroanal. Chem.,* 170 **(1984)** 347

[9] I. Svancara, K. Vytras, K. Kalcher, A. Walcarius, J. Wang, *Electroanal.,* 21 **(2009)**7

[10] R. N. Adams, *Electrochemistry at Solid Electrodes,* (M. Dekker, New York) **1969**

[11] J. Wang, *Electroanalysis* 11 **(1999)** 283

[12] T. Kuwana, W. G. French, *Anal. Chem.*, 36 **(1964)** 241

[13] Z. Q. Zhang, H. Liu, H. Zhang, Y. F. Li, *Anal. Chim Acta.,* 333 **(1996)** 119

[14] S. S. Huang, Y. D. Cheng, B. F. Li, G. D. Liu, *Microchim. Acta.,* 130 **(1998)** 97

[15] R. Metelka, K. V. Vytras, A. Martinez, M. T. Ramirez, I. Gonzalez, *Electroanal.,* 10 **(1998)** 336

[16] Q. L. Chi, W. Goepel, T. Ruzgas, L. Gorton, P. Heiduschka, *Electroanal.,* 9 **(1997)** 357

[17] S. Griveau, J. Pavez, J. H. Zagal, Bedioui, *J. Electroanal. Chem.*, 497 **(2001)** 75

[18] T. F. Kang, G. L. Shen, R. Q. Yu, *Anal. Chim. Acta.,* 356 **(1997)** 245

[19] I. Svancara, K. Kalcher, W. Diewuld, K. Vytras, *Electroanal.,* 8 **(1996)** 336

[20] I. Svancara, M. Pravda, Hvizdalova K. Vytras, K. Kalcher, *Electroanal,* 6 **(1994)** 663

[21] I. Svancara, K. Vytras, *Anal. Chim. Acta.,* 273 **(1993)** 195

[22] K. Kalcher, J.M. Kauffmann, J. Wang, I. Svancara, K. Vytras, C. Neuhold, Z. Yang, *Electroanal,* 7 **(1995)** 5

[23] K. Kalcher, *Electroanal.,* 2 **(1990)** 419

[24] R. N. Adams, *Rev. Polarog.,* 11 **(1963)** 71

[25] M. Sajid, M. K. Nazal, M. Mansha, A. Alsharaa, S. M. S. Jillani, C. Basheer,

Trends in Anal.Chem., 76 **(2016)** 15

[26] K. Kalcher, K. Schachl, I. Svancara, K. Vytras, H. *Alemu, Sci. Pap. Univ. Pardubice, Ser. A* **1997**

[27] R. N. Adams, *Anal. Chem.*, 30 **(1958)** 1576

[28] K. Kalcher, X. H. Cai, G. Kolbl, I. Svancara, K. Vytras, *Sb. Ved. Pr., Vys. Sk. Chemickotechnol, Pardubice*, 57 **(1994)** 5

[29] W. Sun, M. Yang, K. Jiao, *Anal. Bioanal. Chem.*, 389 **(2007)** 1283

[30] L. Gorton, *Electroanal.*, 7 **(1995)** 23

[31] L. Hernandez, P. Hernandez, M. H. Blanco, M. Sanchez, *Analyst.*, 113 **(1988)** 41

[32] W. Diewald, K. Kalcher, C. Neuhold, I. Svancara, X. H. Cai, *Analyst.*, 119 **(1994)** 299

[33] J. Zak, T. Kuwana, *J. Electroanal. Chem.*, 150 **(1983)** 645

[34] R. Banu, B. E. K. Swamy, E. Ebenso, *Inorg. Chem. Commun.*, 145 **(2022)** 110013

[35] G. Altiokka, M. Altiokka, Pharmazie 57 **(2002)** 500

[36] R. N. Goyal, S. Bishnoi, *Bioelectrochem.*, 79 **(2010)** 240

[37] A. M. Bond, P. J. Mahon, J. Schiewe, V. Vicente-Beckett, *Anal. Chim. Acta.*, 345 **(1997)** 67

[38] W. Gao, J. Song, N. Wu, *J. Electroanal. Chem.*, 576 **(2005)** 7

[39] D. Demetriades, A. Economou, A. Voulgaropoulos, *Anal. Chimi. Acta.*, 519 **(2004)** 172

[40] H. Karadeniz, B. Gulmez, F. Sahinci, *J. Pharm. Biomed. Anal.*, 33 **(2003)** 302

[41] A. Levent, Y. Yardim, Z. Senturk, *Electrochim. Acta.*, 55 **(2009)** 195

[42] M. Vestergaard, K. Kerman, E. Tamiya, *Anal. Chim. Acta*, 538 **(2005)** 281

[43] A. Erdem, P. Papakonstantinou, H. Murphy, *Anal. Chem.*, 78 **(2006)** 6659

[44] M. Mazloum-Ardakani, G. Bahram, H. Beitollahi, A. M. Kazem, M. Fakhradin, N. Hossein, *Electrochim. Acta.*, 56 **(2011)** 9113

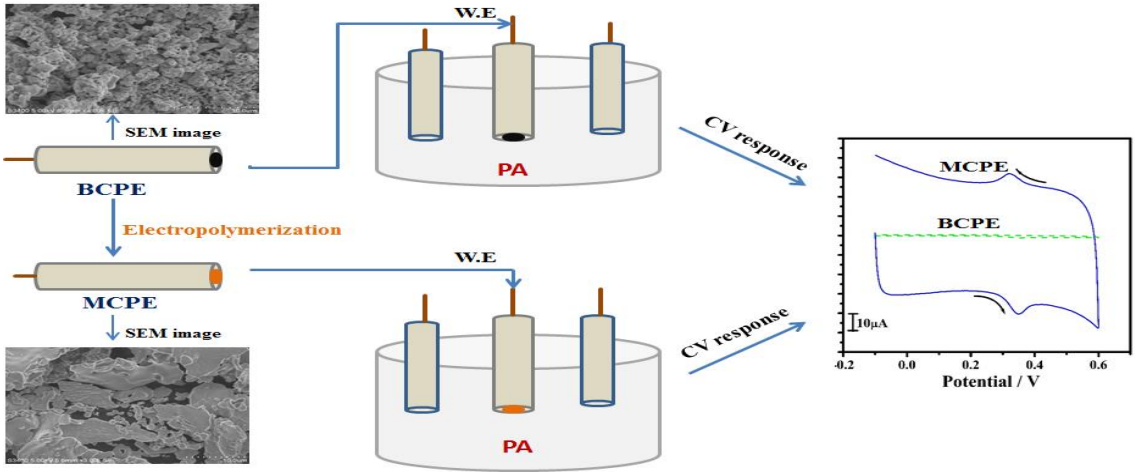
[45] M. S. Vishwanath, B. E. K. Swamy, K. A. Vishnumurthy, *Mater. Chem. Phys.*, 289 **(2022)** 126443

[46] J. K. Shashikumara, B. E. K. Swamy, S. C. Sharma, S. A. Hariprasad, K. Mohanty, *Sci. Rep.*, 11 **(2021)** 14310

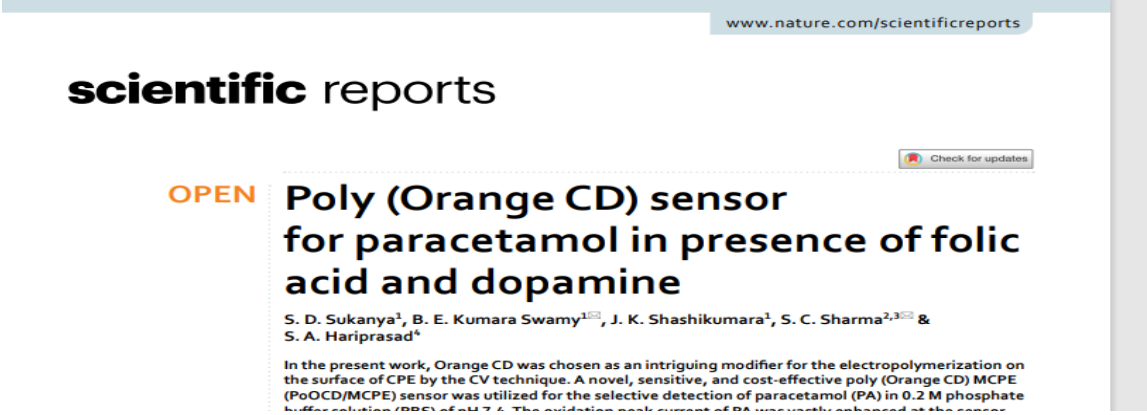
[47] K. Chethankumar, B. E. K. Swamy, *Anal. Bioanal. Electrochem.*,14 **(2022)** 753

Chapter -3

Poly (Orange CD) sensor for paracetamol in presence of folic acid and dopamine



Published in
Scientific Reports 11(2021) 22332


www.nature.com/scientificreports

scientific reports

Check for updates

OPEN

Poly (Orange CD) sensor for paracetamol in presence of folic acid and dopamine

S. D. Sukanya[1], B. E. Kumara Swamy[1]✉, J. K. Shashikumara[1], S. C. Sharma[2,3]✉ & S. A. Hariprasad[4]

In the present work, Orange CD was chosen as an intriguing modifier for the electropolymerization on the surface of CPE by the CV technique. A novel, sensitive, and cost-effective poly (Orange CD) MCPE (PoOCD/MCPE) sensor was utilized for the selective detection of paracetamol (PA) in 0.2 M phosphate

3.1 Introduction

Paracetamol (PA) is one of the most extensively used analgesics and antipyretic drugs in clinical practice [1, 2]. It is a very effective agent recommended for mild to moderate pain alleviation such as flu-induced fever, migraine, arthritis, and extenuates pain (headache, toothache, joint, muscular, chronic, postoperative) [3, 4]. PA relieves pain by inhibiting prostaglandin synthesis in the central nervous system, and it also relieves fever by sedating the hypothalamus heat-regulating center [5]. PA is easily degraded by glucuronidation and sulfation into inactive metabolites, which are excreted in the urine, with just 5% of PA remaining unaltered [6]. In general, PA is known to have an excellent safety profile at approved therapeutic doses. But its toxic metabolite accumulation in case of overdosing and chronic use lead to harmful side effects such as liver problem, kidney damages, trembling, nervousness, seizures, insomnia, and nausea and even death [7-10]. Therefore, developing a simple, fast response, economical, sensitive, accurate, and reliable detection method for the assessment of PA is highly demanded in the medical field. There are lots of methods like capillary electrophoresis [11], titrimetry [12], SEC, LC-MS, HPLC [13-15], chemiluminescence [16], spectrofluorimetric [17], and spectrophotometry [18-20] which have been availed for the assessment of PA. Among all these methods, the electrochemical method stands out with its simplicity, sensitivity, selectivity, modest and fast response.

Folic acid (FA) is water-soluble vitamin B_9 and known as folacin that helps the growth of healthy new cells especially during pregnancy and controls the generation of ferroheme. FA is involved in a variety of biological tasks related to cell metabolism, including DNA replication, repair, and methylation, as well as the production of nucleotides, vitamins, and amino acids. Deficiency of FA causes anemia, leucopoenia, devolution of mentality, neurosis and increases the chances of heart attack and stroke [21-24]. Dopamine (DA) is the neurotransmitter involved in the functioning of the central nervous system. DA is also utilized as an injectable medicine that stimulates the sympathetic nervous system, causing effects such as increased blood pressure and heart rate. Deficiency of DA may cause disorders like Parkinson's disease, Schizophrenia, Alzheimer's disease, and HIV infection [25-28]. When used for a long time, no steroidal anti-inflammatory agent like PA can prevent FA from being absorbed by the human being. The simultaneous measurement of PA and FA is particularly relevant since PA

enhances the need for FA. The usage of PA protects dopaminergic neurons against oxidative stress damage produced by acute exposure to increased amounts of DA, according to in vitro studies. Furthermore, prolonged PA use in in vivo model has been shown to dramatically lower DA levels. Selective or simultaneous detection of PA, FA, and DA have been achieved by the voltammetric method due to their electroactive natures [29-32]. In the electrochemical sensor field, electropolymerized MCPE has made a great contribution to the determination of biomolecules because of their good stability, homogeneity, strong adhesion of polymer film onto the electrode surface, more active sites, fine reproducibility, fine resolution voltammogram, low-cost, and easy preparation method [33-36]. As redox dyes are artificial electron donatives, they are effective to undergo electropolymerization and produce stable redox-active films [37, 38].

This chapter explores, less studied Orange CD (Scheme 3.1) dye [39] as a modifier for the electropolymerization on the CPE surface by CV technique. The performance of PoOCD/MCPE was assessed for the sensitive, selective determination of PA and simultaneous determination of PA, FA, and DA in biological pH 7.4. The sensor displayed higher electrocatalytic activity, as well as a low detection limit and large linear ranges for PA resolution. The practical applicability of the sensor has been tested by determining PA in tablets successfully. This work is intended to pave the way for the development of more efficient, dependable, and generally affordable sensors.

3.2 Experimental

3.2.1. Materials and instrumentation

All analytical grade chemicals such as PA (M_{wt} = 151.16 $gmol^{-1}$, purity 99 %), FA (M_{wt} = 441.40 $gmol^{-1}$, purity 99.5 %), DA (M_{wt} = 189.64 $gmol^{-1}$, purity 99 %), graphite powder, Na_2HPO_4, and NaH_2PO_4. H_2O was procured from Merck Chemicals (Mumbai, India) and Orange CD dye from Astik Dyestuff Pvt. Ltd (Gujarat, India). Stock solutions of orange CD, PA, FA, and DA with a concentration of $25x10^{-4}$ M were prepared in double-distilled water (DDW). The 0.2 M PBS was prepared by Na_2HPO_4 and $NaH_2PO_4.H_2O$.

Voltammetric measurements were conducted in CHI-660c model (CH Instrument-660 electrochemical workstation, USA) analytical system. An

electrochemical cell (25ml) consisting of saturated Calomel electrode (Equip-tronics, Mumbai), platinum wire (Equip- tronics, Mumbai)) and bare CPE or PoOCD/MCPE, were acted as a reference, counter, and working electrodes respectively at room temperature.

3.2.2. Preparation of paracetamol tablet sample

In a mortar, a 500mg of Calpol pill was acquired from local drug stores (Shivamogga, India) was finely pulverized. In a 100ml flask, an adequate amount of homogenous white powder was dissolved in water. The solution was thoroughly agitated to get the appropriate concentration before being utilized in pharmaceutical sample analysis.

3.2.3. Working electrode construction

The bare CPE was prepared as described in the literature [40]. The PoOCD/MCPE was constructed by dipping bare CPE into 1 mM aqueous Orange CD with NaOH (0.1 M) as a supporting electrolyte. The electrochemical polymerization was performed at the potential between -0.6 to 1.6 V with a scan rate (SR) of 100 mV s^{-1} using 10 cycles. Then obtained electropolymerized electrode was rinsed in the DDW to eliminate unreacted molecules.

3.3 Results and discussion

3.3.1 Electrochemical polymerization of Orange CD on bare CPE

Fig.3.1 shows the CVs of electrochemical polymerization of 1 mM aqueous orange CD with NaOH (0.1 M) on the bare CPE surface in the potential cycling between -0.6 to 1.6 V with SR of 100 mVs^{-1} using 10 cycles. The examination of voltammograms by gradually increasing the progressing electropolymerization procedure reveals accumulation and growth of orange CD film on the surface of bare CPE [41]. The polymer film thickness affects the electrochemical response of the modified electrode. The film thickness was easily managed by regulating the number of voltammetric scans from 5 to 25 during electropolymerization. The experimental results analogous to it were obtained for the PA as shown in Fig.3.2. As the current response achieves a maximum at ten multiple cycles, the optimum cycle number of ten was selected for the construction of PoOCD/MCPE and further voltammetric measurements.

3.3.2 Characterization of PoOCD/MCPE

For investigation of electrocatalytic activity of the MCPE, a potassium ferrocyanide system was used. Fig.3.3 displays the electrochemical activity of $K_4[Fe(CN)_6]$ (freshly prepared) at bare CPE (A) and PoOCD/MCPE (B) containing 1 M KCI as supporting electrolyte obtained at an SR of 100 mVs^{-1} was recorded by CV method. The small redox peak current signal corresponds to bare CPE while PoOCD/MCPE shows enhanced peak current showing the dramatic increase in the rate of electron transfer [33]. According to Randles-Sevick's equation (3.1), the electrocatalytic surface area of both bare CPE and MCPE was calculated [42].

$$Ip = (2.69 \times 10^5)\, n^{3/2}\, A\, D^{1/2}\, \nu^{1/2}\, C \ldots\ldots\ldots\ldots\ldots (3.1)$$

The area of bare CPE ($0.0295cm^2$) is less than PoOCD/MCPE area ($0.0499cm^2$) which indicates that Orange CD acts as an effective modifier contributing a large surface and promote the electron transfer between the electrode and the solution.

The surface morphological features of bare CPE and PoOCD/MCPE were characterized by SEM. The SEM of bare CPE (Fig.3.4a) appears to be a rough surface with irregularly shaped and PoOCD/MCPE (Fig.3.4b) appears to be a smooth with consistent ordering of the polymer film of Orange CD on the CPE surface. The remarkable distinction in the surface structure of both electrodes confirms the remarkable modification of the CPE surface by electropolymerized Orange CD.

3.3.3 Voltammetric measurements

The electrochemical response of PA was studied on the bare CPE (A) and PoOCD/MCPE (B) in 0.2 M PBS (pH 7.4) at an SR 100 mVs^{-1} by CV method as displayed in Fig.3.5. An irreversible voltammogram was obtained at bare CPE for PA with an anodic peak potential of 0.357 V indicating the poor response as well as the occurrence of only oxidation. But at the same condition, PoOCD/MCPE exhibited a significant increase in the current signals giving a sharp reversible voltammogram. The anodic and cathodic peak potential for PA were found to be 0.349 V and 0.320 V respectively reveals the occurrence of both oxidation and reduction at proposed PoOCD/MCPE.

The impact of potential scan rate (SR) for the electrochemical studies of 0.1 mM PA in 0.2 M PBS (pH 7.4) from 50-500 mVs^{-1} was investigated by the CV method at PoOCD/MCPE as depicted in Fig.3.6. It is found that the redox peak currents rise with rising scan rates. The electrode phenomenon is controlled by adsorption at PoOCD/MCPE for PA as deduced from the good linearity with regression equations Ipa (µA) = 0.34 υ (mV/s) + 5.56 (µA) (R^2 =0.9998), Ipa (µA) = 1.02 υ (mV/s) – 6.39 (µA) (R^2 = 0.9909) and Ipa (µA) = 0.90 log υ (V/s) – 6.21 (µA) (R^2 = 0.9998) of the Ipa vs SR (Fig.3.7), Ipa vs square root of SR (Fig.3.8) and log Ipa vs log SR (Fig.3.9) plots respectively [43, 44]. The heterogeneous rate constant (k^0) was estimated for such voltammograms whose ΔEp (experimental peak potential difference) values are greater than 10 mV using the equation (3.2) [45] and the results were incorporated in Table 3.1.

$$\Delta Ep = 201.39 \log (\nu / k^0) - 301.78 \ldots\ldots\ldots (3.2)$$

The effect of PA concentration on redox behavior was studied at PoOCD/MCPE. Fig.3.10 depicts the CVs of 10-60 µM PA at PoOCD/MCPE in PBS (pH 7.4) at the SR of 50 mVs^{-1}. By increasing PA concentration, the redox peak current gradually increased. Ipa vs PA concentration (Fig.3.11) plot shows good linearity with regression equation Ipa (µA) = 0.7 (µM) + 6.38 (µA) (R^2 =0.9990). LOD and LOQ were calculated according to the Eqns. (3.3) and (3.4) [6, 46] for PA were found to be 2.64 µM and 8.81 µM respectively. The LOD of this modified electrode for the estimation of PA in comparison to other reported electrodes is given in Table 3.2.

$$LOD = 3S/M \ldots\ldots\ldots\ldots\ldots (3.3)$$

$$LOQ = 10S/M \ldots\ldots\ldots\ldots\ldots (3.4)$$

Where S is the standard deviation, M is the slope.

The pH plays a remarkable role in asses the number of participating electrons and protons in the oxidation mechanisms of the PA. The increase of pH (6.2 to 7.8) over PA (10µM) oxidation at PoOCD/MCPE shifts Epa towards a more negative direction as analyzed by CV are shown in Fig.3.12. Fig.3.13 illustrates the Epa vs pH values of PA graph that are linear with a slope of 0.0601 V/pH (R^2= 0.995). This suggests that during the oxidation of the PA, the same number of protons and electrons are participated [3, 47] and the possible electrooxidation was shown in Scheme 3.2.

3.3.4 Simultaneous resolution of analytes PA, FA, and DA

This study aimed to utilize the developed sensor for the selective and sensitive estimation of PA in the existence of FA and DA. Fig.3.14 illustrates the CVs recorded for the equimolar mixture (0.1 mM) of analytes PA, FA, and DA in 0.2 M PBS (pH 7.4) at SR 50mVs^{-1} at bare CPE (a) and PoOCD/MCPE (b). At bare CPE, a low current signal with poor sensitivity was observed. However, in the same condition, the PoOCD/MCPE has shown a higher current signal with improved sensitivity for oxidation of DA, PA, and FA at 0.134V, 0.408V, and 0.695V, respectively. Hence, the developed PoOCD/MCPE serves as an excellent sensor for the PA.

3.3.5 Interference studies

Studies were conducted by the DPV method in the solution mixture containing PA, FA, and DA at PoOCD/MCPE. The concentration of one analyte was varied, whereas the others were kept constant. Fig.3.15 illustrates the DPVs of PA by increasing the concentration of PA from 10 to 60 µM when holding the concentration of FA and DA constant. The oxidation peak current of PA increased linearly with increasing PA concentration from 10 to 60 µM and anodic peak current for FA and DA remaining constant. Similarly, it was also observed that the peak potentials remain unaltered with any enhancement in the peak current for the other two analytes. Fig.3.16 and Fig.3.17 self illustrates the DVPs of FA (from 10 to 50µM) and DA (from 10 to 60µM) by keeping the other two analytes constant. These observations reveal that the oxidation of PA, FA, and DA has negligible influence on the variation of the other analytes. Therefore, PoOCD/MCPE showed good selectivity and sensitivity for the resolution of PA, FA, and DA.

3.3.6 Repeatability, reproducibility, and stability study

The repeatability of the PoOCD/MCPE for 0.1 mM PA in 0.2 M PBS (pH 7.4) was examined through five successive measurements and the RSD value of 2.3% demonstrates the superior repeatability of the MCPE. The reproducibility of the MCPE was investigated by fabricating five different MCPE under the same conditions. The RSD value obtained to be 4.8% confirms the good reproducibility. The stability of the MCPE was studied by 15 successive cycles remained 98% of its original current

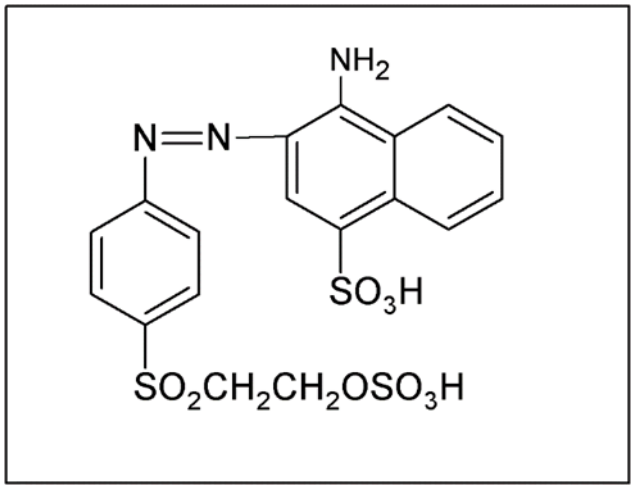
response for PA even after 15 cycles shows the good stability of the PoOCD/MCPE.

3.3.7 Determination of PA in tablet sample

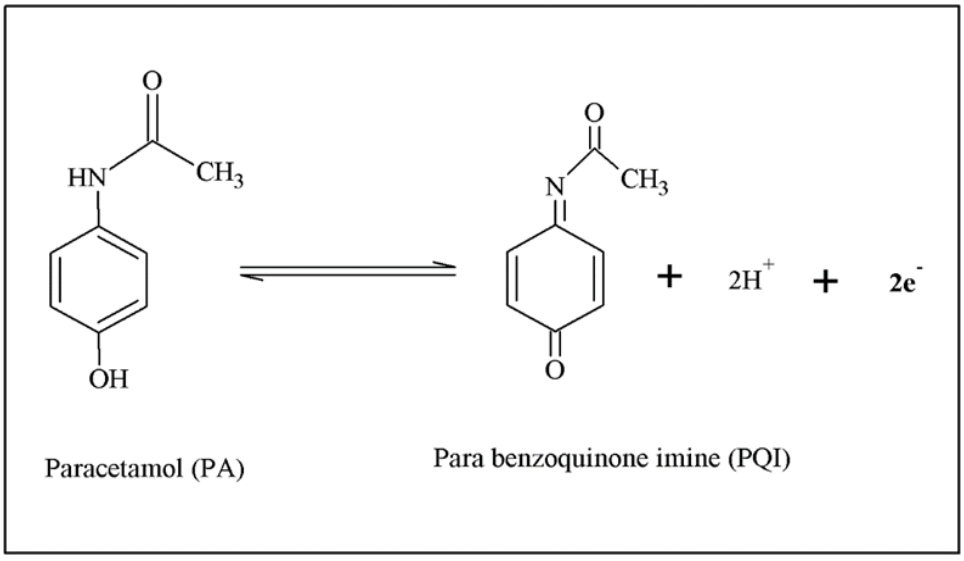
To evaluate the efficacy of PoOCD/MCPE in practical analysis, PA was successfully determined in tablet (Calpol 500mg) by the CV method. The recovery test was done using the standard addition technique and the obtained results for four consecutive PA concentrations in the range from 10 to 40 μM were tabulated in Table 3.3. The acceptable percentage recoveries in the range of 98.28 ± 0.985 to 99.81 ± 0.545 obtained specify that the proposed sensor might be enough for practical application and can be employed for the determination of PA in pharmaceutical formulations.

3.4. Conclusion

This article reports the fabrication of novel, simple, sensitive and less cost sensor PoOCD/MCPE for voltammetric resolution of PA. The sensor shows high sensitivity, selectivity, and anti- interference capability for the electrochemical oxidation of PA. The developed PoOCD/MCPE displayed well separated and resolved peaks for the electro-oxidation of PA, FA, and DA. The sensor can be used for determining the PA individually and simultaneously in the existence of FA and DA. The capability of the sensor was studied by estimating PA in the tablet. The developed sensor can also be applied to estimate some other biomolecules in the pharmaceutical industry.



Scheme 3.1: Structure of Orange CD



Scheme 3.2: Oxidation mechanism of PA

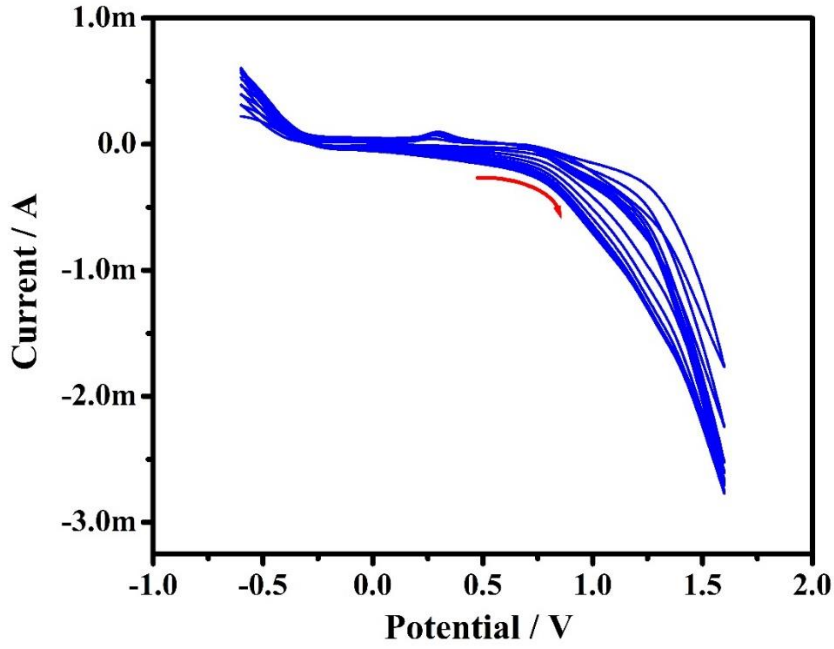


Fig.3.1. CVs of construction of PoOCD/MCPE with 0.1 M NaOH for 10 cycles at SR of 100 mVs^{-1}.

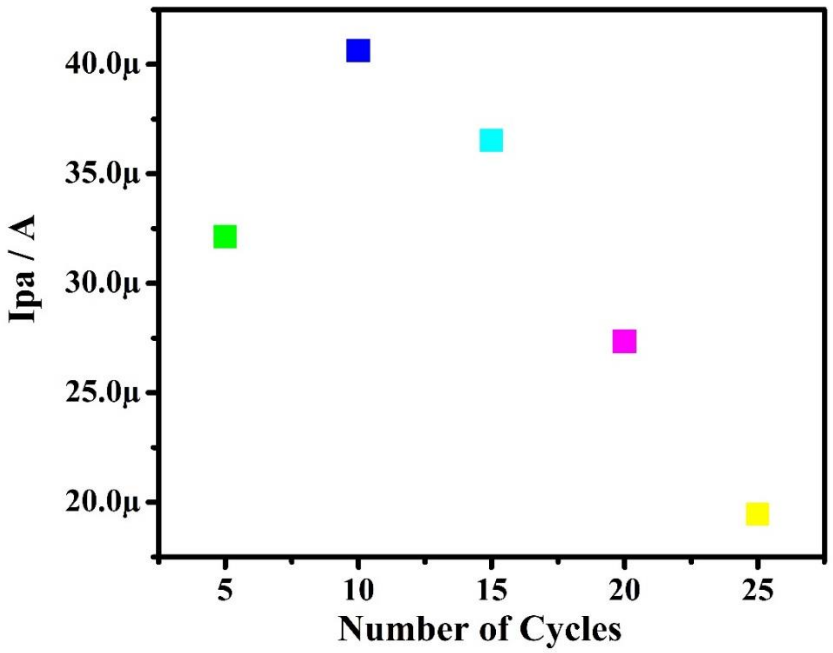


Fig.3.2. Graph of Ipa vs number of voltammetric scans.

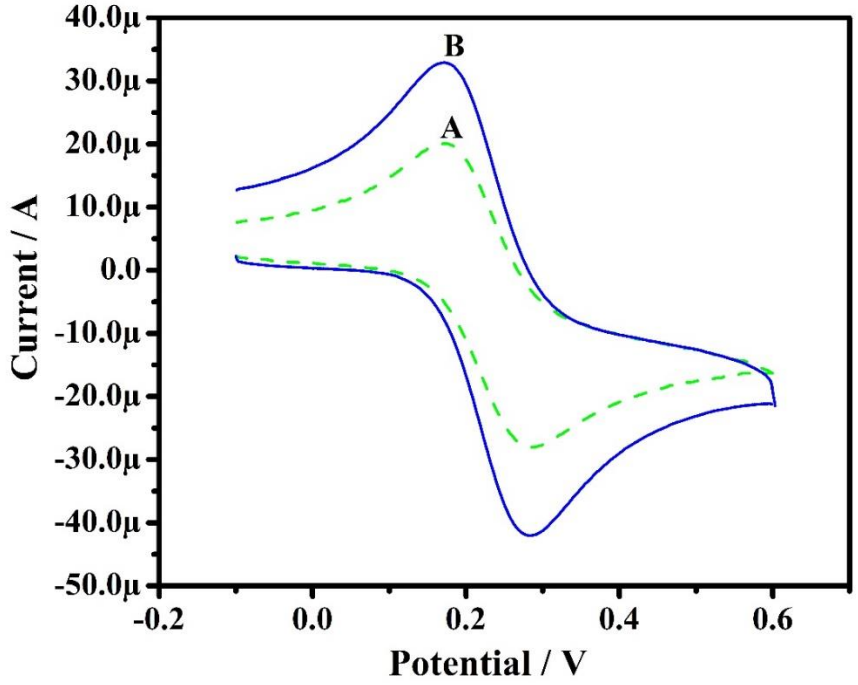


Fig.3.3. CV results of K_4 [Fe $(CN)_6$] at bare CPE (A) and PoOCD/MCPE (B) at a SR 100 mVs^{-1}

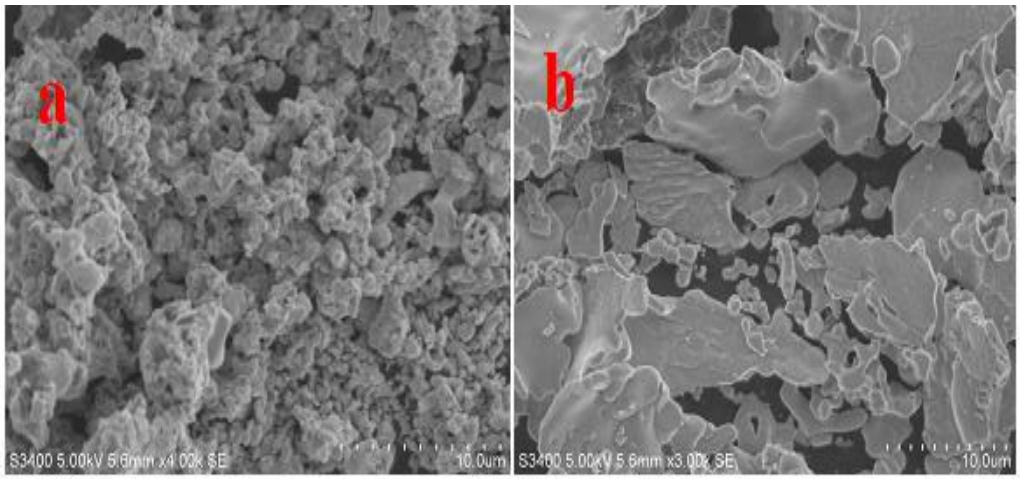


Fig.3.4. SEM of bare CPE (a) and PoOCD/MCPE (b)

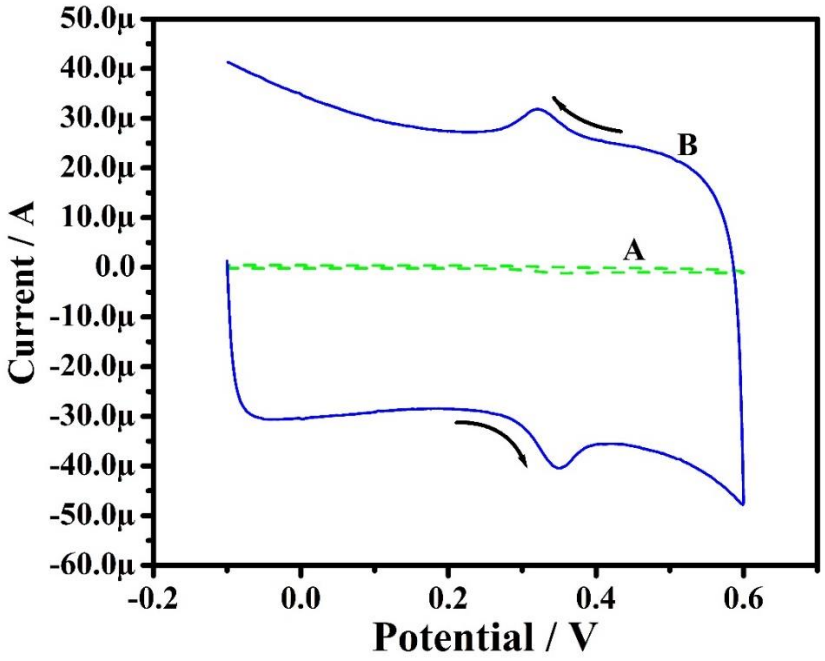


Fig.3.5. CVs for PA (0.1 mM) in 0.2 M PBS (pH 7.4) at bare CPE (A) and PoOCD/MCPE (B) at SR 100 mVs^{-1}

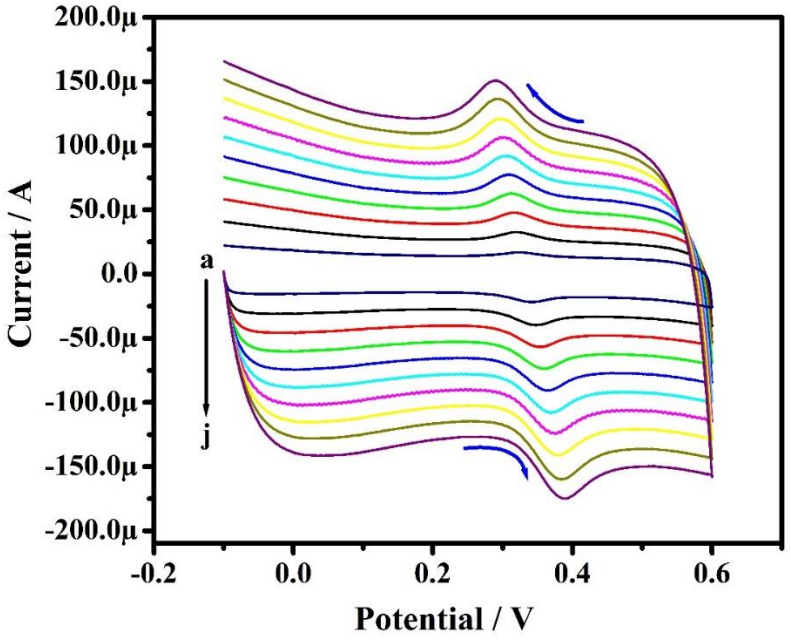


Fig.3.6. CVs obtained for PA (0.1 mM) at PoOCD/MCPE with various SR (50-500) mVs^{-1} in PBS (0.2 M, pH 7.4)

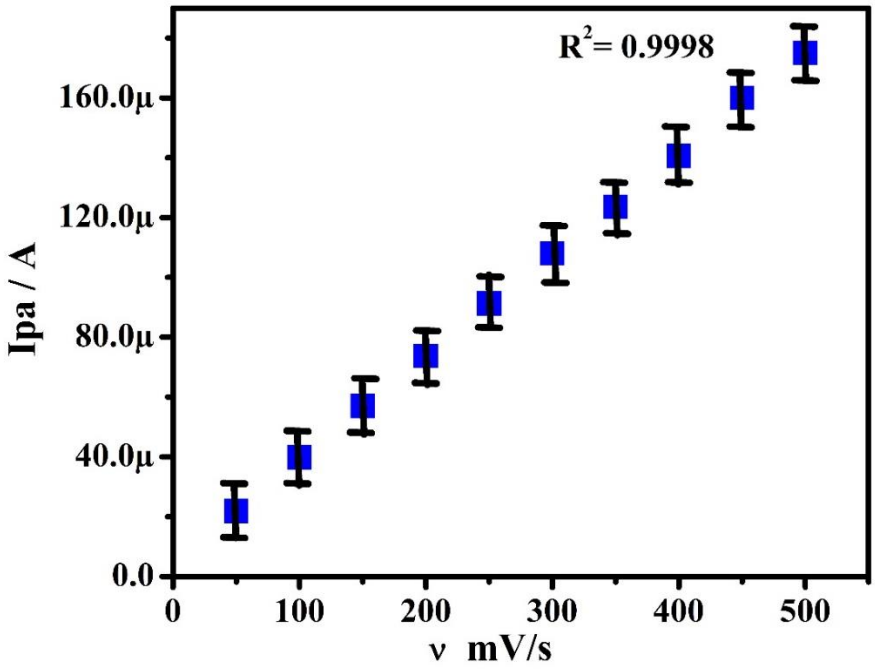


Fig.3.7. Graph of Ipa vs SR of PA (0.1 mM) in PBS (0.2 M, pH 7.4).

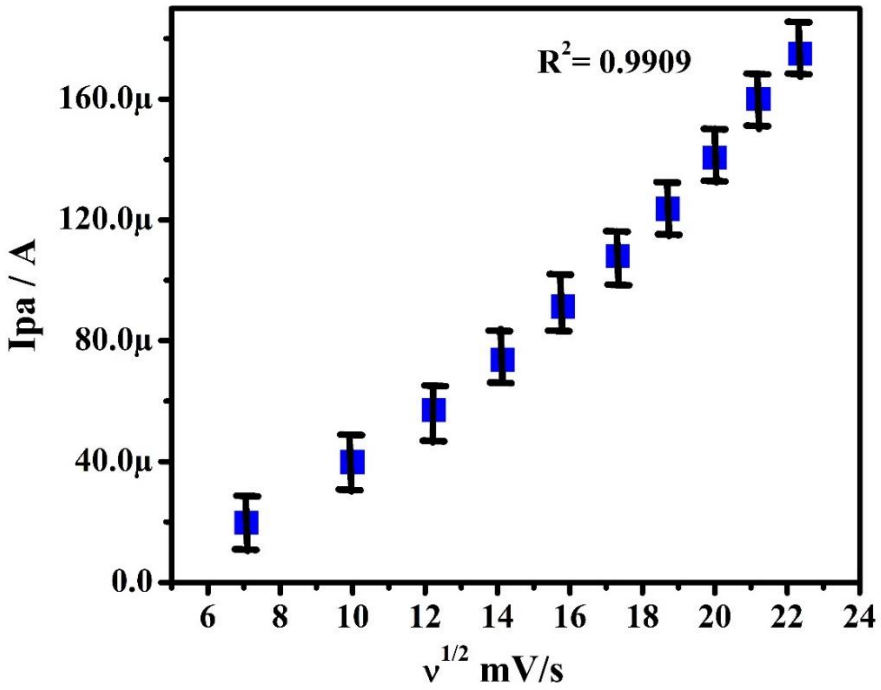


Fig.3.8. Graph of Ipa vs square root of SR of PA (0.1 mM) in PBS (0.2 M, pH 7.4).

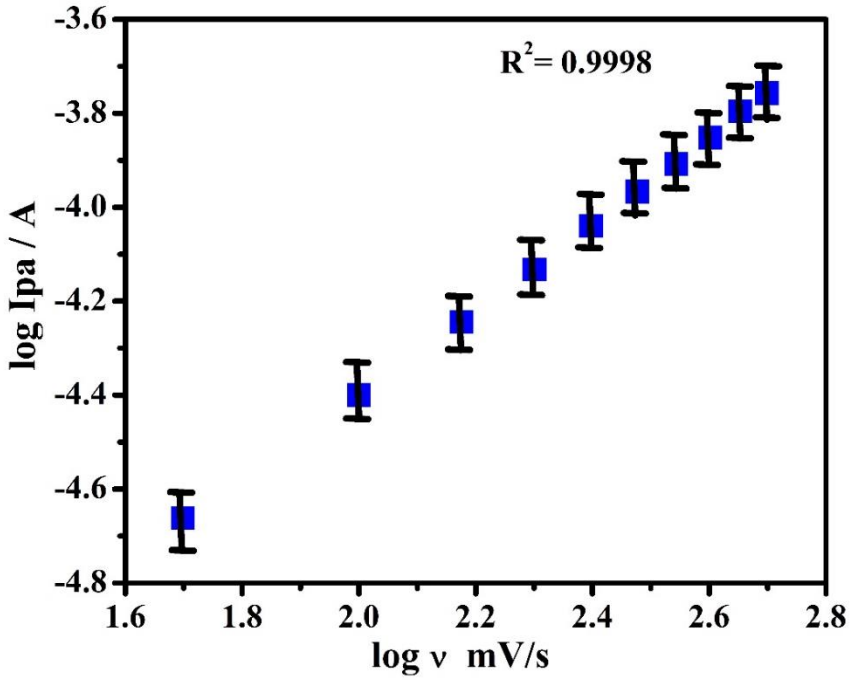


Fig.3.9. Graph of log Ipa vs log SR of PA (0.1mM) in PBS (0.2 M, pH 7.4).

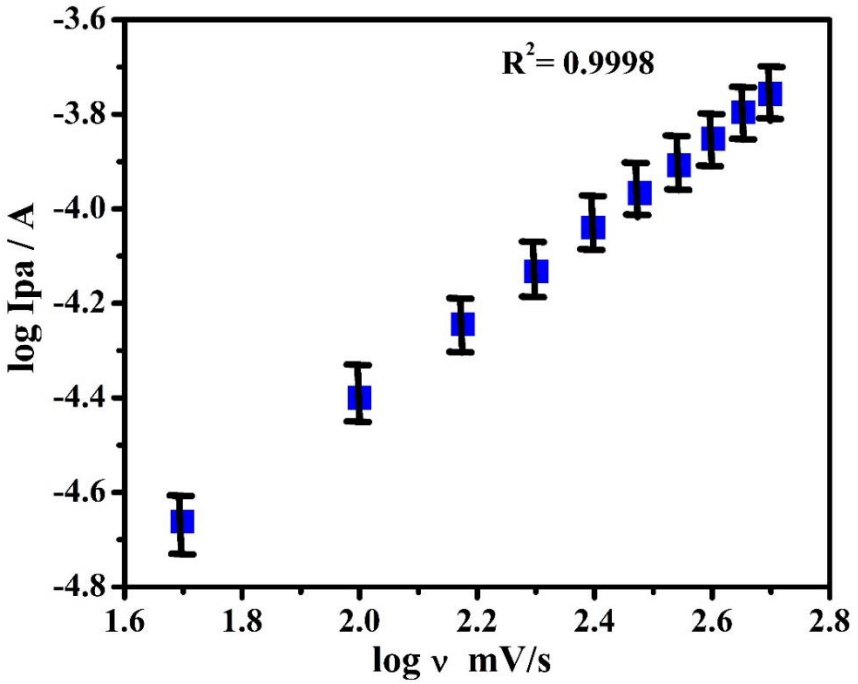


Fig.3.10. CVs for PA at different concentrations (10-60 µM) in PBS (0.2 M, pH 7.4) at PoOCD/MCPE.

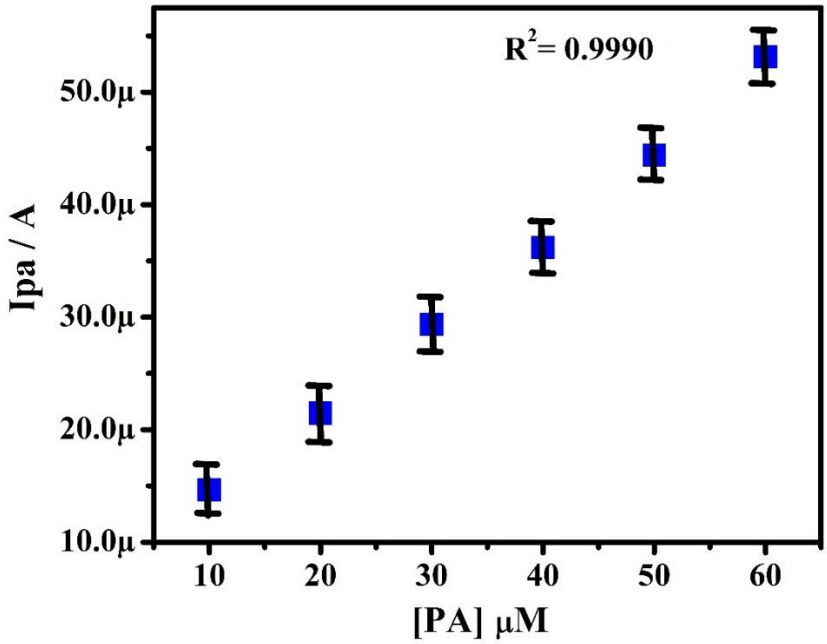


Fig.3.11. Graph of Ipa vs PA concentration

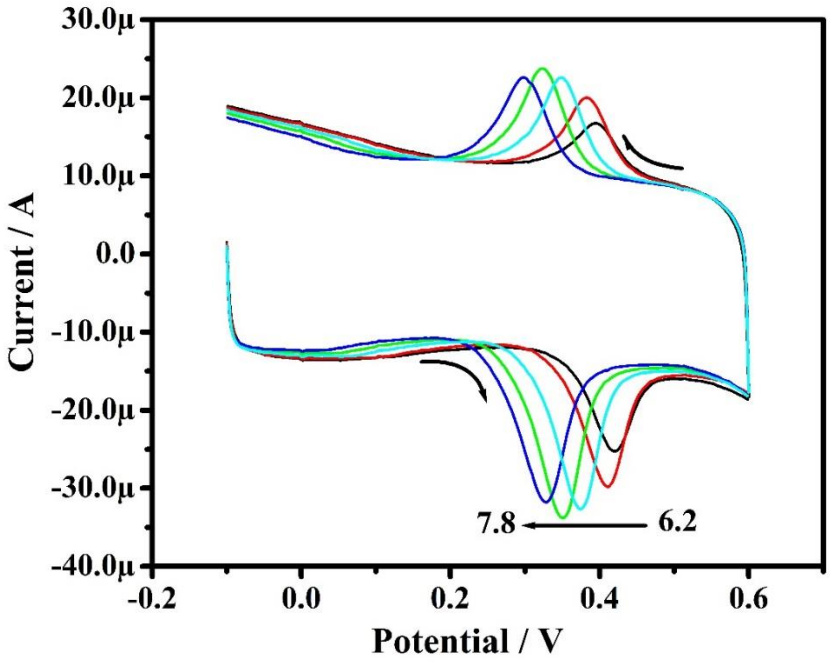


Fig.3.12. CVs for PA with varied pH at PoOCD/MCPE.

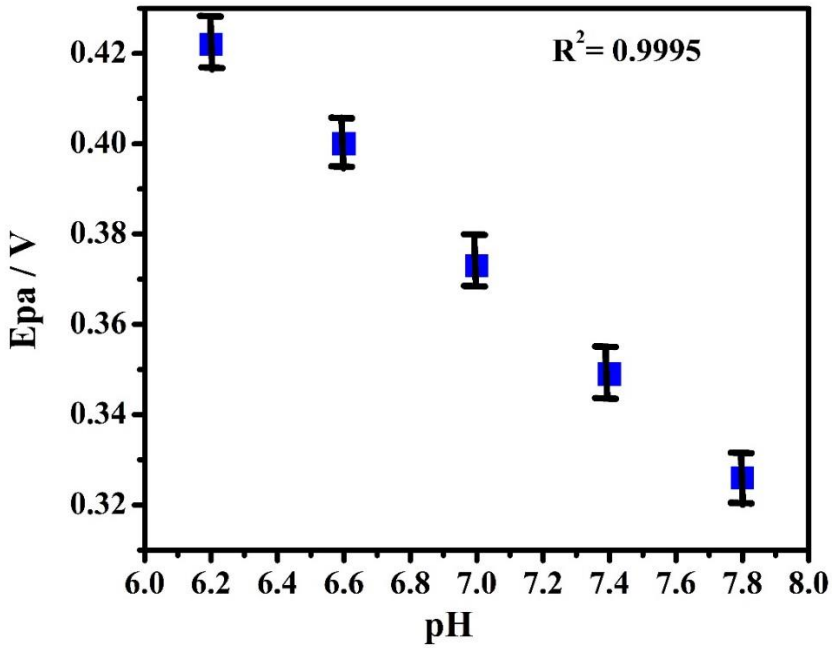


Fig.3.13. Graph of Epa vs varied pH for PA

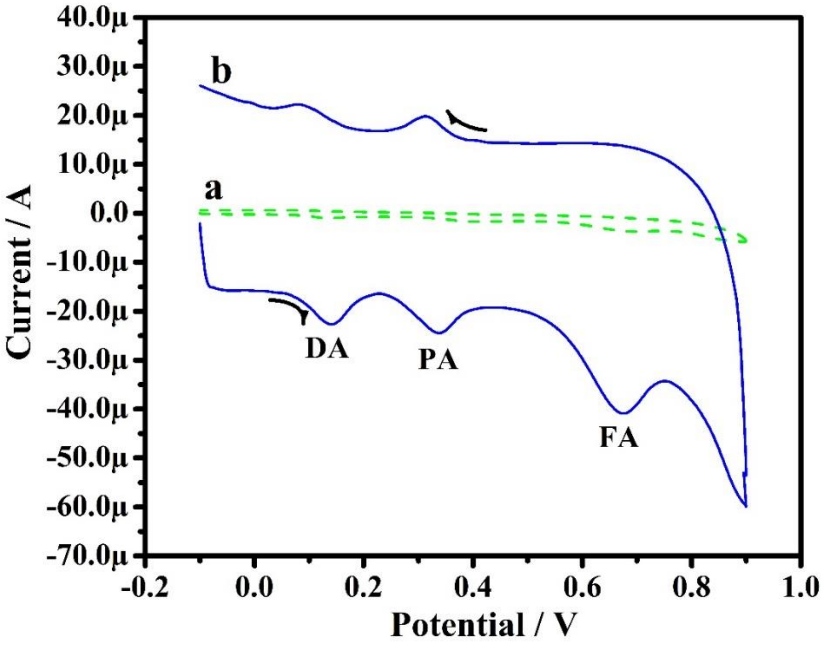


Fig.3.14. CV obtained for simultaneous studies of PA (0.1 M), FA (0.1 M) and DA (0.1 M) on bare CPE (a) and PoOCD/MCPE (b)

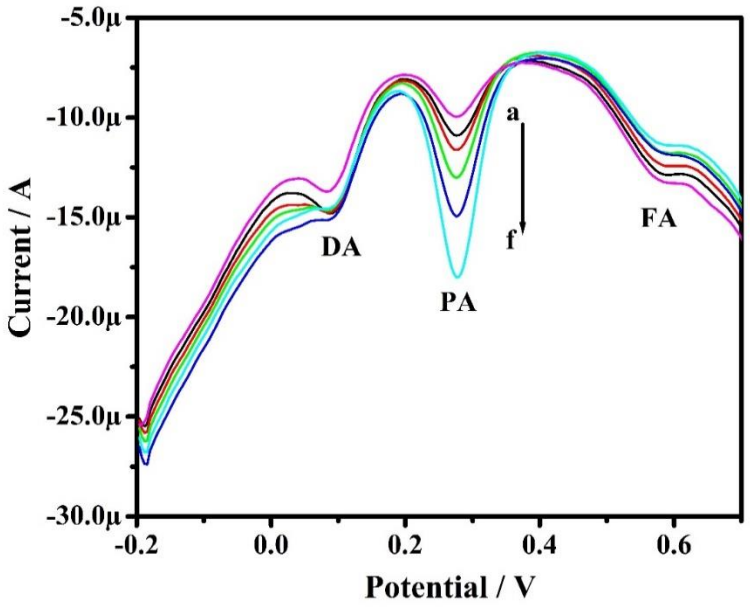


Fig.3.15. DPVs for PA at different concentrations (10-60 µM) in PBS (0.2 M, pH 7.4) at SR 50 mVs^{-1} at PoOCD/MCPE

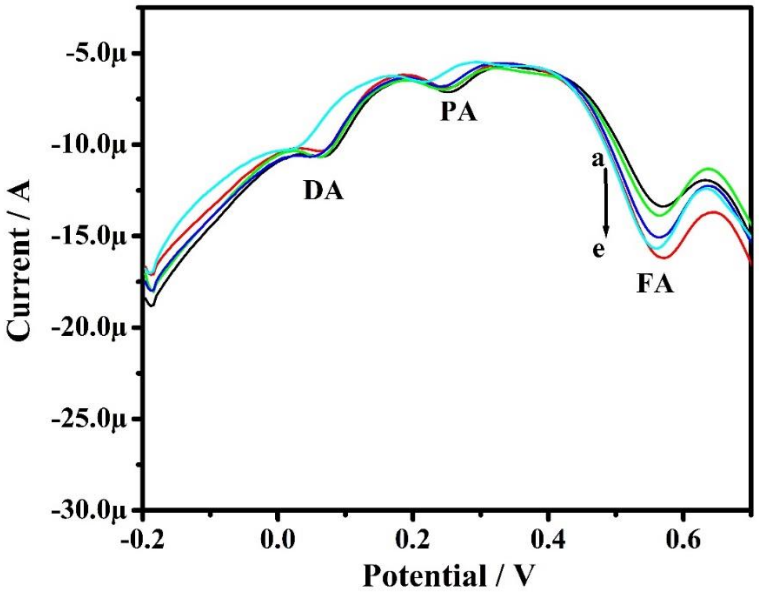


Fig.3.16: DPVs for FA at different concentrations (10-50 µM) in PBS (0.2 M, pH 7.4) at SR 50 mVs^{-1} at PoOCD/MCPE.

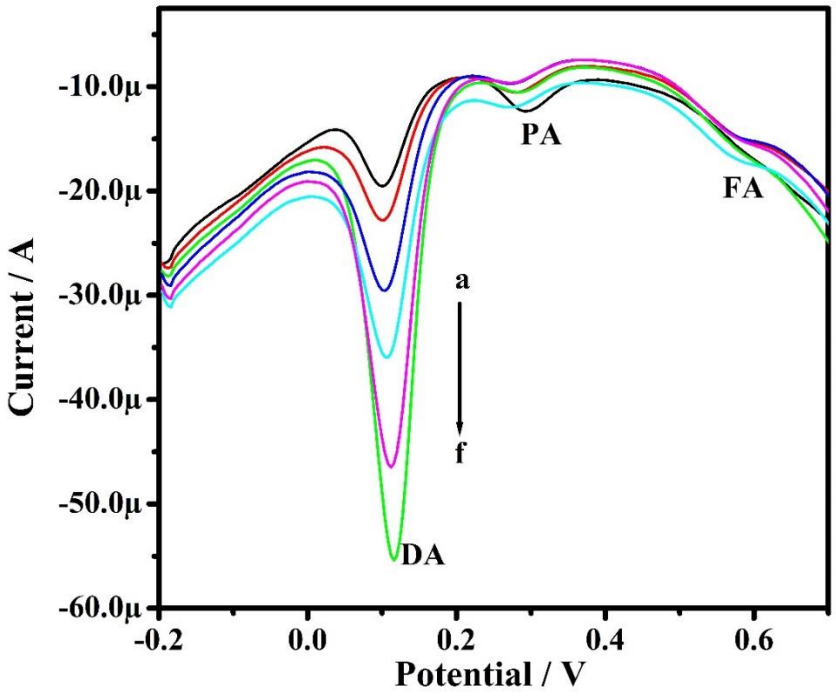


Fig.3.17. DPVs for DA at different concentrations (10-60 µM) in PBS (0.2 M, pH 7.4) at SR 50 mVs^{-1} at PoOCD/MCPE.

Table 3.1. Variation of the voltammetric parameters for PA at different scan rates.

Scan rate (mVs^{-1})	ΔEp (mV)	k^0 (s^{-1})
50	19	1.276
100	29	2.277
150	37	3.118
200	48	3.666
250	51	4.428
300	64	4.579
350	72	4.876
400	83	4.914
450	90	5.103
500	101	5.000

Table 3.2. Comparisons of the LOD of PoOCD/MCPE with other modified electrode reported.

Sl.No	Electrode	Limit of Detection in µM	Method	References
1	poly-NA-MCPE	7.2	CV	[2]
2	Diacerein/MCPE	3.8	DPV	[47]
3	N-DHPB-MWNT /CPE	10.0	DPV	[48]
4	Pd/Al	50.0	DPV	[49]
5	C_{60}/GCE	50.0	DPV	[50]
6	Cu-poly-TTC	5.0	CV	[51]
7	PVA-Fe3O4/MGCE	8.0	DPV	[52]
8	GrRAC sensor	8.36	DPV	[53]
9	TiO2 nanoparticle MCPE	5.25	CV	[54]
10	PoOCD/MCPE	2.64	CV	This work

Table 3.3. Evaluation of PA in Tablet using PoOCD/MCPE.

Content	Added (µM)	Found (µM)	Recovery (%)
500mg Paracetamol tablet	10	9.9394	99.39 ± 0.125
	20	19.6563	98.28 ± 0.985
	30	29.8751	99.58 ± 0.315
	40	39.9242	99.81 ± 0.545

3.5 References

[1] H. Beitollahi, I. Sheikhshoaie, *Mater. Sci. Eng. C.* 32 **(2012)** 375

[2] A. B. Teradale, P. S. Ganesh, B. E. K. Swamy, S. N. Das, *Anal. Bioanal. Electrochem*. 10 **(2018)** 203

[3] S. B. Tanuja, B. E. K.Swamy, K. V. Pai, *J. Electroanal. Chem,* 798 **(2017)** 17

[4] Y. Song, Y. Zhang, J. Li, C. Tan, Y. J. Li, *Electroanal. Chem.,* 865 **(2020)** 114157

[5] B. G. Mahmoud, M. Khairy, F. A. Rashwan, C. E. Banks, *Anal. Chem.* 89 **(2017)** 2170

[6] A, B. Teradale, S.D. Lamani, P. S. Ganesh, B. E. K. Swamy, S. N. Das, *Z. Phys.Chem.* 3 **(2018)** 345

[7] M. Tefera, A. Geto, M. Tessema, S. Admassie, *Food Chemistry,* 210 **(2016)** 156

[8] M. Amare, W. Teklay, *Cogent Chemistry.* 5 **(2019)** 1576349

[9] C. Fernandez, Z. Heger, R. Kizek, T. Ramakrishnappa, A. Boruń, N. H. Faisal, *Int. J. Electrochem. Sci*. 10 **(2015)** 7440

[10] M. A. T. Gilmartin, J. P. Hart, *Analyst*, 119 **(1994)** 2431

[11] M. Lecoeur, G. Rabenirina, N. Schifano, P. Odou, S. Ethgen, G. Lebuffe, G. Foulon, *Talanta* 205 **(2019)** 120108

[12] R. M. Carvalho, R. S. Freire, S. Rath, L.T. Kubota, *J. of Pharma. Biomed. Anal.*, 34 **(2004)** 871

[13] S. H. Youssef, D. Mohamed, M. A. M. Hegazy, A. Badawey, *BMC Chemistry.* 13 **(2019)** 78

[14] N. R. Ahmad, F. K. Omar, *World J. of Pharma. Res.* 7 **(2018)** 15

[15] K. Palur, S. C. Archakam, B. Koganti, Spectrochim. *Acta A Mol. Biomol. Spectrosc.* 243 **(2020)** 118801

[16] M. Iranifam, S. Khodaei, M. Saadati, *Microchem. J.* 146 **(2019)** 850

[17] H. Tavallali, Y. Hamid, *Asian J. of Biochem.and Pharma. Res*. 1 **(2011)** 2

[18] C. Pasha, *Ecletica Quim J.* 45 **(2020)** 37

[19] B. Chefirat, A. Zergui, M. N. Belmessabih, C. Rahmani, H. Rezk-kallah, *Toxicol. Anal. Clin.* 32 **(2020)** 266

[20] B. Doğan, A. Elik, N. Altunay, *Microchem. J.* 154 **(2020)** 104645

[21] P. S. Ganesh, B. E. K. Swamy, *J. Biosens. Bioelectron.* 7 **(2016)** 1

[22] B. Unnikrishnan, Y. L. Yang, S. M. Chen, *Int. J. Electrochem. Sci.* 6 **(2011)** 3224

[23] M. Kumar, B. E. K. Swamy, S. Reddy, W. Zhao, S. Chethana, V. G. Kumar, *J. Electroanal. Chem.* 835 **(2019)** 96

[24] S. Wei, F. Zhao, Z. Xu, B. Zeng, *Microchim. Acta*. 152 **(2006)** 285

[25] B. Demirkan, S. Bozkurt, K. Cellat, K. Arıkan, M. Yılmaz, A. Şavk, M. H. Çalımlı, M. S. Nas, M. N. Atalar, M. H. Alma, F. Sen, *Sci. Rep.*10 **(2020)** 2946

[26] S. Immanuel, T. K. Aparna, R. A. Sivasubramanian, *Surf. Interfaces.* 14 **(2019)** 82

[27] S. Schindler, T. Bechtold, *J. Electroanal. Chem.* 836 **(2019)** 94

[28] M. Kumar, B. E. K. Swamy, D. R. Rekha, *Sci. Lett. J.* 4 **(2015)** 211

[29] C. M. Kuskur, B. E. K. Swamy, H. Jayadevappa, *Anal. Bioanal. Electrochem.* 10 **(2018)** 658

[30] A. Kannan, R. Sevvel, *J. Electroanal. Chem.* 791 8-16

[31] N. B. Ashoka, B. E. K. Swamy, H. Jayadevappa, S. C. Sharma, *J. Electroanal. Chem.* 859 **(2020)** 113819

[32] H. Beitollahi, A. Mohadesi, S. Mohammadi, A. Pahlavan, H. K. Maleh, A. Akbari, *J. Molecular Liq.* 169 **(2012)** 130

[33] K. Chetankumar, B. E. K. Swamy, S. C. Sharma, *J. Electroanal. Chem.* 849 **(2019)** 113365

[34] A. Volkov, G. Tourillon, P. Lacaze, J. Dubois, *J. Electroanal. Chem.* 115 **(1980)** 279

[35] N. Hareesha, J. G. Manjunatha, *Sci. Rep.*11 **(2021)** 12797

[36] J. K. Shashikumara, B. E. K. Swamy, S. C. Sharma, S. A. Hariprasad, K. Mohanty, *Sci. Rep.* 11 **(2021)** 14310

[37] S. Chitravathi, B. E. K. Swamy, G. P. Mamatha, B. S. Sherigara, *J. Electroanal. Chem.* 667 **(2012)** 66

[38] C. M. Kuskur, B. E. K. Swamy, K. Shivakumar, H. Jayadevappa, S. C. Sharma, *J. Electroanal. Chem.* 840 **(2019)** 52

[39] J. K. Shashikumara, B. E. K. Swamy, K. Chetankumar, *Chem. Data Coll.* 32 **(2021)** 100661

[40] K. Chetankumar, B. E. K. Swamy, S. C. Sharma, S. A. Hariprasad, *Sci.Rep.*11 **(2021)** 15064

[41] Q. Qin, X. Bai, Z. Hu, *J. Electroanal. Chem.*782 **(2016)** 50

[42] R. Sainz, M. Pozo, M. Vilas-Varela, J. Castro-Esteban, M. P. Corral, L. Vázquez, E. Blanco, D. Peña, J. A. Martin-Gago, G. J Ellis, M.D. Petit-Dominguez, C. Quintana, E. Casero, *Sci.Rep*.10 **(2020)** 14614

[43] R. N. Goyal, V. K. Gupta, S. Chatterjee, *Sens. Actuat. B.* 149 **(2010)** 252

[44] K. V. Harisha, B. E. K. Swamy, P. S. Ganesh, H. Jayadevappa, *J. Anal. Bioanal. Tech.,*10 **(2018)** 1273

[45] N. Hareesha, J. G. Manjunatha, *J. Electroanal. Chem.* 878 **(2020)** 114533

[46] Y. V. M. Reddy, V. P. Rao, A. V. B. Reddy, M. Lavanya, M. Venu, G. Madhavi, *Mater. Sci. Eng. C,* 57 **(2015)** 378

[47] A. Sathisha, B. E. K. Swamy, *Anal. Bioanal. Electrochem*. 10 **(2018)** 1437

[48] A. A. Ensafi, H. Karimi-Maleh, S. Mallakpour, M. Hatami, Sens. *Actuat. B.* 155 **(2011)** 464

[49] M. H. Pournaghi-Azar, A. Saadatirad, *Electroanalysis*. 22 **(2010)** 1592

[50] R. N. Goyal, S.P. Singh, *Electrochim. Acta*. 51 **(2006)** 3008

[51] M. Boopathi, M. Won, Y. Shima, *Anal. Chim. Acta*. 12 **(2004)** 191

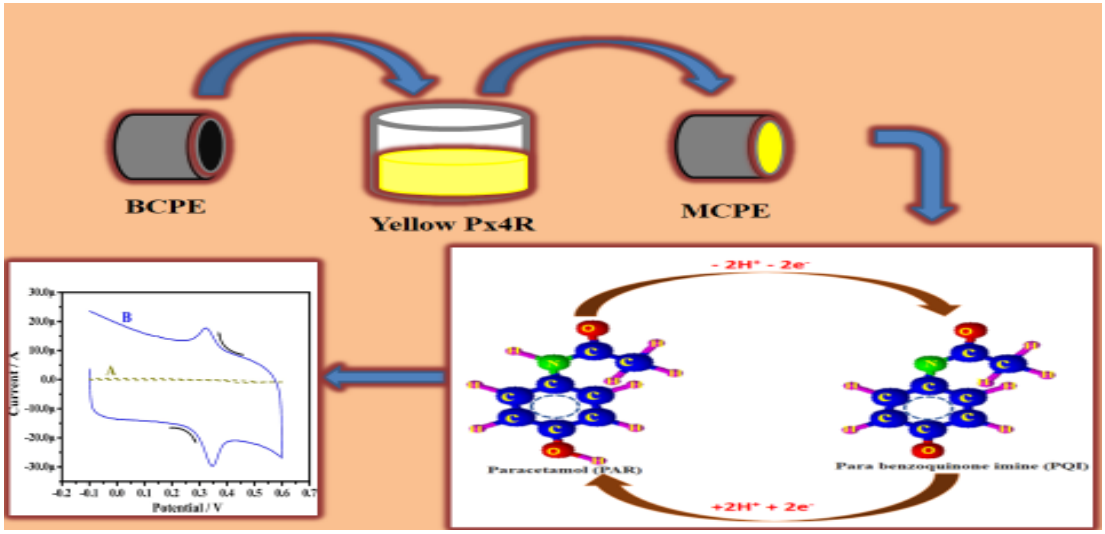
[52] R. Andawiyah, A. Mulyasuryani, H. Sulistyarti, *Mater. Sci. Eng.,* 833 **(2020)** 012059

[53] M. K. S. Monteiro, E. C. M. M. Santos, D. R. Silva, C. A. Martínez-Huitle, E. V dos Santos, *J. Solid State* Electrochem. 24 **(2020)** 1789

[54] K. G. Manjunatha, B. E. K. Swamy, H. D. Madhuchandra, K. A. Vishnumurthy, *Chem. Data Coll.* 31 **(2021)** 100604

Chapter -4

Poly (yellow PX4R) carbon paste electrode sensor for paracetamol: A voltammetric study



Published in
Inorganic Chemistry Communications 140 (2022) 109394

Inorganic Chemistry Communications 140 (2022) 109394

Contents lists available at ScienceDirect

Inorganic Chemistry Communications

journal homepage: www.elsevier.com/locate/inoche

Short communication

Poly (yellow PX4R) carbon paste electrode sensor for paracetamol: A voltammetric study

Sukanya [a], B.E. Kumara Swamy [b,*], J.K. Shashikumara [b], S.C. Sharma [c,d,e]

[a] Department of P.G. Studies and Research in Analytical Chemistry, Alva's College, Moodubidire, Dakshina Kannada, Karnataka 574227, India
[b] Department of P.G. Studies and Research in Industrial Chemistry, Kuvempu University JnanaSahyadri, Shankaraghatta, Shimoga, Karnataka 577451, India
[c] National Assessment and Accreditation Council (NAAC), Nagarabhavi, Bengaluru 560072, Karnataka, India
[d] Jain University, Bengaluru 560069, Karnataka, India
[e] Schoolof Energy Science and Engineering, Indian Institute of Technology Guwahati, Guwahati, India

4.1 Introduction

Paracetamol (PAR) is a significant antipyretic and analgesic drug of the first choice worldwide [1, 2]. PAR is a key component in most cold and flu reversal medications. PAR is administered to treat mild to moderate pain, including headaches, muscle aches, rheumatic pain, joint pain, backaches, toothaches, relief of fever, osteoarthritis, and cancer pain [3, 4]. At the proper therapeutic dose, PAR is easily metabolized. Overdosing on PAR causes toxic metabolite buildup, which leads to acute liver necrosis and increased morbidity and mortality [5]. Frequently intake of PAR by pregnant women is linked to a higher risk of wheezing and eczema in their baby in early life [6]. Owing to in vitro studies, the use of PAR protects dopaminergic neurons against oxidative stress caused by acute exposure to high levels of dopamine (DA). Conversely, in an in vivo model, chronic PAR usage has been found to significantly decrease DA levels [7, 8]. PAR monitoring becomes critical to control and avoid these issues [9]. PAR research is important in the life sciences and medicine, hence quick, easy, and accurate method for the determination of PAR in physiological pH circumstances is of greater interest. Numerous methods including chemiluminescence [10], chromatography [11], LC-MS, HPLC [12-14], spectrophotometric [15-17], spectrofluorimetric [18] etc. availed for the quantification of PAR. Since PAR has an electrochemically oxidizable phenolic hydroxyl group, it will be easier to quantify it using an electrochemical approach [19].

Electrochemical sensors are the most well-known promising devices in modern days because of their high sensitivity and selective properties, fast response, ease of automation, eco-friendly and has been widely employed in analytical strategies for drug molecule testing, clinical diagnosis, agriculture, and food sector quality control, and environmental monitoring [20-23]. In this area, the kind of working electrode material chosen has a significant impact on the practical application of the fabricated sensor. From the literature, it is noteworthy that, carbon paste electrodes (CPEs) are popular electrochemical sensors that have a broad range of applications in CV studies. CPEs have many advantages over other materials due to the rapid renewability surface, high selectivity, reduced cost, porous surface, low residual current, and a higher level of biological compatibility [24-27]. The most key aspect in resolving the selectivity and sensitivity issues associated with CPEs has been electrode modification [28].

Herein, we have described an effectual modification of CPE using yellow (PX4R) (Scheme 4.1) through electropolymerization using CV techniques. This fabricated Po-YPX4R-MCPE sensor performs a crucial role in the determination of PAR and simultaneous determination of PA and DA in biological pH 7.4. The sensor displayed higher electrocatalytic activity, as well as a low detection limit and large linear ranges for PA resolution. The practical applicability of the sensor has been tested by determining PA in tablets successfully. This research aims at developing efficient, robust, and less expensive sensors.

4.2. Experimental

4.2.1 Equipment and Chemicals

A CH Instrument-660 electrochemical workstation (CHI-660c model, USA) was used to acquire CVs at room temperature. An electrochemical cell was used to accommodate three electrodes such as saturated calomel electrode reference electrode, platinum wire counter electrode, BCPE, and Po-YPX4R-MCPE working electrodes.

PAR, DA, Na_2HPO_4, $NaH_2PO_4.H_2O$, graphite powder was bought from Nice Chemicals Pvt. Ltd Bengaluru India and yellow (PX4R) was procured from Astik Dyestuffs Pvt. Ltd Gujarat India. All analytical grade compounds were utilized exactly as received. Standard solutions of yellow (PX4R), PA, and DA of concentrations $25x10^{-4}$ M were prepared with double-distilled water (DDW). 0.2 M PBS was made by mixing Na_2HPO_4 and $NaH_2PO_4.H_2O$.

4.2.2 Preparation of PA tablet solution

A mortar was used to finely pulverize a 500mg Calpol tablet. The required amount of Calpol powder was dissolved in 100ml DDW and vigorously agitated to achieve the desired concentration.

4.2.3 Setting up of a BCPE and Po-YPX4R-MCPE

BCPE was made by mixing graphite powder with silicone oil (70:30) in an agate mortar, then packing it into a cavity of a homemade Teflon tube with a current collector. To get a smooth surface, it is then smoothed with a tissue paper [29]. By dipping BCPE into 1 mM aqueous yellow (PX4R) with NaOH (0.1 M) as a supporting electrolyte, the

Po-YPX4R-MCPE was constructed through electropolymerization. The electro-chemical polymerization was carried out in 10 cycles at a gradient of -0.6 to 1.6 V with a scan speed (SP) of 100mVs^{-1}. The electropolymerized electrode was then rinsed in DDW to clear away residual molecules.

4.3 Results and Discussion

4.3.1 Electrochemical polymerization of yellow (PX4R) on BCPE

The CVs of electrochemical polymerization of 0.1mM aqueous yellow (PX4R) with NaOH (0.1 M) on the BCPE surface at a gradient of – 0.6 to 1.6 V with SP of 100 mVs^{-1} during 10 cycles are shown in Fig. 4.1a. Voltammograms taken as the ongoing electropolymerization is increased demonstrate the aggregation and proliferation of yellow (PX4R) layer on the surface of BCPE [30]. The electrochemical reactivity of the MCPE is impacted by the thickness of the polymer sheet. During electropolymerization, sheet thickness was conveniently tuned by varying the number of voltammetric scans from 5 to 25. As demonstrated in insect Fig. 4.1b, similar experimental results were obtained for the PAR. Since the current response peaks at 10 multiple cycles, the ideal cycle number of 10 was adopted for the Po-YPX4R-MCPE fabrication and subsequent voltammetric measurements.

4.3.2 Electro active surface area of BCPE and Po-YPX4R-MCPE

A potassium ferrocyanide system was utilized to investigate the electrocatalytic activity of the Po-YPX4R-MCPE. Fig. 4.2 depicts CVs for $K_4[Fe(CN)_6].3H_2O$ at BCPE (Line 'A') and Po-YPX4R-MCPE (Line 'B') in supporting electrolyte KCl (1M) was monitored by CV technique at SP of 100 mVs^{-1}. The modest redox peak current correlates to BCPE, whereas Po-YPX4R-MCPE exhibits amplified peak current, indicating a substantial rise in electron transfer rate [31]. The electro-active surface area of both BCPE and Po-YPX4R-MCPE was computed using Randles-Sevick's equation 4.1 [32].

$$Ip = 2.69 \times 10^5 n^{3/2} A D^{1/2} \upsilon^{1/2} C \quad \text{---------- (4.1)}$$

BCPE (0.031 cm^2) utilized less electroactive surface area than Po-YPX4R-MCPE (0.048 cm^2), implying that yellow (PX4R) functions as a potent modifier by contributing a wide surface area and promoting electron transport between solution and

electrode.

SEM was used to characterize the surface morphological aspects of BCPE and Po-YPX4R-MCPE. The SEM of BCPE (Fig. 4.3a) shows hard texture, asymmetrically designed flakes with randomly oriented polymer films, and Po-YPX4R-MCPE (Fig. 4.3b) shows smooth, uniformly modified, and consistent layering of the polymer matrix of yellow PX4R. The striking difference in the surface morphology between the two electrodes proves an enormous change of BCPE surface by electropolymerized yellow PX4R.

4.3.3 Electrochemical response of PAR at BCPE and Po-YPX4R-MCPE

The electrochemical response of PAR was tested on BCPE (Line 'A') and Po-YPX4R-MCPE (Line 'B') in PBS (0.2 M and 7.4 pH) by CV technique at SP of 100 mVs^{-1}, as shown in Fig.4.4. At BCPE, an irreversible voltammogram for PAR was produced with only an anodic peak potential of 0.355 V, showing an inadequate response including the occurrence of only oxidation. However, under the same circumstances, Po-YPX4R-MCPE showed a considerable incremental signal, resulting in a sharp reversible voltammogram. The anodic and cathodic peak potentials for PAR were determined to be 0.345 and 0.322 V respectively, indicating that both oxidation and reduction occur at the tailored Po-YPX4R-MCPE. As a result, the Po-YPX4R-MCPE established itself as an excellent sensor for PAR analysis.

4.3.4 Influence of electrolyte pH on PAR at Po-YPX4R-MCPE

The influence of supporting electrolyte pH was investigated by CV techniques for PAR (10 µM) in 0.2 M PBS of pH series (6.2 to 7.8) on the surface of Po-YPX4R-MCPE at the SP of 50 mVs^{-1}. As depicted in Fig.4.5a, the peak potential of PAR was transferred to the negative side as the pH was hiked. This attained result demonstrated the proton's direct involvement in the redox process. Fine linearity between Epa and altered pH (insect Fig.4.5b) of PAR and the attained linear regression equation is expressed as Epa (V) = 0.773 pH + 0.573 (R^2= 0.997). The slope value of 0.573 V/pH was very close to the theoretical value of 0.59 V/pH indicating that an equal number of proton and electrons are associated in electro-oxidation of PAR at Po-YPX4R-MCPE [33, 34]. The electro-oxidation of PAR is portrayed in Scheme 4.2. The PBS of pH 7.4

was optimized as the best for further electrochemical investigation by assessing maximal peak current and sensitivity.

4.3.5 Scan speed impact on PAR at Po-YPX4R-MCPE

The CVs documented for PAR (0.1mM) in PBS (0.2M and 7.4 pH) with varying scan speeds from 50 to 500 mVs^{-1} at Po-YPX4R-MCPE were depicted in Fig.4.6a. The redox peak currents are shown to increase with increasing scan speeds. The kinetic behavior of the MCPE towards the redox reaction of PAR was validated using acceptable linear plots of Ipa Vs square root of SP (Fig.4. 6b) and log Ipa Vs log of SP (Fig. 4.6c). The linear regression equation established as Ipa (µA) = 6.71 υ (mVs^{-1}) - 4.27 (µA) (R^2= 0.990) (Fig. 4.6b) and Ipa (µA) = 0.95 logυ (mVs^{-1}) - 6.51(µA) (R^2= 0.999) (Fig. 4.6c) narrates the kinetic property of PAR at the surface of Po-YPX4R-MCPE was adsorption controlled [35, 36]. Equation 4.2 was used to compute the heterogeneous rate constant k^0 of Po-YPX4R-MCPE (Table 4.1) [20].

$$\Delta Ep = 201.39 \log (\upsilon / k^0) - 301.78 \text{ ---------- (4.2)}$$

4.3.6 Impact of PAR's concentration

Fig.4.7a details the CVs of PAR concentrations in the range of 10 to 60 µM in PBS (0.2M and 7.4 pH) at Po-YPX4R-MCPE. The redox peak current of PAR significantly inflates when concentration was raised. The regression equation Ipa (A) = 0.57 (µM) + 2.03 (µA) (R^2 = 0.986) exhibits good linearity when plotted against PAR concentration (Fig.4.7b). Based on equations 4.3 and 4.4, the LOD and LOQ were estimated to be 0.084 µM and 0.280 µM, respectively [37, 38]. Table 4.2 discusses the LOD of the proposed electrode in comparison to earlier PAR sensors.

$$LOD = 3S / M \quad \text{---------- (4.3)}$$

$$LOQ = 10S / M \quad \text{---------- (4.4)}$$

4.3.7 Simultaneous analysis of PAR and DA

The purpose of this analysis was to use the fabricated electrode for the selective and sensitive assessment of PAR in the existence of DA. The CVs were measured for the mixture of PAR (0.1 µM) and DA (0.1µM) in PBS (0.2 M and 7.4 pH) at SP of 50

mVs^{-1} at bare CPE (A) and Po-YPX4R-MCPE (B) as shown in Fig.4.8. Low current intensities with inadequate sensitivity and selectivity were recorded at bare CPE. Furthermore, under the same conditions, Po-YPX4R-MCPE demonstrated large current intensities with greater sensitivity and selectivity for PAR and DA oxidation at 0.137 and 0.339 V, respectively. As a result, the built Po-YPX4R-MCPE acts as an ideal sensor for PAR.

4.3.8 Interference study

Finally, to assess the efficiency of the proposed approach, the interfering of possible chemicals in the detection of PAR was studied using DPV techniques in the mixture of PAR and DA samples at Po-YPX4R-MCPE. The Po-YPX4R-MCPE (Fig. 4.9) reveals that Ipa of DA increased with increasing concentration from 10 to 50 µM while maintaining a constant concentration of PAR. Likewise, Ipa of PAR increased with increasing its concentration from 10 to 60 µM at DA remaining constant (Fig.4.10). These findings show that the accurate and precise estimation of PAR at Po-YPX4R-MCPE since the oxidation of PAR and DA does not effect on the variance of the other analytes.

4.3.9 Reproducibility, repeatability, and stability

The reproducibility of the Po-YPX4R-MCPE was verified by producing four distinct Po-YPX4R-MCPE under uniform conditions and the RSD value of 4.5 percent achieved supports the remarkable reproducibility. The repeatability of the Po-YPX4R-MCPE for PAR (0.1mM) in PBS (0.2 M and 7.4 pH) was tested over six consecutive trials. The RSD value of 2.1 percent illustrates the higher repeatability of the Po-YPX4R-MCPE. The stability was examined by 10 successive cycles, and it remained 97.9 percent of its original current response for PAR despite 10 cycles, demonstrating the good stability of Po-YPX4R-MCPE.

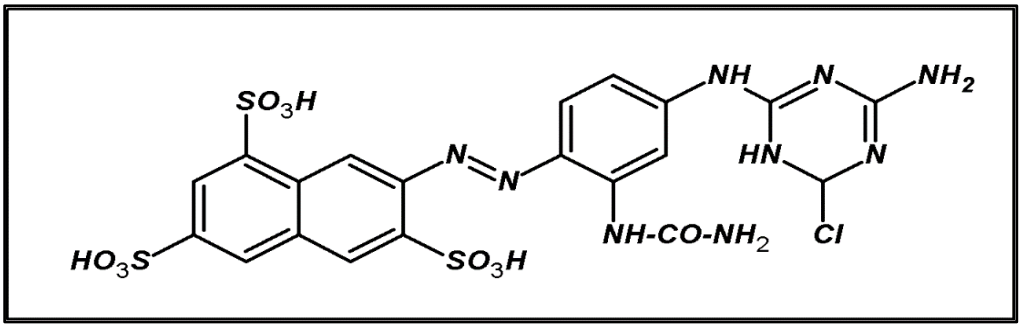
4.3.10 Analytical application

PAR was successfully assessed in tablet form utilizing the CV approach to evaluate the real-time application of Po-YPX4R-MCPE. Table 4.3 shows the results of the recovery test, which was performed using standard addition methodology. The satisfactory recoveries of 99.97 to 100.88 percent indicate that the proposed sensor has

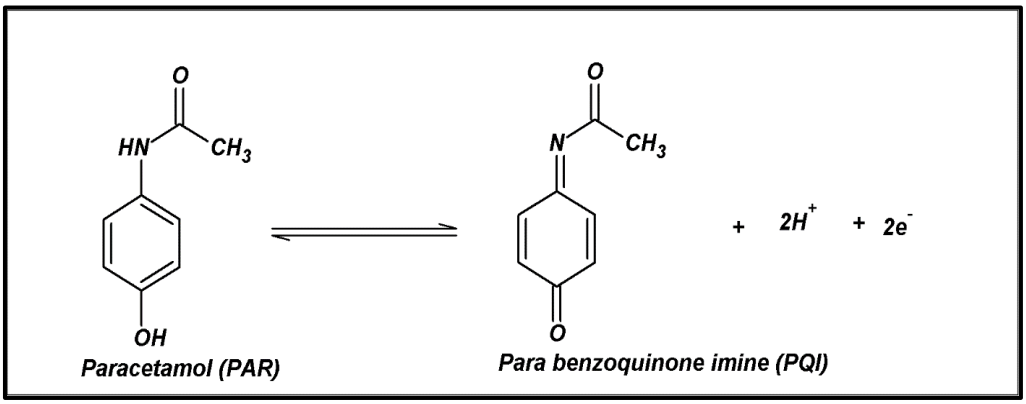
the potential to be a promising choice for PAR analysis in drug substances.

4.4 Conclusion

In conclusion, we proposed a simple and efficient way for fabricating Po-YPX4R-MCPE. The core attractive features of Po-YPX4R-MCPE include the easy modification process, increased sensitivity, high stability, and low detection limits. The developed sensor was used for individual and simultaneous detection of PAR and DA. The oxidation peak currents of PAR presented a good linear relationship with the concentrations in the range from 10µM to 60 µM with a detection limit of 0.084 µM. Furthermore, the results of simultaneous detection showed that the response of PAR is independent of the presence of DA. In real sample analysis, the developed sensor recovered PAR with high accuracy. The developed sensor may also be explored in the analysis of other electro-active molecules and pharmaceutical samples. As a result, Po-YPX4R-MCPE will have high opportunities in the sensor field.



Scheme 4.1. Structure of yellow (PX4R)



Scheme 4.2. Mechanism of PAR

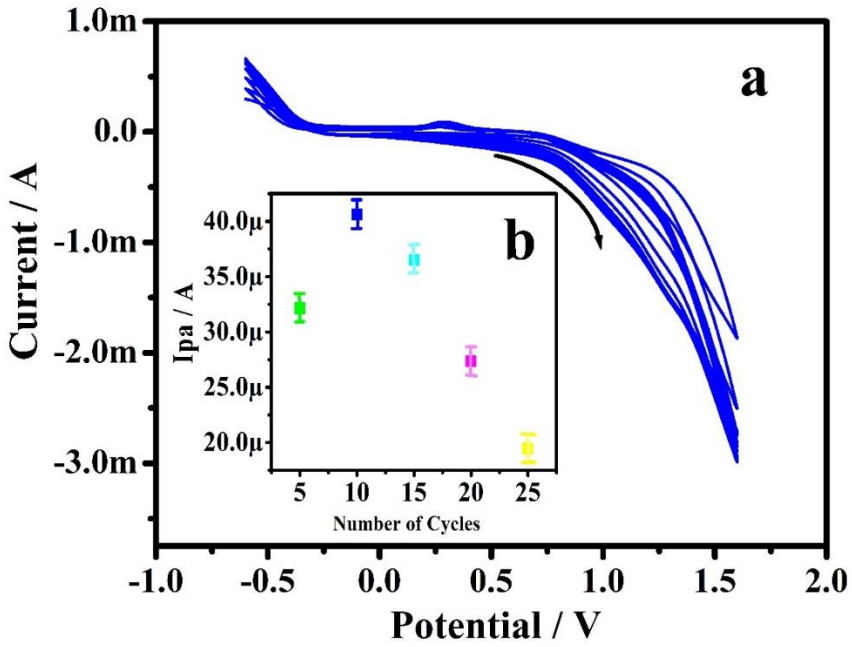


Fig. 4.1. a) CVs of developed Po-YPX4R-MCPE with NaOH (0.1 M) for 10 cycles at SP of 100 mVs^{-1}. b) Graph of Ipa Vs number of voltammetric scans.

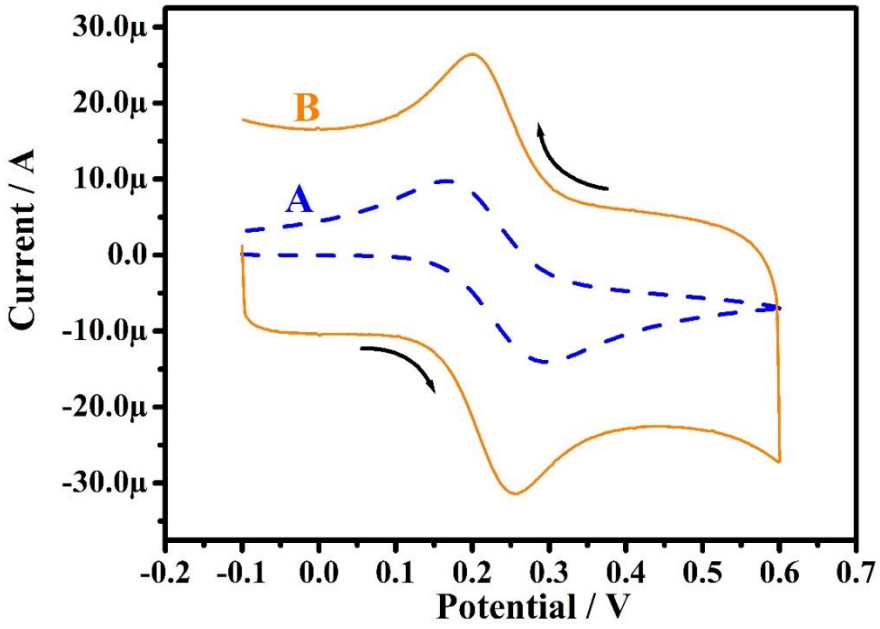


Fig. 4.2. CVs for $K_4[Fe(CN)_6].3H_2O$ at BCPE (Line 'A') and Po-YPX4R-MCPE (Line 'B') at SP of 100 mVs^{-1}.

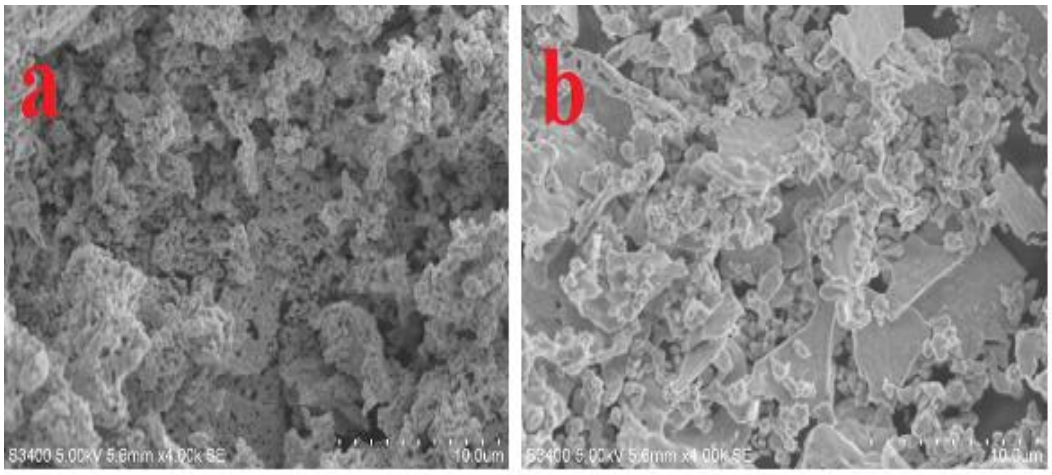


Fig. 4.3. SEM of BCPE (a) and Po-YPX4R-MCPE (b)

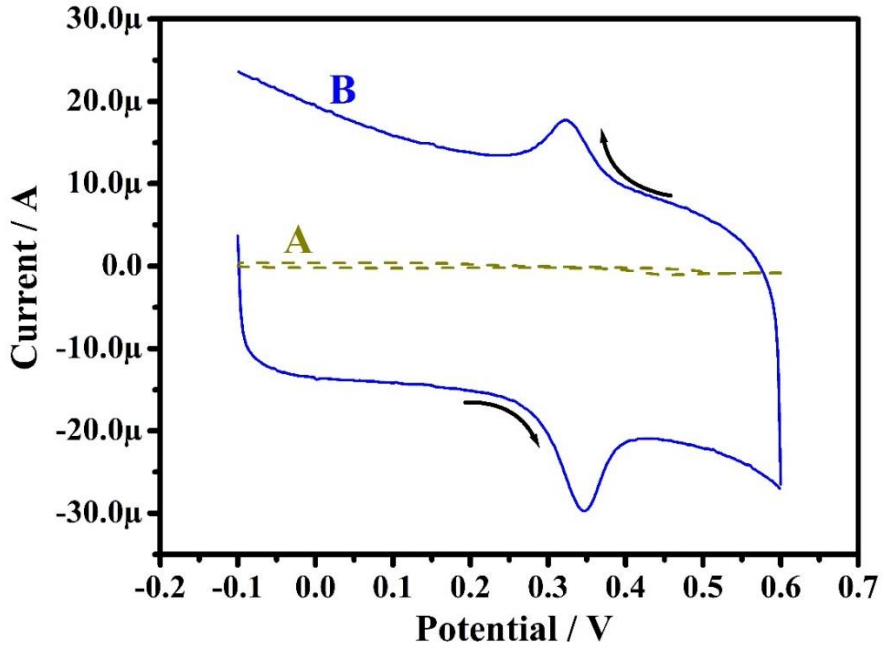


Fig.4.4. CVs of PAR in PBS (0.2 M, 7.4 pH) at BCPE (Line 'A') and Po-YPX4R-MCPE (Line 'B') at SP of 100 mVs^{-1}.

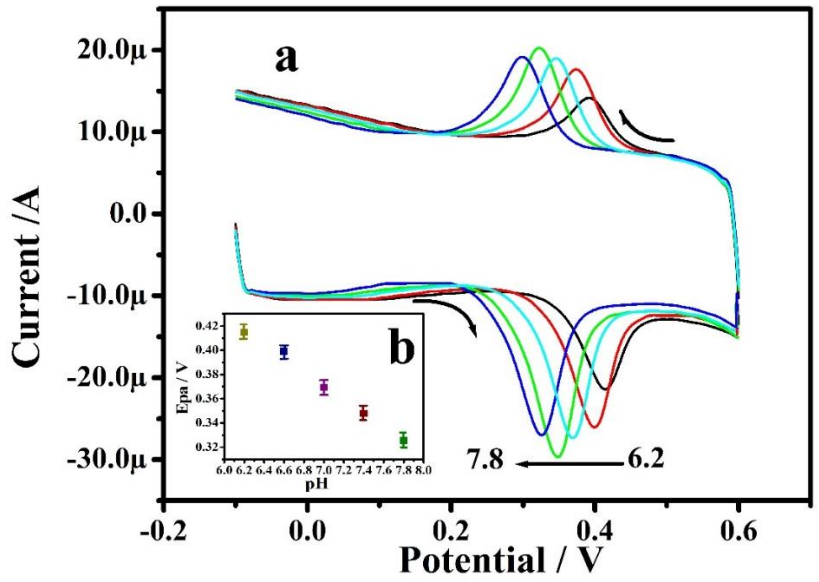


Fig.4.5. a) CVs for PAR with pH series at Po-YPX4R-MCPE. b) Graph of Epa Vs altered pH for PAR.

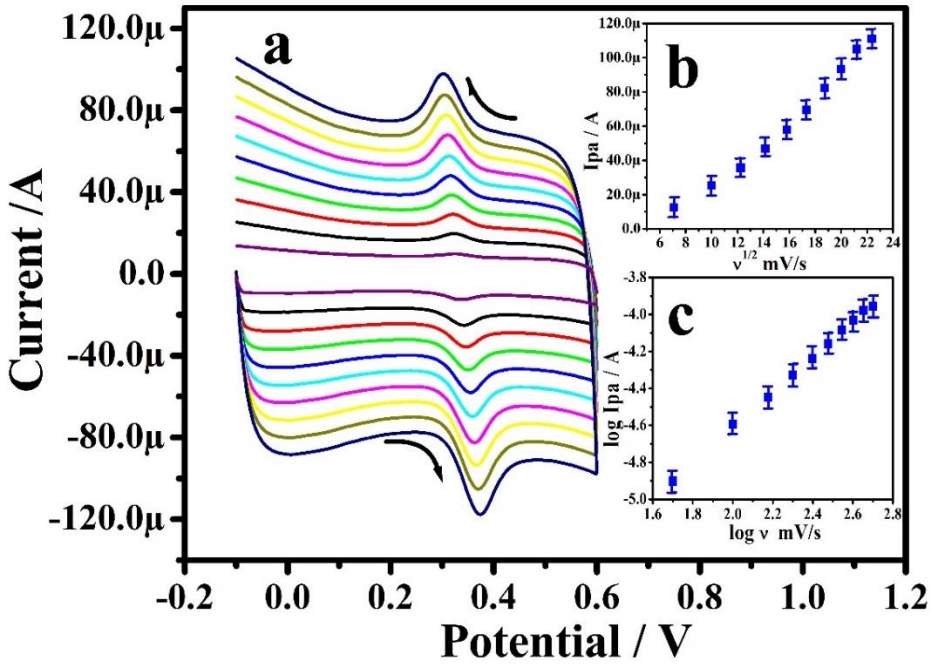


Fig. 4.6. a) CVs documented for PAR (0.1mM) in PBS (0.2M and 7.4 pH) at Po-YPX4R-MCPE with various SP (50-500 mVs^{-1}). b) Graph of Ipa Vs square root of SP of PAR (0.1mM) in PBS (0.2M and 7.4pH). c) Graph of Ipa Vs log of SP of PAR (0.1mM) in PBS (0.2M and 7.4pH).

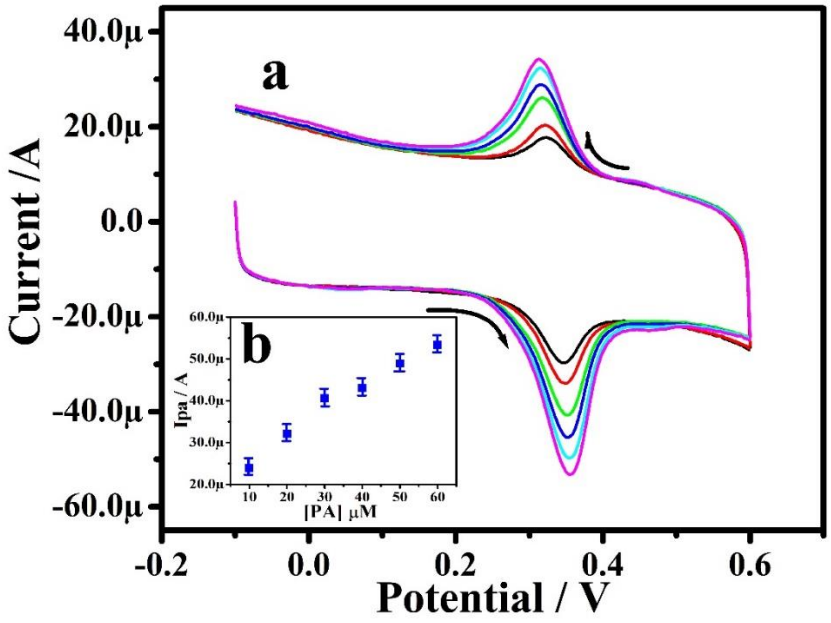


Fig. 4.7. a) CVs documented for different concentration of PAR (10 – 60 µM) using PBS (0.2M and 7.4 pH) at Po-YPX4R-MCPE. b) Graph of Ipa Vs PAR concentrations.

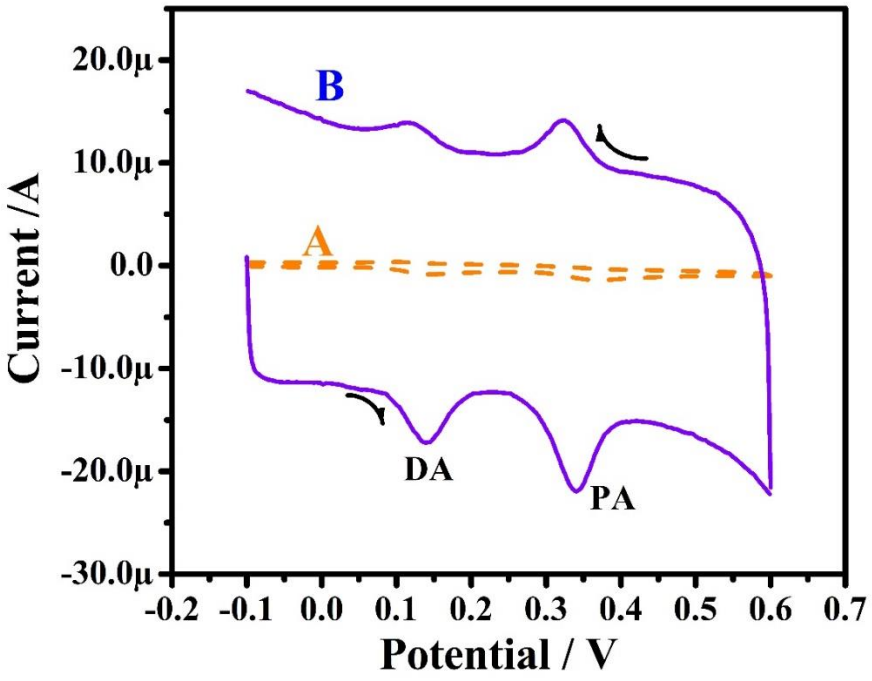


Fig. 4.8. Resulted CVs for simultaneous studies of PAR (0.1mM) and DA (0.1mM) at BCPE (A) and Po-YPX4R-MCPE (B).

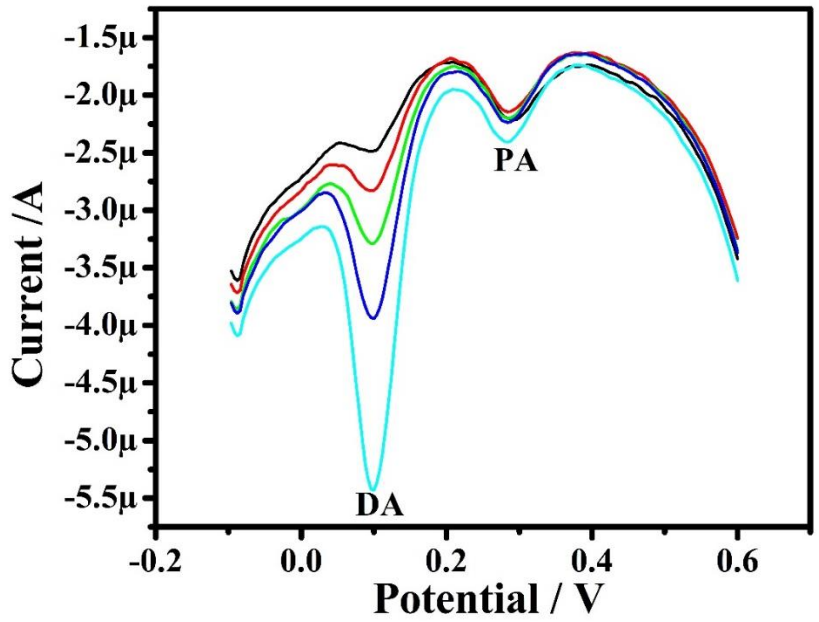


Fig. 4.9. DPVs of DA at altered concentrations (10 - 50 µM) in PBS (0.2 M and 7.4 pH) on Po-YPX4R-MCPE.

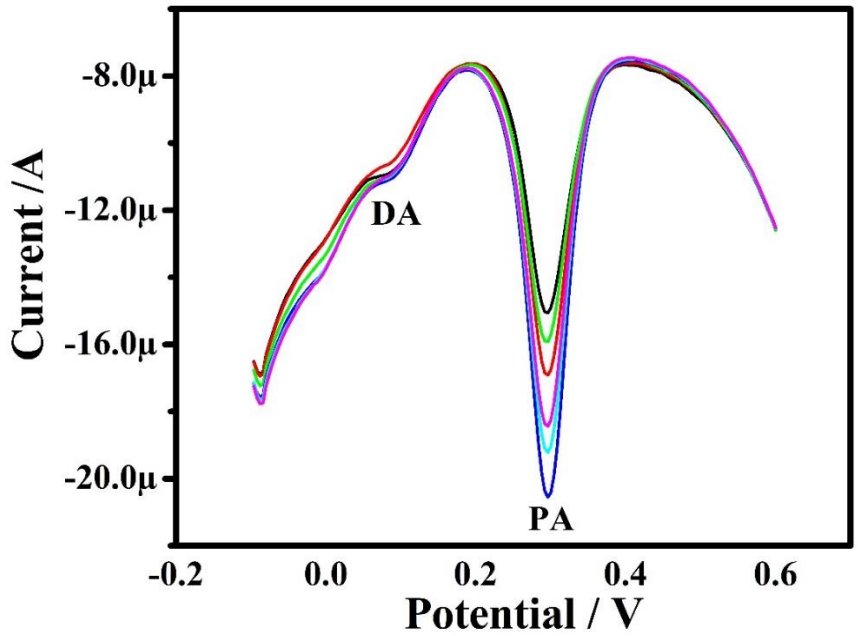


Fig. 4.10. DPVs of PAR at altered concentrations (10 – 60µM) in PBS (0.2M and 7.4 pH) on Po-YPX4R-MCPE.

Table 4.1. Heterogeneous rate constant for PAR at Po-YPX4R-MCPE.

Scan Speed mVs^{-1}	**ΔEp** **mV**	k^0 s^{-1}
50	12	1.383
100	20	2.524
150	27	3.495
200	33	4.351
250	38	5.137
300	46	5.626
350	52	6.128
400	59	6.465
450	65	6.791
500	71	7.045

Table 4.2. LOD of the proposed electrode in comparison to earlier PAR sensors.

Sl.No	Electrode	LOD in µM	Method	References
1	Diacerein/MCPE	3.8	DPV	[34]
2	poly-NA-MCPE	7.2	CV	[39]
3	N-DHPB-MWNT /CPE	10.0	DPV	[40]
4	Pd/Al	50.0	DPV	[41]
5	C_{60}/GCE	50.0	DPV	[42]
6	Cu-poly-TTC	5.0	CV	[43]
7	PVA-Fe_3O_4/MGCE	8.0	DPV	[44]
8	GrRAC sensor	8.36	DPV	[45]
9	TiO_2nanoparticle MCPE	5.25	CV	[46]
10	TiO_2/PB/AuNPs/CMK-3/Nafion/GE	0.21	CV	[47]
11	Nevirapine MCPE	0.77	DPV	[48]
12	Po-YPX4R-MCPE	0.084	CV	This work

Table 4.3. Result of recoveries of PAR at Po-YPX4R-MCPE in a Tablet.

Content	Added (µM)	Found (µM)	Recovery (%)
500mg paracetamol tablet	10	9.9794	99.97
	20	19.7563	98.78
	30	29.8787	99.59
	40	39.9532	100.88

4.5 References

[1] H. V. Dang, H. T. T. Thu, L. D. T. Ha, and H. N. Mai, *J. Anal. Methods Chem.,* 2020 **(2020)** 13

[2] H. Beitollahi, I. Sheikhshoaie, *Mater. Sci. Eng. C,* 32 **(2012)** 375

[3] C. J. Nikles, M. Yelland, C. D. Mar, D. Wilkinson, *Am. J. Ther*., 12 **(2005)** 80

[4] Y. Song, Y. Zhang, J. Li, C. Tan, Y. Li, *J. Electroanal. Chem.,* 865 **(2020)** 114157

[5] R. N. Goyal, V. K. Gupta, M. Oyama, N. Bachheti, *Electrochem. Commun.,* 7 **(2005)** 803

[6] S. O. Shaheen, R. B. Newson, *Thorax*, 57 **(2002)** 958

[7] A. Kannan, R. Sevvel, *J. Electroanal. Chem.* 791 **(2017)** 8

[8] H. Beitollahi, A. Mohadesi, S. Mohammadi, A. Pahlavan, H. K. Maleh, A. Akbari, *J. Molecular Liq.* 169 **(2012)** 130

[9] M. M. Charithra, J. G. Manjunatha, *J. Electrochem. Sci. Eng.* 10(1) **(2020)** 29

[10] M. Iranifam, S. Khodaei, M. Saadati, *Microchem. J.* 146 **(2019)** 850

[11] M. E. C. Peir´o, D. Bose, M. F. Rubert, J. E. Romero *J. Chromatogr. B,* 839 **(2006)** 95

[12] S. H. Youssef, D. Mohamed, M. A. M. Hegazy, A. Badawey, *BMC Chemistry.* 13 **(2019)** 78

[13] N. R. Ahmad, F. K. Omar, *World J. of Pharma. Res.* 7 **(2018)** 15

[14] K. Palur, S. C. Archakam, B. Koganti, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 243 **(2020)** 118801

[15] C. Pasha, *Ecletica Quim J.* 45 **(2020)** 37

[16] B. Chefirat, A. Zergui, M. N. Belmessabih, C. Rahmani, H. Rezk-kallah, *Toxicol. Anal. Clin.* 32 **(2020)** 266

[17] B. Doğan, A. Elik, N. Altunay, *Microchem. J.* 154 **(2020)** 104645

[18] H. Tavallali, Y. Hamid, *Asian J. of Biochem.and Pharma. Res*. 1 **(2011)** 2

[19] B. Avinash, C. R. Ravikumar, M. R. A. Kumar, H. P. Nagaswarupa, M. S. Santosh, A. S. Bhatt, D. Kuznetsov, *J. Phys. Chem. Solids,* 134 **(2019)** 193

[20] J. K. Shashikumara, B. E. K. Swamy, S. C. Sharma, *Chem. Data Coll.,* 27 **(2020)** 100366

[21] T. A. Saleh, *Detection*, 2 **(2014)** 27

[22] J. K. Shashikumara, B. E. K. Swamy, H. D. Madhuchandra, *Mater. Sci. Technol*.3 **(2020)** 390

[23] S. Tajik, H. Beitollahi, *Anal. Bioanal. Chem. Res.*, 6 **(2019)** 171

[24] S. Tajik, M. B. Askari, S. A. Ahmadi, F. G. Nejad, Z. Dourandish, R. Razavi, H. Beitollahi, A. D. Bartolomeo, *Nanomaterials,* 12 **(2022)** 491

[25] M. Shahsavari, M. Mortazavi, S. Tajik, I. Sheikhshoaie, H. Beitollahi, *Micromachines,* 13 **(2022)** 88

[26] Y. Tadesse, A. Tadese, R. C. Saini, and R. Pal, *J. Electroanal. Chem.*, 2013 **(2013)** 7

[27] J. M. Flores-Álvarez, D. Cortés-Arriagada, J. Reyes-Gómez, Z. Gómez-Sandoval, J.C. Rojas Montes, K. Pineda-Urbina, *J. Electroanal. Chem.*, 888 **(2021)** 115208

[28] P. S. Ganesh, B. E. K. Swamy, *J. Biosens. Bioelectron.* 7 **(2016)** 1

[29] K. Chetankumar, B. E. K. Swamy, S. C. Sharma, S. A. Hariprasad, *Sci.Rep.*11 **(2021)** 15064

[30] Q. Qin, X. Bai, Z. Hu, *J. Electroanal. Chem.*782 **(2016)** 50

[31] K. Chetankumar, B. E. K. Swamy, & S. C. Sharma, *J. Electroanal. Chem.* 849 **(2019)** 113365

[32] T. Teker, M. Aslanoglu, *Microchem. J.,* 158 **(2020)** 105234

[33] M. Haroon, I.Abdulazeez, T. A. Saleh, A. A. Al-Saadi, *Electrochimica Acta,* 387 **(2021)** 138463

[34] A. Sathisha, B. E. K. Swamy, *Anal. Bioanal. Electrochem*. 10 **(2018)** 1437

[35] R. N. Goyal, V. K. Gupta, S. Chatterjee, *Sens. Actuat. B.* 149 **(2010)** 252

[36] N. Hareesha, J. G. Manjunatha, *J. Electroanal. Chem.* 878 **(2020)** 114533

[37] A. R.Younus, J. Iqbal, N. Muhammad, F. Rehman, M. Tariq, A. Niaz, S. Badshah, T. A. Saleh, Abdur Rahim, *Microchim Acta*., 186 **(2019)** 471

[38] P. A. Pushpanjali, J. G. Manjunatha, M. T. Shreenivas, *ChemistrySelect,* 4 **(2019)** 13427

[39] A. B. Teradale, P. S. Ganesh, B. E. K. Swamy, S. N. Das, *Anal. Bioanal. Electrochem*. 10 **(2018)** 203

[40] A. A. Ensafi, H. Karimi-Maleh, S. Mallakpour, M. Hatami, *Sens. Actuat. B.* 155 **(2011)** 464

[41] M. H. Pournaghi-Azar, A. Saadatirad, *Electroanalysis*. 22 **(2010)** 1592

[42] R. N. Goyal, S. P. Singh, *Electrochim. Acta*. 51 **(2006)** 3008

[43] M. Boopathi, M. Won, Y. Shima, *Anal. Chim. Acta*. 12 **(2004)** 191

[44] R. Andawiyah, A. Mulyasuryani, H. Sulistyarti, *Mater. Sci. Eng.* 833 **(2020)** 012059

[45] M. K. S. Monteiro, E. C. M. M. Santos, D. R. Silva, C. A. Martínez-Huitle, E. V. dos Santos, *J. Solid State* Electrochem. 24 **(2020)** 1789

[46] K. G. Manjunatha, B. E. K. Swamy, H. D. Madhuchandra, K.A. Vishnumurthy, *Chem. Data Coll.* 31 **(2021)** 100604

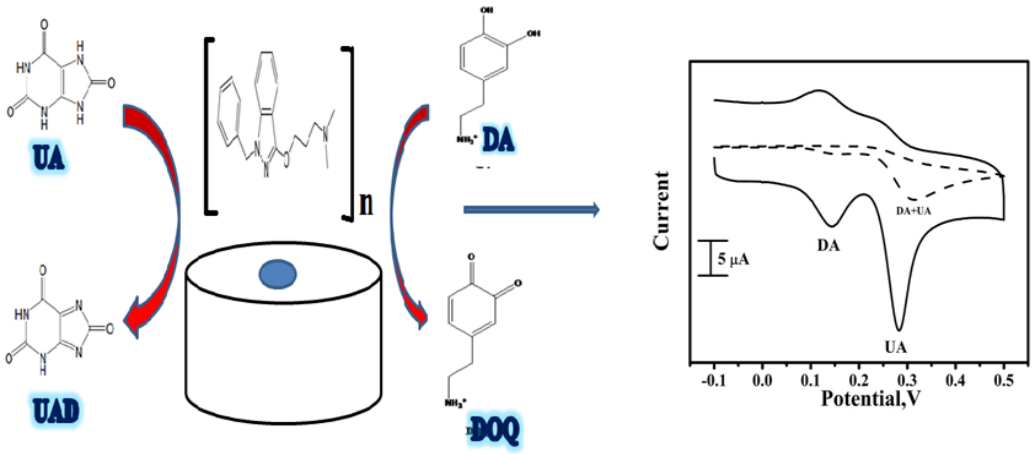
[47] Pollap, K. Baran, N. Kuszewska, J. Kochana, *J. Electroanal. Chem.* 878 **(2020)** 114574

[48] S. B. Tanuja, B. E. K. Swamy, K. V. Pai, *J. Electroanal. Che,* 798 **(2017)** 17

Chapter -5

Part -A

Voltammetric investigation of uric acid in existence of dopamine at Poly (benzydamine) modified carbon paste electrode



Published in
Sensors International 1 (2020) 100045

Sensors International 1 (2020) 100045

Contents lists available at ScienceDirect

Sensors International

journal homepage: www.keaipublishing.com/en/journals/sensors-international

Voltammetric investigation of uric acid in existence of dopamine at Poly(benzydamine) modified carbon paste electrode

Sukanya, B.E. Kumara Swamy *, J.K. Shashikumara

Department of P.G. Studies and Research in Industrial Chemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shimoga, Karnataka, 577451, India

ARTICLE INFO

ABSTRACT

5.1 Introduction

Uric acid (UA) is a most important biological molecule present in body fluids [1]. It is a major nitrogenous compound in the urine. UA is the final product of a many exogenous purines and endogenous purine metabolism. The diet as well as animal proteins alters exogenous pool remarkably. Endogenous production of uric acid predominantly occurs in the intestines, liver, kidneys, muscles, and vascular endothelium [2-4]. In healthy person, the normal UA concentration in urine is around 2 mM and in plasma depending on the age and gender, an average value of UA in healthy 19-year adult blood ranges from 2.6 to 7.2 mg/dL (male 3.5 – 7.2 mg/dL, female 2.6 – 6 mg/dL) [5] The abnormality of it in human body leads to symptoms of several physiological diseases such as, hyperuricaemia, gout, Lesch–Nyan disease and heavy hepatitis [6]. Pneumonia and leukemia are also accompanied with increased urate levels [7]. UA is also a sign for renal failure as well as toxicity [8]. Therefore, accurate determination of UA concentration in human body fluids has become important for the treatment of diseases. In general, irreversible oxidation of electroactive UA in aqueous solution can be utilized for this purpose with the major product being allantoin [9] (Scheme 5.1).

DA, a catecholamine is well known as a neurotransmitter widely distributed in mammalian central nervous system. [10]. High concentration level of DA is found in caudate nucleus of brain and very low concentration of it is observed in extra cellular fluids (0.01-1μM) [11]. DA is used as a medication for Parkinson's disease, Schizophrenia and Alzheimer's disease [12]. It has been observed that the dopamine is an electrochemical active, which gives dopamine-o-quinone as oxidation product [13] (Scheme 5.2). It is well known that UA and DA commonly co-exist in human body fluids [14]. Several methods like fluorescence [15-17], colorimeter method [18, 19], HPLC [20], capillary electrophoresis [21], gas chromatography [22] have been employed for the selective or simultaneous investigation of UA and DA. But, because of the high sensitivity and selectivity, modest and fast response, the electrochemical methods have attained a great influence [23]. However, resolution of UA in existence of DA is always challenging, as they have similar oxidation potential [24, 25]. The variously modified electrodes have been fabricated to overcome this problem. But, in sensor field, CPEs are a good choice for electrochemical investigation, because of their

easy preparation, wider potential window, fast response, renewability of the electrode surface and lower residual currents than glassy carbon electrodes. The CPEs can be modified to enhance their properties by different modifiers [26, 27]. Now a days, the electropolymer film coated electrode plays important role as they are highly selective, sensitive, and homogeneous in deposition, strong and chemically stable adhesion occurs at the electrode surface [28] towards the determination of UA, DA, ascorbic acid etc., [29].

Benzydamine (Scheme 5.3) is a tertiary amine; a basic drug with analgesic, anti-inflammatory, antipyretic and local anesthetic effects [30-32]. It can be used both topically and systemically for the treatment of primary or normoreactive types of inflammation [33].

In the present study, for the first time we have reported fabrication of a poly (benzydamine) MCPE and its application to investigation of UA in existence of DA using CV and DPV techniques. The novel poly (benzydamine) MCPE exhibited an excellent electrocatalytic behavior of uric acid. The oxidation peaks of UA and DA are clearly separated by poly (benzydamine) MCPE. This modified CPE showed high sensitivity, selectivity, stability, and reproducible results for the investigation of UA in existence of DA. The good detection limit for UA was reported to be 11.33nM. The performance of the modified electrode was compared with the other reported modified electrodes for UA shows good result. The poly (benzydamine) MCPE could have a great influence in biomedical, chemical, biological and pharmaceutical research.

5.2. Experimental part

5.2.1. Chemicals and Solutions

Analytical grade chemicals such as UA, DA, NaOH (Nice chemicals) and benzydamine (Sigma Aldrich), graphite powder (Himedia), silicon oil (Lobo Chemie) was used without any further purification. 25 mM UA was prepared in NaOH (0.1M) solution, 25 mM DA in perchloric acid (0.1M), and 25 mM benzydamine in double distilled water. Different pH of 0.2 M PBS was prepared by relevant amount of Na_2HPO_4 and NaH_2PO_4.

5.2.2. Apparatus

The voltammetric measurements were carried out on CHI-660c (CH Instrument-660 electrochemical workstation) consisting of standard three electrode cell such as working (bare CPE or poly (benzydamine) MCPE), reference (saturated Calomel electrode) and auxiliary (platinum) electrode at room temperature.

5.2.3. Construction of bare and modified CPE

The preparation of homogenous bare CPE was carried out by mixing of graphite powder (70%) and silicone oil (30%). Cavity of Teflon tube having a current collector was packed by prepared carbon paste and surface smoothened by a tissue paper [34]. The poly (benzydamine) MCPE was achieved using CV method by electrochemical polymerization of 1mM aqueous benzydamine in 0.1 M NaOH as supporting electrolyte.

5.3. Results and discussion

5.3.1 Electropolymerization of benzydamine on bare CPE

The poly (benzydamine) MCPE was constructed by electropolymerization of 1 mM aqueous benzydamine in 0.1 M NaOH as supporting electrolyte at the scan rate of 100 mV/s for 15 cycles over the potential range between -0.6 V to 1.6 V as illustrated in Fig.5.1A. During the process of cyclic scanning CV was gradually increased at first, after some successive sweeps a steady state voltammogram was observed. It implies that poly (benzydamine) film formed was adhered on the surface of bare CPE [35].

As the electrocatalytic performance of the MCPE related to the polymer film thickness, thickness was well controlled by varying the cycle scans (from 5 to 25). The corresponding electrocatalytic behavior towards the resolution of 0.1mM UA in PBS (pH 7.4) was examined and showed in the inset Fig.5.1B. At 15 multiple cycles, it was observed that maximum enhancement of Ipa, therefore, 15 multiple cycles was considered as a standard for the electropolymerization of benzydamine for all electrochemical analysis.

5.3.2 Electrochemical characterizations

Fig.5.2 shows the electrochemical performance from $K_3[Fe(CN)_6]$ (freshly prepared) at both bare CPE (dotted line) and poly (benzydamine) MCPE (Solid line) in a supporting electrolyte 1 M KCl at a sweep rate of 100mV/s. The redox peaks were observed at bare CPE and poly (benzydamine) MCPE. The low redox peak current signal was seen at bare CPE. At poly (benzydamine) MCPE significant enhancement in the redox peak was observed. ΔEp values are low when compared to ΔEp value for bare CPE. The result shows that MCPE surface morphology was drastically changed, changing the outcome at poly (benzydamine) MCPE. The surface area was available for the electron transfer was calculated by the Randles-Sevick's equation (5.1) [36].

$$I_p = (2.69 \times 10^{-5})\, n^{3/2}\, A\, D_0^{1/2}\, C_0\, \square^{1/2} \text{---------- (5.1)}$$

The surface area (A) of poly (benzydamine) MCPE was found to be maximum (0.047 cm^2) compared to bare CPE (0.030 cm^2), which showed that poly (benzydamine) MCPE as an effective modifier.

5.3.3. Electrochemical performance of UA at poly (benzydamine) MCPE

The electrocatalytic oxidation of 0.1 mM UA at bare CPE (dotted line) and poly (benzydamine) MCPE (solid line) was studied in 0.2 M PBS (pH 7.4) with a sweep rate of 100 mV/s as illustrated in Fig.5.3. The bare CPE exhibits a broad peak of voltammogram at about 282 mV which imply slow electron transfer kinetics because of fouling of the electrode surface as a result of the oxidation process [37]. However, the proposed modified electrode showed a sharp peak at 300mV which is shifted positively by 18mV compared to bare CPE. These phenomena suggest electron transfer kinetics of UA is faster at poly (benzydamine) MCPE [38].

5.3.4. Effect of scan rate

The influence of scan rate for UA (0.1mM) was investigated at poly (benzydamine) MCPE in 0.2 M PBS (pH 7.4) by CV technique. The illustrated Fig. 5.4A showed the small positive shift in the oxidation peak potential with increased peak current when the scan rate varied from 20 to 200 mV/s according to Randles-Sevick's relationship. The graph of I_{pa} Vs scan rate (υ) (Inset Plot 5.4B) and log I_{pa} Vs log of

scan rate (Inset Plot 5.4C) plotted exhibits good linearity with correlation coefficients of 0.9987 and 0.9866, respectively. The slope 0.684 found from the graph log I_{pa} Vs log of scan rate is near to the theoretical value of 0.5 for a diffusion-controlled electrode process. These results indicate that the electrode process is diffusion –controlled [39].

5.3.5. Influence of concentration and pH

The effect of variation of UA concentration was carried out by CV and DPV techniques at poly (benzydamine) MCPE. Fig.5.5A illustrates the CV recorded for UA at different concentrations from 10 to 60 μM at poly (benzydamine) MCPE with a sweep rate 100 mV/s. By increasing concentration of UA, Ipa of UA was found to increase. The graph of Ipa versus UA concentration exhibits good linearity with correlation coefficient value r^2 =0.9939 as illustrated in Fig.5.5B (Inset). LOD of UA was found to 7.19μM using the equation 3S/M, where S is standard deviation and M is slope [40]. Fig.5.6A shows the DPVs obtained for UA with different concentrations from 5-30 nM. By increasing the UA concentration, the current response corresponding to the oxidation of UA goes on increasing. Fig.5.6B (Inset) shows the plot of Ipa Vs UA concentration having good linearity. LOD of UA was found to 11.33 nM by DPV method. The LOD of poly (benzydamine) MCPE for UA was compared with other reported MCPE is given in Table. 5.1

The influence of pH at the poly (benzydamine) MCPE on the investigation of UA in PBS solution at the poly (benzydamine) MCPE was studied in the pH range from 6.2 to 7.8. A shift to less positive potential of anodic peak potential of UA was observed as the pH increased as shown in Fig.5.7A. Fig.5.7B (Inset) illustrate the plot of E_{pa} versus different pH having a good linearity and slope value obtained is very close to the Nernstian value (59 mV) for an equal number of electron and proton transfer reaction [36].

5.3.6. Simultaneous electroanalysis of Uric acid and Dopamine

To establish a selectivity and sensitivity, the electrochemical behavior of UA in existence of interference like DA at poly (benzydamine) MCPE was investigated. The Fig.5.8 showed the CVs obtained for the binary mixture of UA (50 μM) in existence of equal concentration of DA at both bare CPE and poly (benzydamine) MCPE. At bare

CPE (dashed line), the voltammetric response obtained was broad, less sensible for the oxidation of two analytes and overlapped at a potential of 306 mV. However, in the same environment poly (benzydamine) MCPE (solid line) resolved oxidation potential peaks of UA and DA was observed. Well-distinct oxidation peak potentials of UA at 262 mV and DA at 150 mV were observed. These results proved the higher sensitivity for the simultaneous determination of UA in existence of DA at poly (benzydamine) MCPE.

5.3.7. Interference study

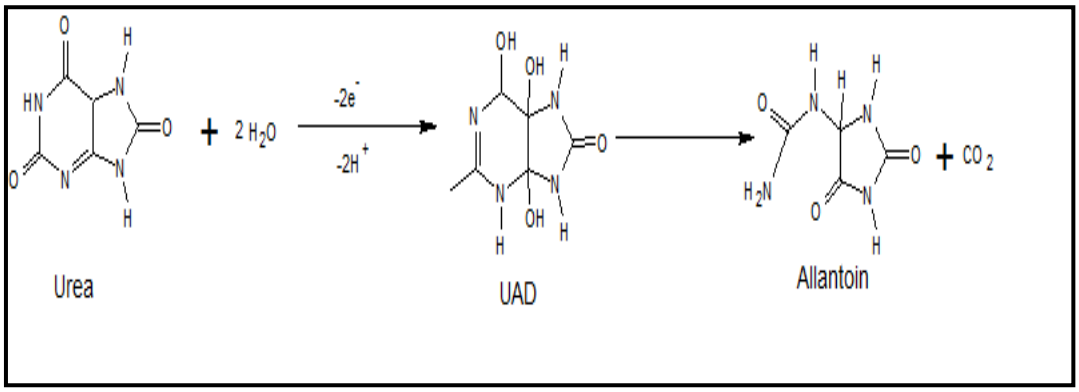
The interference in the sample of a mixture containing UA and DA was studied at poly (benzydamine) MCPE by DPV technique. Fig.5.9 shows the DPVs of UA and DA mixture in which only peak current of UA was increased as UA concentration being varied from 10 to 60 µM while the DA concentration (10 µM) was constant. No change in the DA peak current. Fig.5.10 illustrates only the DA peak current was increased when the concentrations of DA was varied from 10 to 60 µM while keeping the UA (10 µM) concentration constant. These results suggest that oxidation of UA and DA occurs independently. Thus, at poly (benzydamine) MCPE the difficulty of interference was effectively resolved, and it can be used to identify them simultaneously.

5.3.8 Reproducibility and stability study

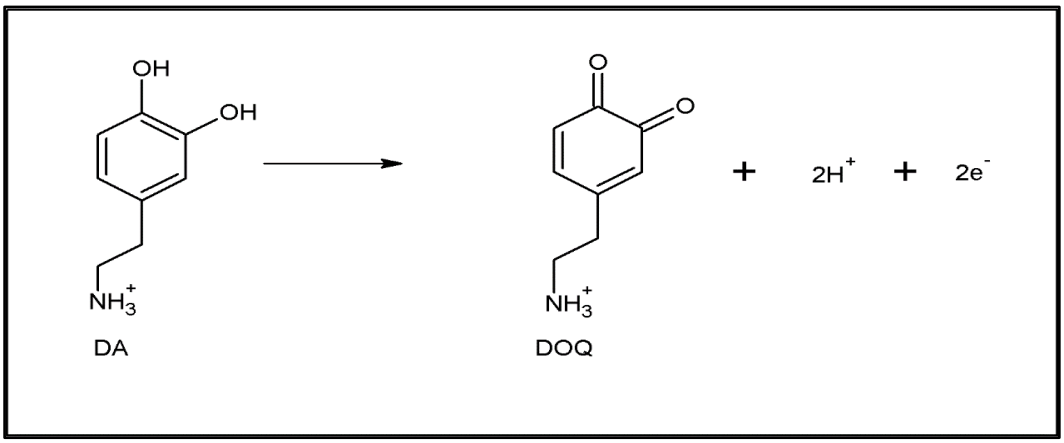
The reproducibility of the modified electrode was investigated by four different poly (benzydamine) MCPE were fabricated individually in the same manner by CV method. The RSD value observed was of 2.8 indicating good reproducibility of the modified electrode. The stability of the modified electrode was studied by continuously scanning for 10 cycles in 0.1 mM UA. When the percentage degradation (= Ipn / Ip_1, where Ipn and Ip_1 are the n^{th} and first cycle anodic peak current respectively) of the modified electrode was calculated, it was less than 2 % indicating that the peak current remains almost constant even after multiple cycling confirming that the modified electrode has good stability.

5.4. Conclusion

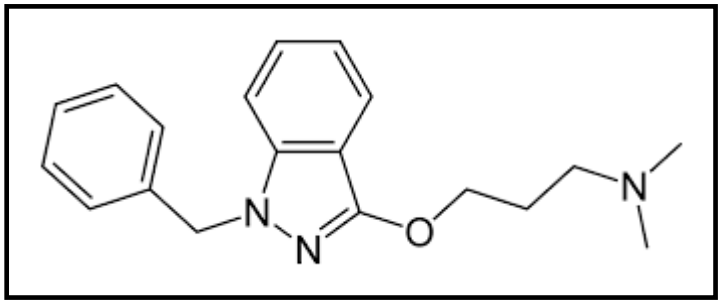
In the present work, the modification of the CPE electrode with benzydamine is an anti-inflammatory drug has led to the fabrication of a most stable sensor for uric acid with excellent selectivity and sensitivity. The developed of poly (benzydamine) MCPE was highly reproducible and repeatable. The electrode process was diffusion controlled. The newly investigated sensor showed good sensitivity, selectivity, stability, and fast electron transfer in the identification of UA and DA simultaneously with separation peak potential value of 112 mV and 118mV from CV and DPV techniques, respectively. Thus, poly (benzydamine) MCPE can be applied into biological, pharmaceutical, and biomedical field for the studies of other biological molecules.



Scheme 5.1. Electrocatalytic oxidation of UA



Scheme 5.2. Electrochemical oxidation of DA



Scheme 5.3. Structure of Benzydamine

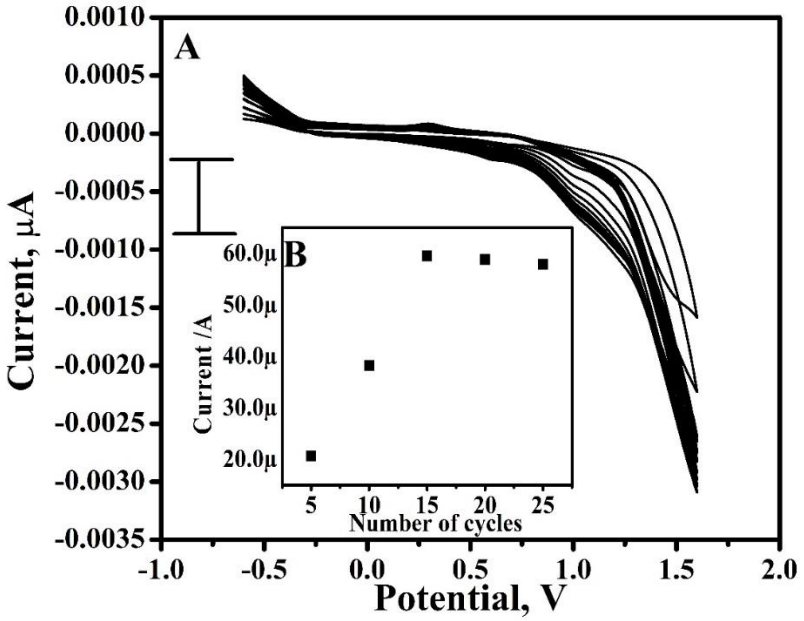


Fig.5.1. A) CVs of 1mM developed poly (benzydamine) MCPE in 0.1 NaOH at 15 cycles with a sweep rate of 100mV/s and insert is graph (B) Plot Ipa Vs number of cycles.

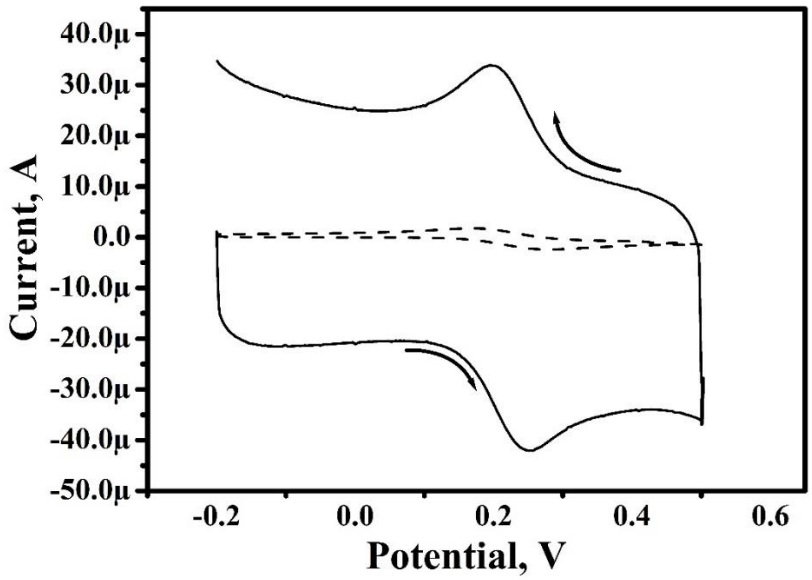


Fig.5.2. CVs of 1mM K_4 [Fe $(CN)_6$] at bare CPE (dotted line) and poly (benzydamine) MCPE (solid line) at a sweep rate of 100 mV/s.

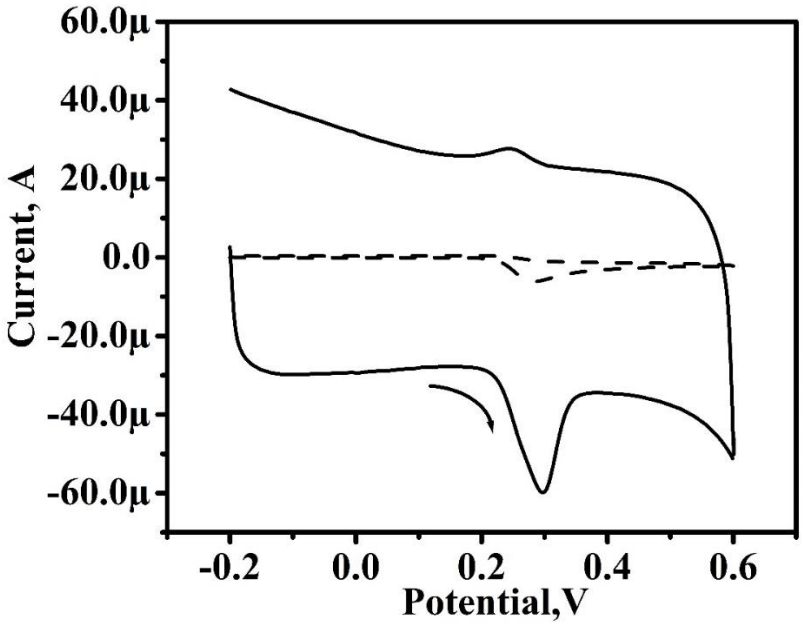


Fig.5.3. CVs of 0.1mM UA bare CPE (dotted line) and poly (benzydamine) MCPE (solid line) in 0.2 M PBS (pH 7.4) at sweep rate of 100 mV/s.

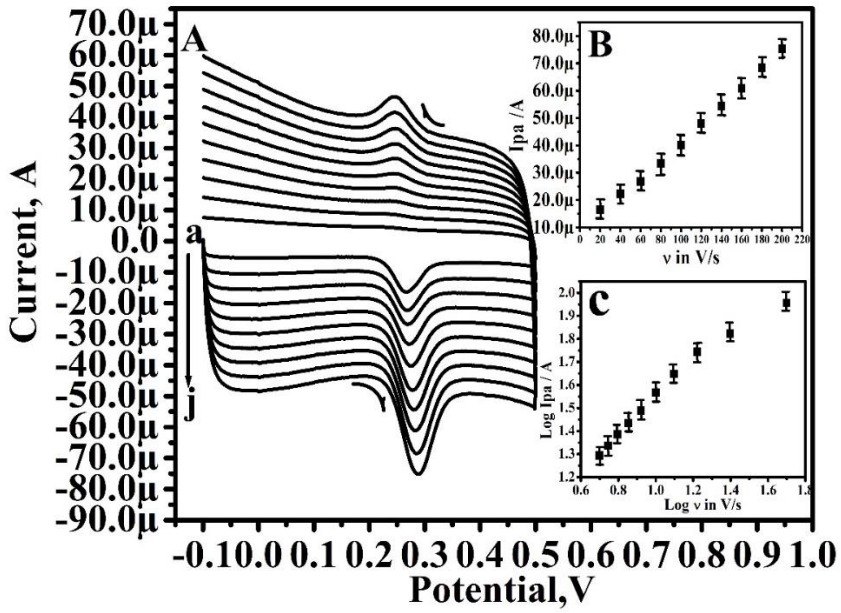


Fig.5.4. A) CVs of 0.1mM UA at poly (benzydamine) MCPE in 0.2M PBS (pH 7.4) at varied scan rates. (a–j; -0.1 to 0.5 V/s); B) Plot of Ipa Vs scan rate; C) Plot of log Ipa Vs log of scan rate.

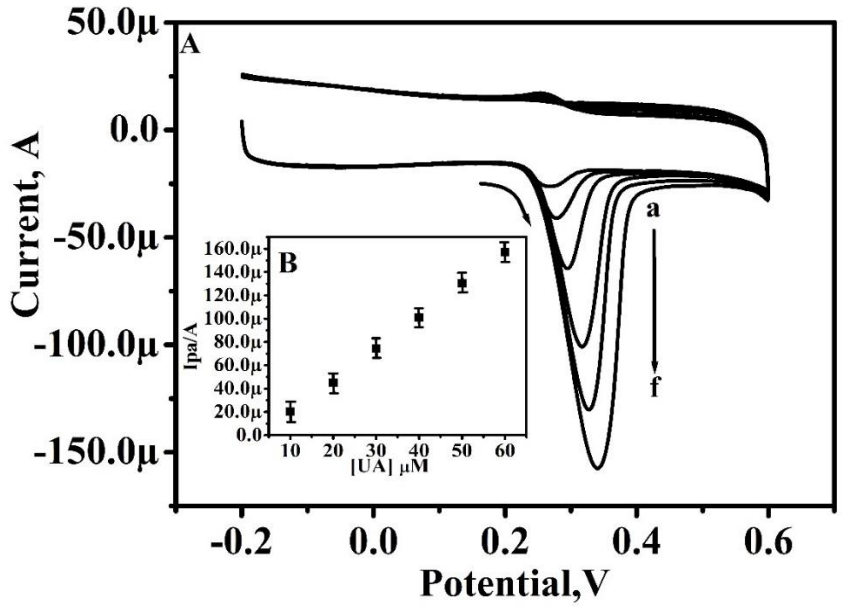


Fig.5.5. A) CVs of UA in 0.2M PBS (pH 7.4) at MCPE at a sweep rate of 50 mV/s with varied concentrations (10µM, 20µM, 30µM, 40µM, 50µM, 60µM; a–f); B) Plot of Ipa Vs UA concentration.

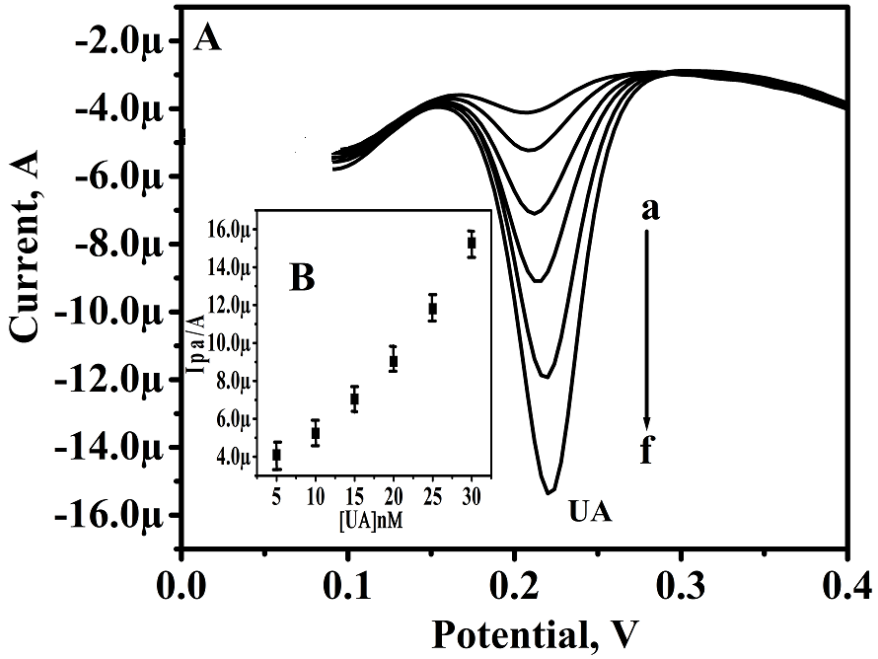


Fig.5.6. A) DPVs of UA in 0.2M PBS (pH 7.4) at MCPE at a sweep rate of 50 mV/s with varied concentrations (5nM, 10nM, 15nM, 20nM, 25nM, 30nM; a–f); B) Plot of Ipa Vs UA concentration.

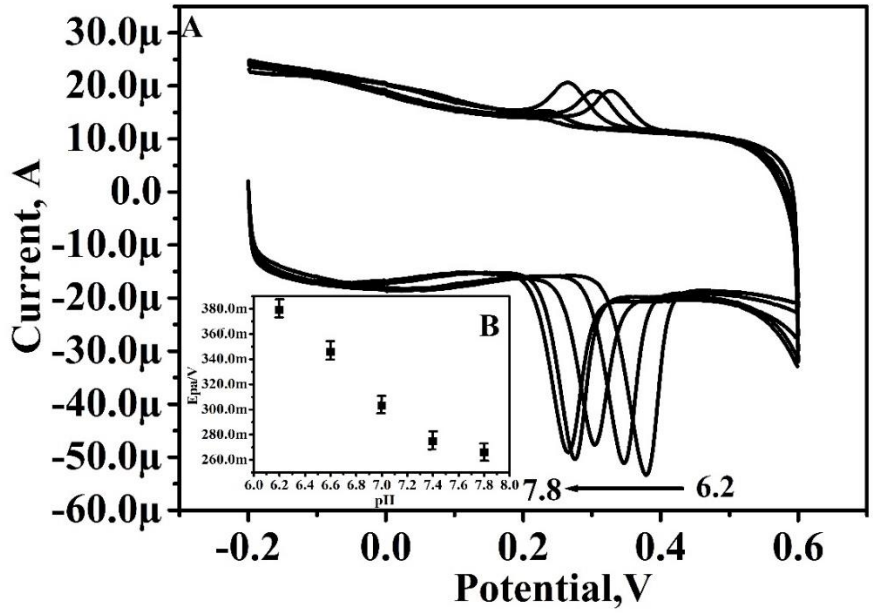


Fig.5.7. A) Cyclic voltammograms of 0.1mM UA with varied pH (6.2 to 7.8 pH; a-e) at poly (benzydamine) MCPE. (B) Plot of Epa Vs varied pH.

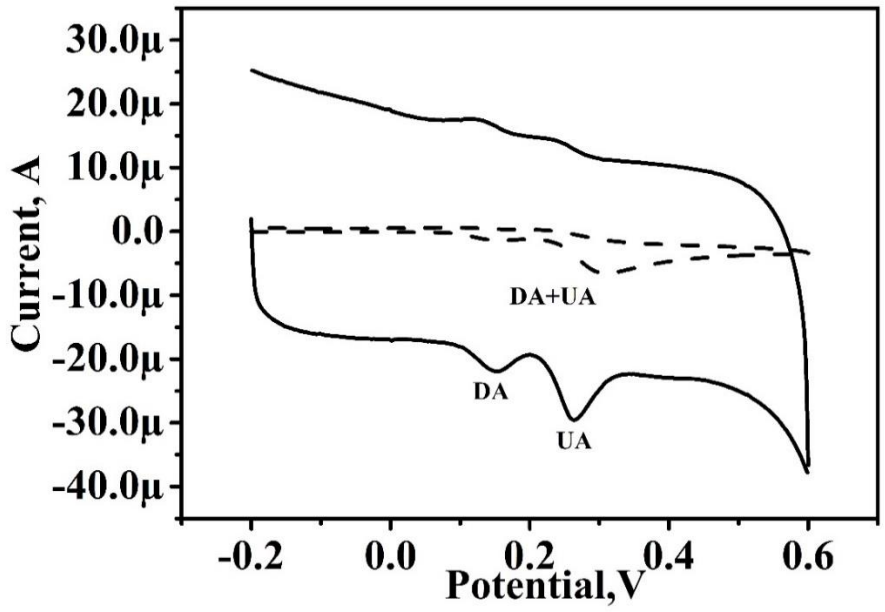


Fig.5.8. A) CVs for simultaneous analysis for 50µM UA, and 50µM DA at bare CPE (dashed line) and MCPE (solid line) at a sweep rate of 100 mV/s.

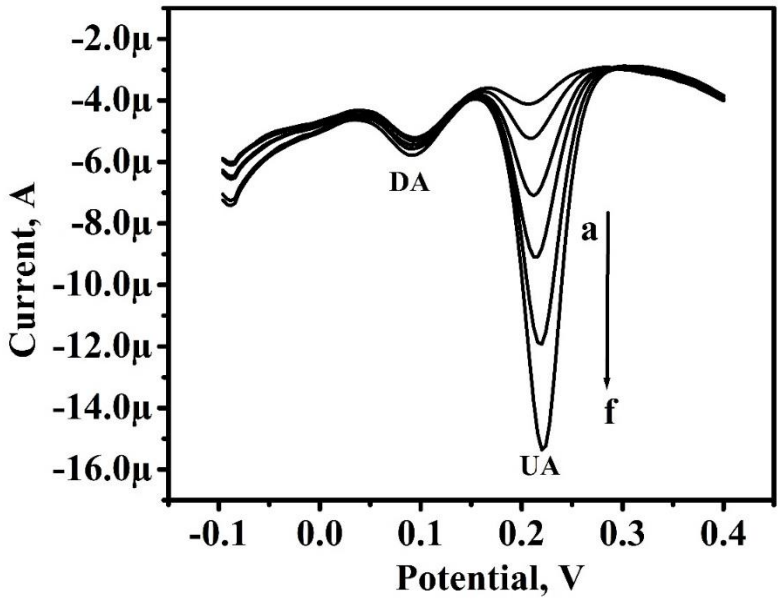


Fig.5.9. DPVs recorded for different of UA concentration (10 µM-60 µM (a-f)) in 0.2M PBS (pH 7.4) with sweep rate of 100 mV/s in existence of 10 µM DA at poly (benzydamine) MCPE.

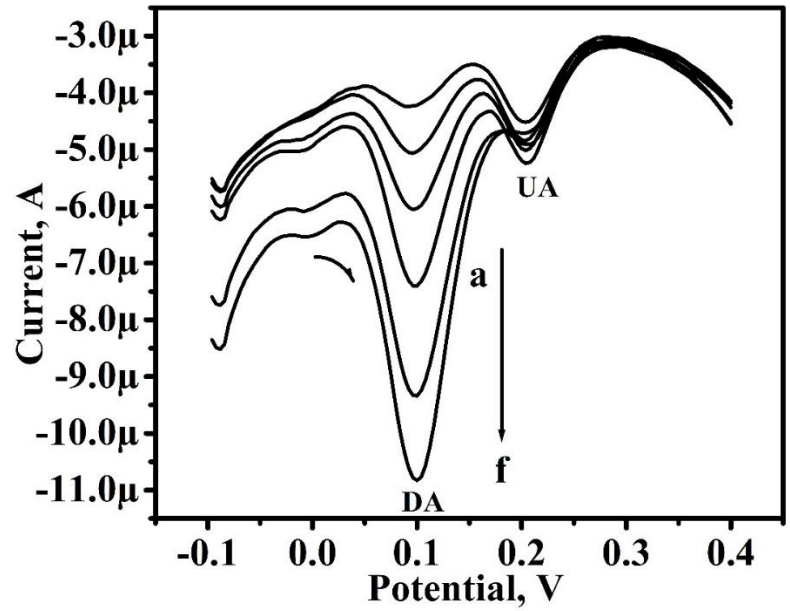


Fig.5.10. DPVs recorded for different of DA concentration (10 µM-60 µM (a-f)) in 0.2M PBS (pH 7.4) with sweep rate of 100 mV/s in existence of 10 µM UA at poly (benzydamine) MCPE.

Table.5.1. Comparison of the LOD of the poly (benzydamine) MCPE with other reported MCPE.

Sl.No	Electrode	Detection limit of UA	Method	References
01	Poly (o -aminophenol)-MCPE	3.0 µM	CV	[7]
02	PPS/SAOS/MCPE	4.18 µM	CV	[9]
03	R-GO/MCPE	0.12 µM	CV	[11]
04	Au/Fc-ac-PPP	0.01 µM	DPV	[12]
05	p-ProH/GCE	0.5 µM	CV	[14]
06	MWCNT-COOH/SWCNT-OH	0.61 µM	DPV	[24]
07	PAIUCPE	0.11 µM	LSV	[27]
08	β-CD/rGO/SPE	0.026 µM	DPV	[35]
09	Pd/RGO/GCE	1.6 µM	DPV	[41]
10	Glu/GCE	1.1 µM	DPV	[42]
11	Au-Cu_2O/rGO/GCE	6.5 µM	DPV	[43]
12	Pdop@GR/MWCNTs	15.0 µM	DPV	[44]
13	PG/GCE	4.82 µM	CV	[45]
14	poly (Benzydamine)MCPE	11.33 nM	DPV	This work

5.5. References

[1] K. Shi, K. Shiu, *Electroanalysis*, 13 **(2001)** 1319

[2] H. Kaur, B. Halliwell, *Chem. Biol. Interactions*, 73 **(1990)** 235

[3] K. Chaudhary, K. Malhotra, J. Sowers, A. Aroor, *Cardiorenal. Med*, 3 **(2013)** 208

[4] J. Maiuolo, F. Oppedisano, S. Gratteri, C. Muscoli, V. Mollace, *Int. J. Cardiol.*, 213 **(2016)** 8

[5] D. Plausinaitis, L. Sinkevicius, U. S. Bubniene, V. Ratautaite, A. Ramanavicius, *Talanta*, 220 **(2020)** 121414

[6] C. R. Raj, F. Kitamura, T. Ohsaka, *Analyst*, 127 **(2002)** 1155

[7] E. Miland, A. J. M. Ordieres, P. T. Blanco, M. R. Smyth, C. O. Fagain, Talanta, 43 **(1996)** 765

[8] J. G. Manjunatha, B. E. K. Swamy, G. P. Mamatha, O. Gilbert, B. S. Sherigara, *Anal. Bioanal. Electrochem*.,3 **(2011)** 146

[9] T. S. N. S. K. Naik, B. E. K. Swamy, *Anal. Bioanal. Electrochem*., 9 **(2017)** 424

[10] Y. Oztekin, M. Tok, E. Bilici, L. Mikoliunaite, Z. Yazicigil, A. Ramanaviciene, *Electrochim. Acta.*, 76 **(2012)** 201

[11] H. Vidya, B. E. K. Swamy, *Science Jet*, 4 **(2015)** 77

[12] Z. Hsine, S. Blili, R. Milka, H. Dorizon, *Anal. Bioanal. Chem.*, 412 **(2020)** 4433

[13] U. Chandra, B. E. K. Swamy, O. Gilbert, B. S. Sherigara, *Electrochim. Acta.*, 55 **(2010)** 7166

[14] D. Kong, Q. Zhuang, Y. Han, L. Xu, Z. Wang, L. Jiang, J. Su, C. Lu, Y. Chi, *Talanta*, 185 **(2018)** 203

[15] D. Jin, M. Seo, B. T. Huy, Q.T. Pham, M. L. Conte, D. Thangadurai, Y. Lee, *Biosens. Bioelectron.*,77 **(2016)** 359

[16] J. Zhao, L. Zhao, C. Lan, S. Zhao, *Sens. Actuators, B: Chem.*, 222 **(2016)** 246

[17] N. E. Azmi, A. H. A. Rashid, J. Abdullah, N. A. Yusof, H. Sidek, *J. Lumin.*, 202 **(2018)** 309

[18] K. Tan, G. Yang, H. Chen, P. Shen, Y. Huang, Y. Xian, *Biosens. Bioelectron.*,59 **(2014)** 227

[19] J. Wang, X. Fang, Y. Zhang, X. Cui, H. Zhao, X. Li, Z. Li, *Colloid. Surface. A.* 555 **(2018)** 565

[20] R. Zuo, S. Zhou, Y. Zuo, Y. Deng, *Food Chem.,* 182 **(2015)** 242

[21] H. Lee, S. Chen, *Talanta*, 64 **(2004)** 750

[22] Y. Sun, J. Chen, H. Qi, Y. Shi, *J. Chromatogr. B.*, 1004 **(2015)** 53

[23] S. Selvarajan, A. Suganthi, M. Rajarajan, *Surf. Interfaces.,* 7 **(2017)** 146

[24] J. Guan, J. Zou, Y. Liu, X. Jiang, J. Yu, *Ecotoxicol. Environ. Saf.,* 201 **(2020)** 110872

[25] U. Chandra, B. E. K. Swamy, O. Gilbert, S. Reddy, B. S. Sherigara, *Am. J. Chem.,* 2 **(2011)** 262

[26] M. Arvand, A. Pourhabib, M. Giahi, *J. Pharm. Anal.,* 7 **(2017)** 110

[27] J. Huo, E. Shangguan, Q. Li, *Electrochim. Acta.,* 89 **(2013)** 600

[28] Y. V. M. Reddy, V. P. Rao, A. V. B. Reddy, M. Lavanya, M. Venu, M. Lavanya, G. Madhavi, *Mater. Sci. Eng. C,* 57 **(2015)** 378

[29] K. R. Mahanthesha, B. E. K. Swamy, U. Chandra, *JETIR*, 5 **(2018)** 520

[30] D. S. Jesus, C. M. C. M. Couto, A. N. Araujo, M. C. B. S. M. Montenegro, *J. Pharm. Biomed.,* 33 **(2003)** 983

[31] V. Sarveiya, J. F. Templeton, H. A. E. Benson, *Eur. J. Pharm. Sci.,* 26 **(2005)** 39

[32] B. Silvestrini, P. S. Barcellona, A. Garau, B. Catanese, *Toxicol. Appl. Pharm.,* 10 **(1967)** 148

[33] G. A. Baldock, R. R. Brodie, L. F. Chasseaud, T. Taylor, *J. Chromatogr.,* 529 **(1990)** 113

[34] M. Kumar, B. E K. Swamy, S. Reddy, W. Zhao, S. Chetana, V. G. Kumar, *J. Electroanal. Chem.,* 835 **(2019)** 96

[35] Q. Qin, X. Bai, Z. Hua, *J. Electroanal. Chem.,* 782 **(2016)** 50

[36] N. P. Shetti, S. J. Malode, R. S. Malladi, S. L. Nargund, S. S. Shukla, T. M. Aminabhavi, Microchem. *J.,* 146 (2019) 387

[37] T. S. S. K. Naik, M. M. Mwaurah, B.E. K. Swamy, *J. Electroanal. Chem.,* 834 **(2019)** 71

[38] O. Gilbert, B. E. K. Swamy, U. Chandra, B. S. Sherigara, *J. Electroanal. Chem.,* 636 **(2009)** 80

[39] K. R. Mahanthesha, B. E. K. Swamy, JETIR, 5 **(2018)** 16

[40] M. D. Meti, J. C. Abbar, S. T. Nandibewoor, S. A. Chimatadar, *Cogent Chem.,* 2 **(2016)** 1235459

[41] J. Wang, B. Yang, J. Zhong, B. Yan, K. Zhang, C. Zhai, Y. Shiraishi, Y. Du, P. Yang, *J. Colloid Interface Sci.,* 497 **(2017)** 172

[42] L. Zhang, X. Lin, *Analyst*, 126 **(2001)** 367

[43] T. K. Aparna, R. Sivasubramanian, M. A. Dar, *J. Alloys Compd.,* 741 **(2018)** 1130

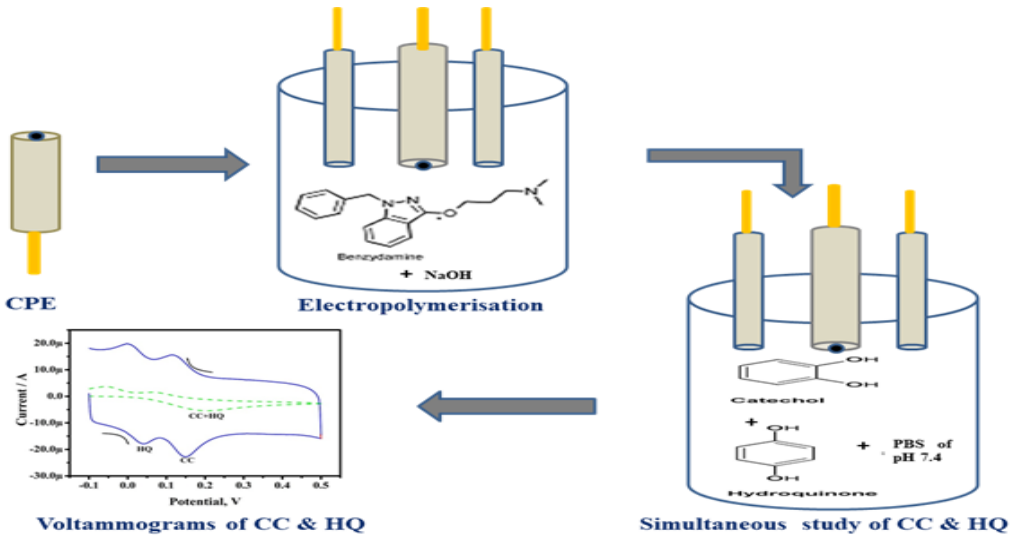
[44] C. Wang, J. Li, K. Shi, Q. Wang, X. Zhao, Z. Xiong, X. Zou, Y. Wang, *J. Electroanal. Chem.,* 770 **(2016)** 56

[45] S. Qi, B. Zhao, H. Tang, X. Jiang, *Electrochim. Acta*, 161 **(2015)** 395

Chapter -5

Part -B

Poly (benzydamine) sensor for electrochemical resolution of catechol and hydroquinone



Published in
Materials Science for Energy Technologies 3 (2020) 640–647

Materials Science for Energy Technologies 3 (2020) 640–647

KeAi
CHINESE ROOTS
GLOBAL IMPACT

Contents lists available at ScienceDirect

Materials Science for Energy Technologies

journal homepage: www.keaipublishing.com/en/journals/materials-science-for-energy-technologies

Poly (benzydamine) sensor for electrochemical resolution of catechol and hydroquinone

Sukanya, B.E. Kumara Swamy *, J.K. Shashikumara

Department of P.G. Studies and Research in Industrial Chemistry, Kuvempu University, JnanaSahyadri, Shankaraghatta, Shimoga, Karnataka 577451, India

ARTICLE INFO

ABSTRACT

5.6 Introduction

In analytical chemistry, the simultaneous and interference less analysis of organic isomers is a fascinating aspect for many researchers [1, 2]. The electrochemical techniques such as voltammetric method have drawn much more attention due to their fast response, high selectivity and separation, low cost, and simple operation [3-7]. Recently, in the electroanalysis of biologically important oxidizable compounds, the MCPE has attracted ample recognition due to its low cost, high sensitivity, simple preparation, reproducibility with low detection limit and its eco-friendly nature [8-13]. The two positional isomers of a dihydroxybenzene moiety namely, CC and HQ [14-15] are widely used in cosmetics, dyes, photostabilizers, plasticizers, pesticide, oil refineries, antioxidants, leather, coal tar, and pharmaceutical industries [16-19]. Both CC and HQ are difficult to degrade and are found to be extremely harmful to humans and animals and are listed as the possible human carcinogens by the International Agency for Research on Cancer [20-22]. Hence, US Environmental Protection Agency (EPA) and European Union (EU) have considered these isomers as the serious environmental pollutants [23, 24]. Moreover, Both CC and HQ usually co-exist due to their structural and property resemblance [25, 26]. These factors necessitate the development of simple and efficient qualitative and quantitative analytical methods for these phenolic isomers [27]. There are many research reports in this regard, such as pH-based flow injection analysis [28], Electrochemiluminescence [29], Synchronous fluorescence [30], fluorescence [31], Spectrophotometry [32], mass spectrometry [33], HPLC [34,35]. All these techniques are complicated, time consuming and expensive. Owing to the same physico- chemical properties of these isomers, they show overlapped and broad voltammograms at BCPE [36]. Electropolymerization leading to the modification of carbon paste electrode would be interesting because of their simplicity, chemical stability, good sensitivity, strong adherence of polymer film; promote electron transfer rates and broad potential window [37, 38]. This paper describes the preparation of poly (benzydamine) MCPE for the electrochemical sensing of CC and HQ in binary mixtures using CV and DPV techniques. Benzydamine (Scheme.5.4), a tertiary amine is a basic drug with analgesic, anti-inflammatory, antipyretic and local anesthetic effects [39-41], used both topically and systemically for the treatment of primary or normoreactive types of inflammation [42]. The poly (benzydamine) MCPE revealed

excellent electrocatalytic activity, sensitivity, stability, selectivity and reproducibility, low detection limit, wide linear ranges towards the resolution of CC and HQ.

5.7. Experimental

5.7.1. Chemicals and solutions

Analytical grade chemicals such as CC, HQ (Himedia), graphite powder (Lobo Chemie) and benzydamine (Sigma Aldrich) were used as received without any further purification. $25x10^{-3}$ M benzydamine and $25x10^{-4}$ M stock solutions of CC and HQ were prepared using double distilled water. The solution of 0.2M PBS of different pH was prepared using Na_2HPO_4 and $NaH_2PO_4.H_2O$.

5.7.2. Apparatus

For the electrochemical measurements CHI-660c model (CH Instrument-660 electrochemical workstation) comprising of a standard three electrode cell at room temperature was made use of. The working electrode was BCPE or poly (benzydamine) MCPE while saturated Calomel (SCE) electrode and platinum wire were used as reference and counter electrodes, respectively.

5.7.3. Construction of working electrodes

The homogenous BCPE was prepared by mixing graphite powder with silicone oil (70:30) in an agate mortar followed by packing it into a cavity of homemade Teflon tube having a current collector. It is then smoothened by a tissue paper to get a smooth surface [43, 44]. The poly (benzydamine) MCPE was successfully developed using CV method by electropolymerization of 1mM aqueous benzydamine in presence of supporting electrolyte, 0.1M NaOH.

5.8. Results and discussion

5.8.1 Electropolymerization of benzydamine on BCPE

The poly (benzydamine) MCPE was optimized by the electropolymerization of 1 mM aqueous benzydamine in 0.1 M NaOH (supporting electrolyte) on the BCPE surface in the potential window between -0.6 to 1.6 V (Fig.5.11A) at a sweep rate of 100 mVs^{-1} for 10 cycles. During multiple scanning processes, the voltammogram

gradually increased at first and after some successive sweeps the voltammogram becomes virtually constant. This suggests the benzydamine film formation and growth on the surface of BCPE [45]. So obtained electropolymerized electrode was dipped in the demineralized water to remove any physically adsorbed material.

As the thickness of the polymer film influences the electrocatalytic performance of the modified electrode, it was effectively controlled by varying the number of cycles (5 to 25). The electrocatalytic activity corresponding to it was investigated for the resolution of CC and HQ as shown in the inset Fig.5.11B. At ten multiple cycles, the current response was found to be maximum. Hence, ten cycles were considered as a standard for fabrication of poly (benzydamine) MCPE. On further increasing the polymerizing cycles, the peak current begins to decrease. This may be attributed to the growing thickness of the film that prevents the electron transfer process and reduces the oxidation process [14, 46]. Thus, for the electropolymerization of benzydamine, 10 cycles were considered as a standard in all further electrochemical analysis.

5.8.2 Characterization of poly (benzydamine) MCPE

Using potassium ferrocyanide system, the electrocatalytic response of the MCPE was investigated by CV method. The electrochemical response of freshly prepared $K_4[Fe(CN)_6]$ at BCPE (dotted line) and poly (benzydamine) MCPE (Solid line) in 1 M KCl (supporting electrolyte) at a sweep rate of 100 mVs^{-1} was observed as shown in Fig.5.12. The lower redox peak current signal corresponds to BCPE whereas MCPE exhibits sharp increase in peak current indicating the fast rate of electron transfer kinetics [47]. This suggests the considerable alteration in the surface morphology of the MCPE. By using Randles-Sevick's equation (5.1), the total active surface area of MCPE was estimated [48]. For poly (benzydamine) MCPE the electroactive surface area (A) was found to be maximum (0.0479 cm^2) when compared to BCPE (0.030 cm^2). This results in the enhancement of electrocatalytic property of poly (benzydamine) MCPE.

The structural morphology of BCPE and poly (benzydamine) MCPE was characterized by SEM. SEM of BCPE (Fig.5.13A) has shown the rough surface with irregular shaped flake- like structures [39]. While the SEM of poly (benzydamine) MCPE (Fig.5.13B) shows the uniform arrangement of a thin film of benzydamine on

the CPE surface [50]. The significant differences in the surface structures of the two electrodes indicate the successful alteration of the surface of CPE by electropolymerized benzydamine [51].

5.8.3. Electrochemical behavior of analytes

The electrochemical response of CC and HQ at BCPE (dotted line) and poly (benzydamine) MCPE (solid line) was studied in PBS (0.2 M, pH 7.4) with a sweep rate of 50 mVs^{-1} by CV technique as illustrated in Fig.5.14A and Fig.5.14B. A broad voltammogram was observed at BCPE for both CC and HQ at 0.201 V and 0.142 V respectively indicating the poor response. But at the identical environment, poly (benzydamine) MCPE showed a massive enhancement in the current signals giving a sharp and resolved voltammogram. The electrochemical oxidations were positioned at 0.141 V for CC and 0.037 V for HQ. The observed reduction of over potential as well as progress in the current response reveals the electrocatalytic capability of the proposed poly (benzydamine) MCPE.

5.8.4. Sweep rate study

The influence of sweep rate variation for the electrochemical oxidation of 0.1 mM CC and 0.1 mM HQ in PBS (0.2 M, pH 7.4) was investigated in the range 20-200 mVs^{-1} at poly (benzydamine) MCPE by CV technique as shown in Fig.5.15A and 15B. For both CC and HQ, the obtained result shows increase in the anodic and cathodic peak currents on increasing scan rates. The graph of Ipa vs sweep rate of CC and HQ (Fig.6A and 6B) and Ipa vs square root of sweep rate of CC and HQ (Fig.5.16C and 5.16D) were plotted to evaluate the electrode process. There was good linearity and correlation coefficient (R^2) of 0.999 and 0.991 for CC and for HQ, 0.999 and 0.992, respectively. This indicates electrode phenomenon was adsorption-controlled at poly (benzydamine) MCPE [52, 53]. From the ΔEp (experimental peak potential difference) data, the heterogeneous rate constant (k^0) was calculated using the equation (5.2) [54, 55] and the results were tabulated in Table 5.2

$$\Delta Ep + 301.78 = 201.39 \log (\nu / k^0) \ldots\ldots\ldots (5.2)$$

5.8.5. Role of analytes concentration at poly (benzydamine) MCPE

Fig.5.17A reveals the CVs of CC at poly (benzydamine) MCPE with varied concentrations from 10-60 µM in presence of PBS (pH 7.4) at the sweep rate of $50mVs^{-1}$. By increasing CC concentration, Ipa and Ipc gradually enhanced with a slight shift in the redox peak potentials. Ipa vs CC concentration (Inset Fig.5.17B) plot shows linear variation and correlation coefficient value (R^2) of 0.9957.

Fig.5.18A illustrates the CVs of HQ at different concentrations (10-60 µM) in presence of PBS (pH 7.4) with sweep rate of 50 mVs^{-1}. By increasing HQ concentration, the redox peak currents increased with a slight shift in the peak potentials. The plot of Ipa vs HQ concentration (Inset Fig.5.18B) gives linear change and correlation coefficient value (R^2) of 0.9981. LOD and LOQ were evaluated using the equations (5.3) and (5.4) [56-60]. The LOD and LOQ for CC found at 5.79 µM and 19.32 µM and for HQ were found at 3.78 and 12.62 µM, respectively. The LOD of the studied modified electrode and other modified electrodes reported earlier, for the resolution of CC and HQ are given in Table 5.3.

$$LOD = 3S/M \quad \text{(5.3)}$$

$$LOQ = 10S/M \quad \text{(5.4)}$$

Where S is the standard deviation, M is the slope.

5.8.6. Influence of a solution pH

The pH plays an important role in the electron transport behavior of the electroactive molecules at modified electrodes. The increase of pH (6.2 to 7.8) shifts oxidation peak potential towards more negative direction as examined by CV at poly (benzydamine) MCPE for oxidation of 10 µM CC and 10 µM HQ are shown in Fig. 5.19A and 5.20A. The inset Fig. 5.19B and 5.20B shows the Epa vs pH values of CC and HQ graphs that are linear with a slope of 0.0524 V/pH (R^2= 0.9976) for CC and 0.06 V/pH (R^2=0.9953) for HQ, respectively. This suggests that its oxidation was followed by equal number of electron and protons [61-64]. Scheme.5.5 depicts the mechanism of oxidation of CC and HQ.

5.8.7. Simultaneous resolution of analytes CC and HQ

Because of the isomorphic nature and similar oxidation potentials, the simultaneous electroanalysis of CC and HQ gives broad as well as overlapped voltammetric response at BCPE. This remains a task for electroanalytical based research. The Fig.5.21 depicts the CVs obtained for the equimolar mixture (0.1mM) of analytes CC and HQ in PBS (0.2 M, pH 7.4) at sweep rate 50 mVs^{-1} at BCPE (dotted line) and poly (benzydamine) MCPE (solid line). At BCPE, broad and overlapped peaks for CC and HQ at 0.206 V were observed. However, in the similar environment the poly (benzydamine) MCPE has shown well separated peaks for CC and HQ at 0.149 V and 0.040 V respectively. The potential difference between the peaks was 0.11 V; this was more than adequate for the resolution of CC and HQ simultaneously. Hence, the proposed poly (benzydamine) MCPE acts as an excellent sensor for the CC and HQ resolution.

5.8.8. Interference study

The interference study was executed by DPV technique in the sample of a mixture containing CC and HQ at poly (benzydamine) MCPE. DPVs of CC and HQ mixture was illustrated in Fig.5.22A which indicates as the CC concentration was increased from 10 to 60 µM, the oxidation peak current of CC increased linearly when the concentration of HQ (10 µM) was kept constant with anodic peak current for HQ remaining the constant. In the same way, Fig.5.22B illustrates the DVPs of CC and HQ mixture, during which HQ concentrations was varied from 10 to 60 µM while keeping the CC (10 µM) concentration constant, only the peak current of HQ was increased. This result clears that the oxidation of CC and HQ did not interfere with each other. Therefore, poly (benzydamine) MCPE exhibited excellent selectivity and sensitivity for the resolution of CC and HQ.

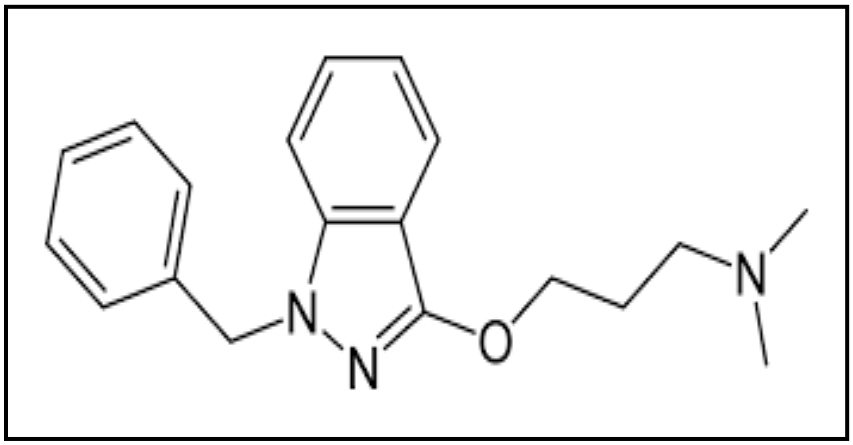
5.8.9 Reproducibility, Repeatability, and stability study

Three different modified electrodes were prepared by the same procedure and the responses toward the CC and HQ were recorded to evaluate the reproducibility. There was not any observable change in the sensitivity or the resolution of the CC and HQ peaks. Similarly, we also studied the same electrode for 5 different measurements

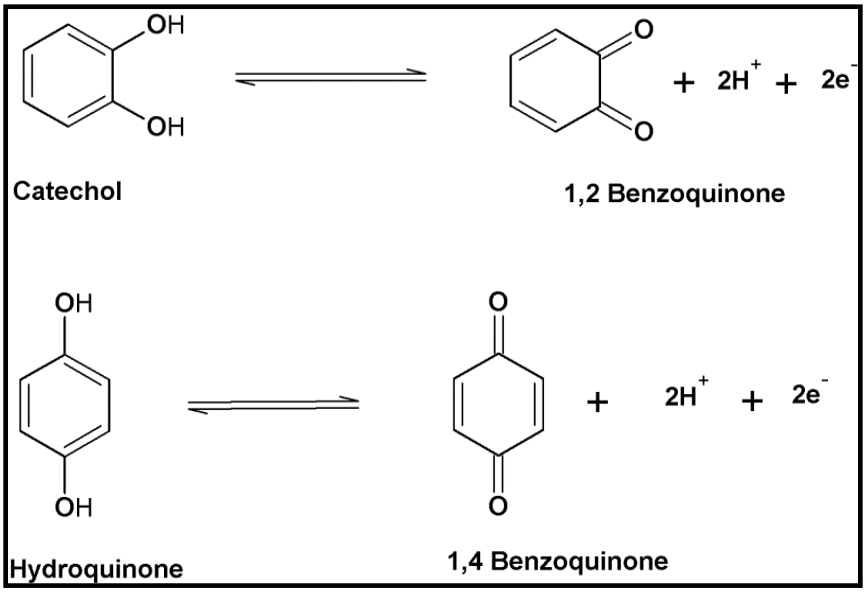
during the same day and also the next day, which also showed almost consistent result. In addition, the stability of the modified electrode was investigated. The modified electrode was not deteriorated for seven days and remained 97% and 94% of its original current response for CC and HQ, respectively.

5.9. Conclusion

At the present work, the modification of the CPE electrode with benzydamine has led to the fabrication of a simple and sensitive sensor for voltammetric study of CC and HQ. The sensor poly (benzydamine) MCPE exhibits high sensitivity, selectivity, stability, and reproducibility, antiinterference ability, electrochemical activity for the electrooxidation of CC and HQ. The fabricated poly (benzydamine) MCPE can be used as the sensor for determining the two dihydroxybenzene isomers individually and simultaneously. The same sensor can also be applied to identify some other bioactive molecules in environmental control.



Scheme.5.4. Structure of benzydamine



Scheme.5.5: Electrochemical oxidation of CC and HQ

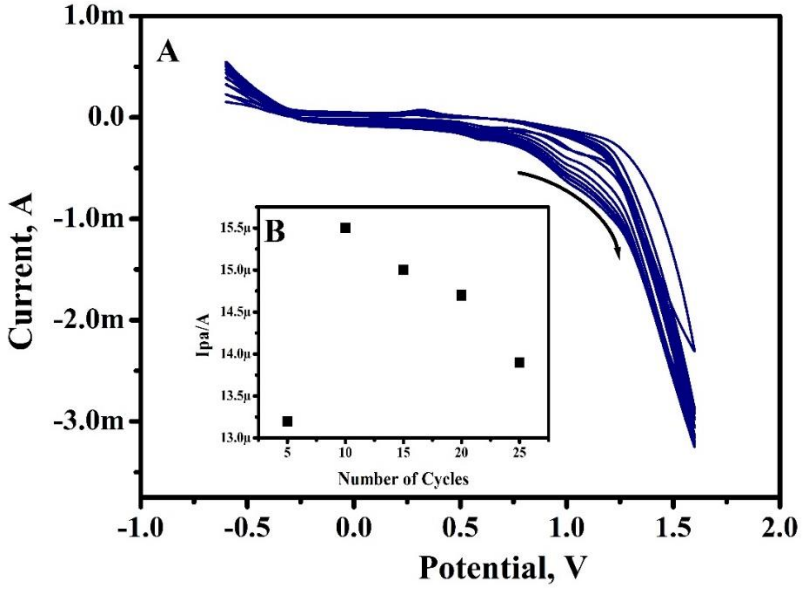


Fig.5.11. (A) CVs of fabrication of poly (benzydamine) MCPE in 0.1 M NaOH for 10 cycles at sweep rate of 100 mVs^{-1}. (B) Plot of Ipa vs number of multiple cycles

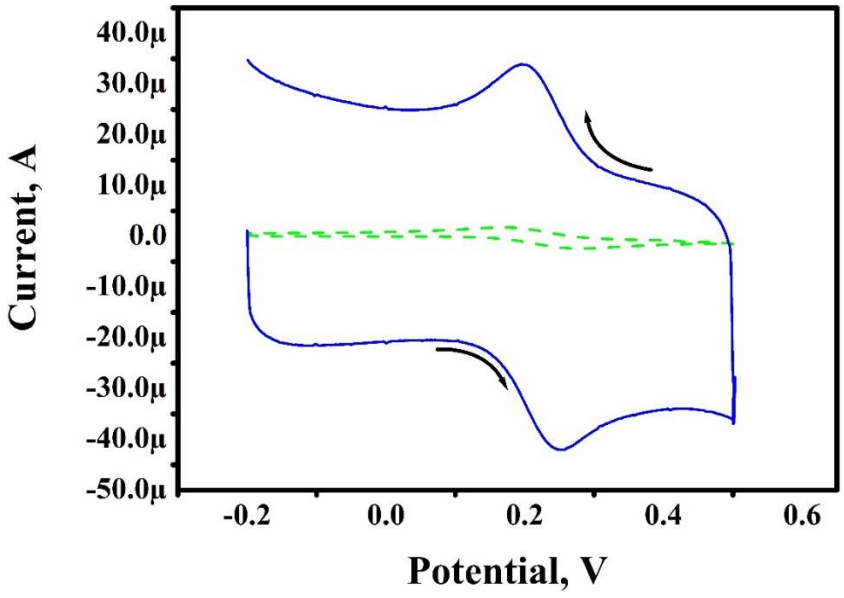


Fig.5.12. CVs obtained for $K_4[Fe(CN)_6]$ (1 mM) using 1 M KCl as supporting electrolyte at BCPE (dotted line) and poly (benzydamine)MCPE (solid line) at sweep rate of 50 mVs^{-1}

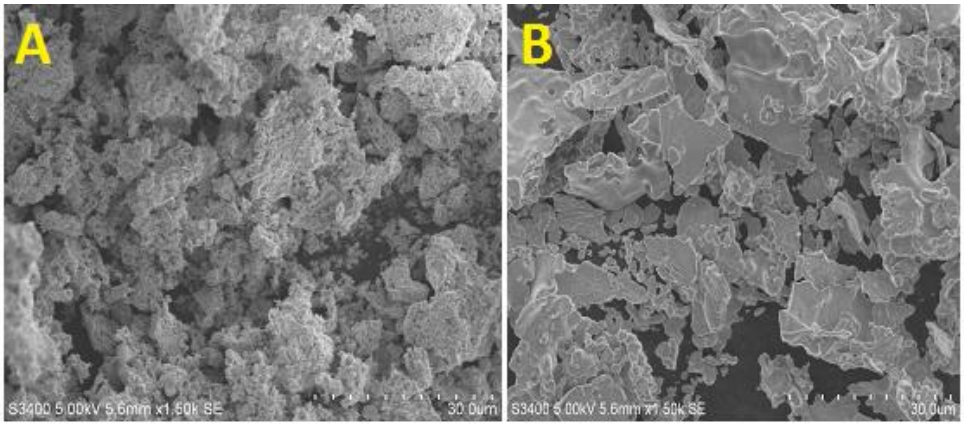


Fig.5.13. SEM images of BCPE (A) and poly (benzydamine)MCPE (B)

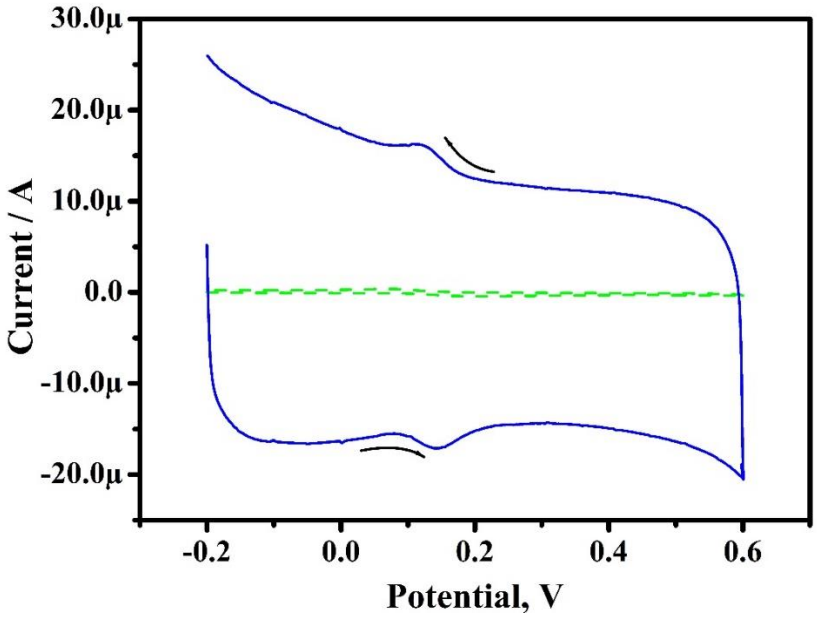


Fig.5.14A. CVs of CC (0.1 mM) in PBS (0.2 M, pH 7.4) at BCPE (dotted line) and poly (benzydamine) MCPE (solid line) at sweep rate 50 mVs^{-1}

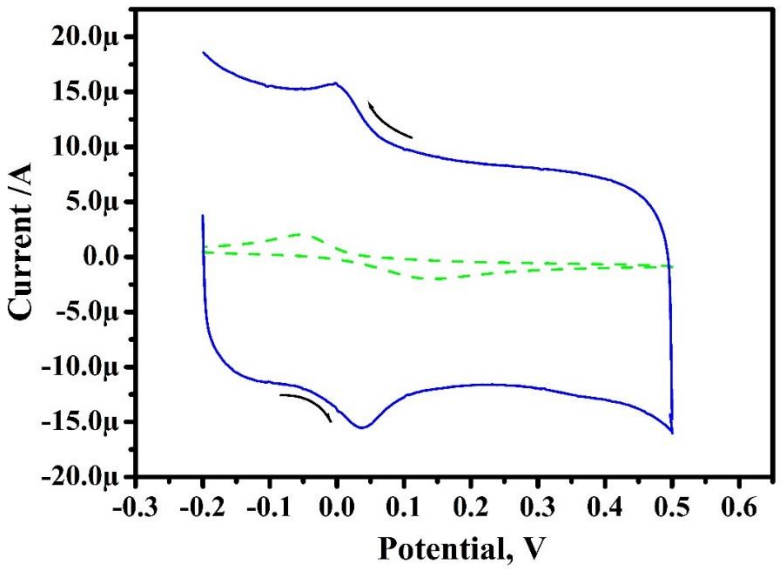


Fig.5.14B. CVs of HQ (0.1 mM) in PBS (0.2 M, pH 7.4) at BCPE (dotted line) and poly (benzydamine) MCPE (solid line) at sweep rate 50 mVs^{-1}

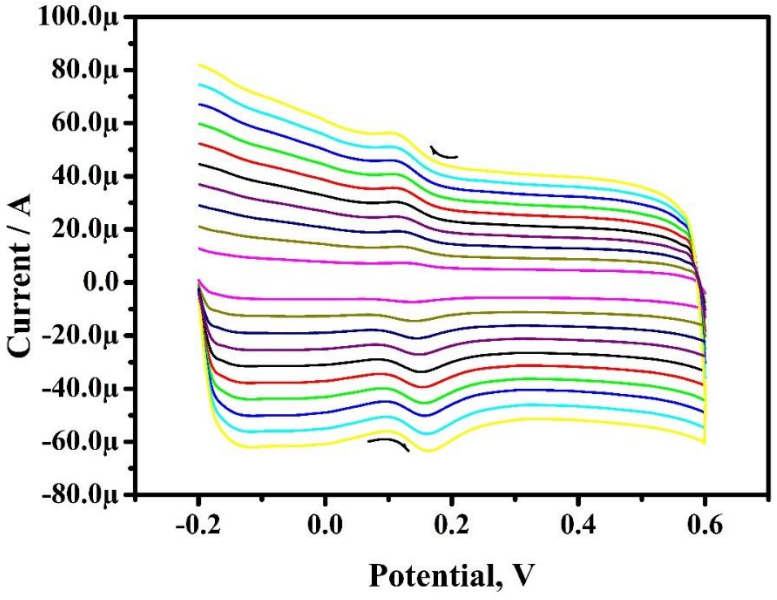


Fig.5.15A. CVs recorded for CC (0.1 mM) at poly (benzydamine) MCPE with varied sweep rates (20-200) mVs^{-1} in PBS (0.2 M, pH 7.4)

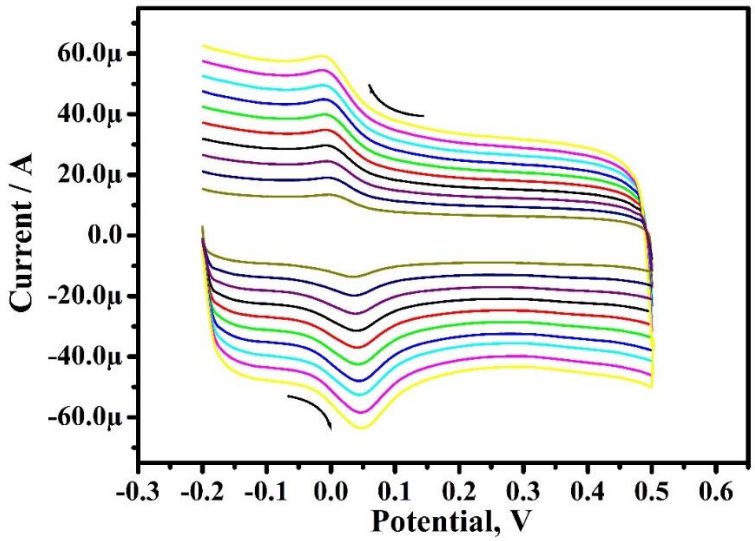


Fig.5.15B. CVs recorded for HQ (0.1 mM) at poly (benzydamine) MCPE with varied sweep rates (20-200) mVs^{-1} in PBS (0.2 M, pH 7.4)

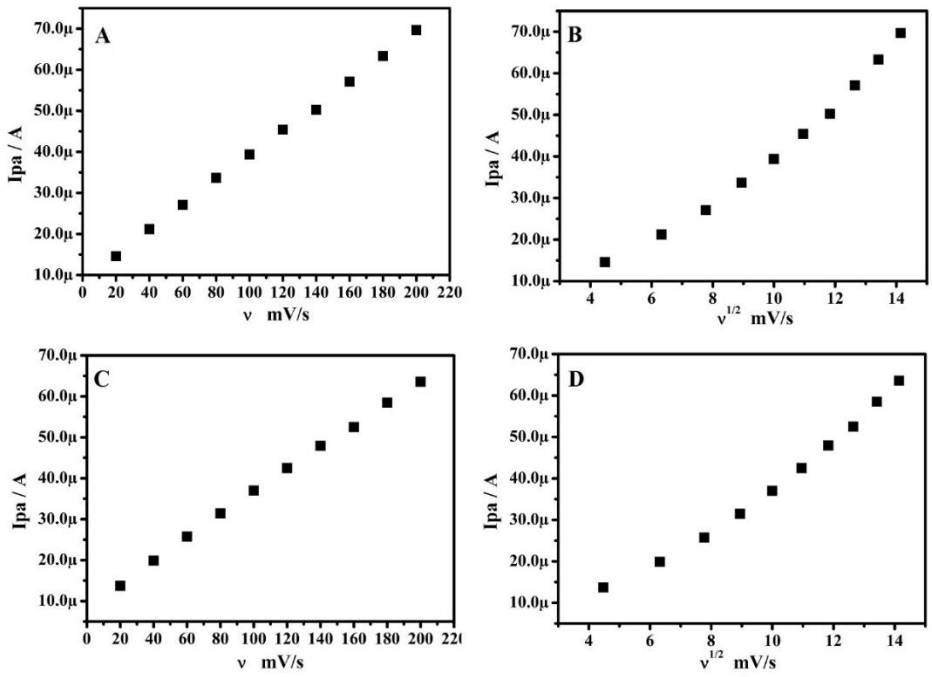


Fig.5.16. (A) Plot of Ipa vs scan rate of CC (0.1 mM) in PBS (0.2 M, pH 7.4). (B) Plot of Ipa vs square root of scan rate of CC (0.1 mM) in PBS (0.2 M, pH 7.4). (C) Plot of Ipa vs scan rate of HQ (0.1 mM) in PBS (0.2 M, pH 7.4). (D) Plot of Ipa vs square root of scan rate of HQ (0.1 mM) in PBS (0.2 M, pH 7.4)

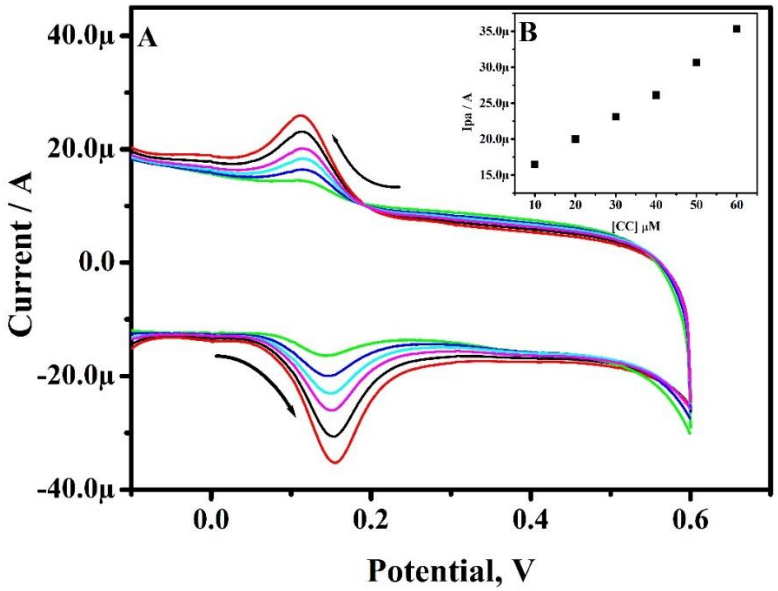


Fig.5.17. (A) CVs for different concentrations of CC (10-60 µM) in PBS (0.2 M, pH 7.4) at poly (benzydamine) MCPE. B) Plot of Ipa vs CC concentration

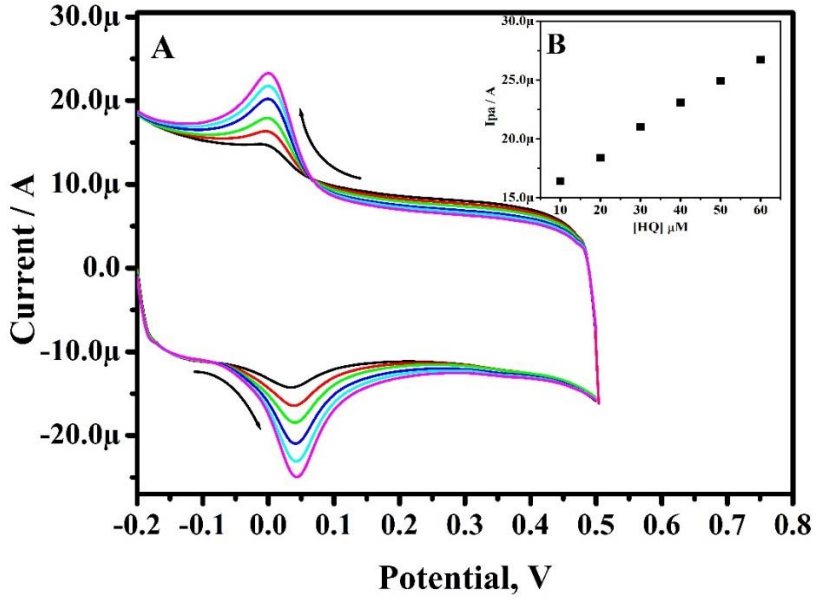


Fig.5.18. (A) CVs for different concentrations of HQ (10-60 µM) in PBS (0.2 M, pH 7.4) at poly (benzydamine) MCPE. B) Plot of Ipa vs HQ concentration

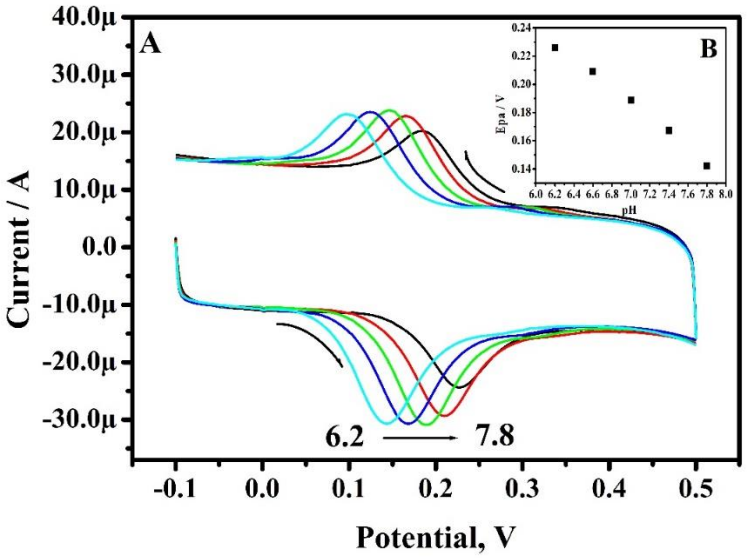


Fig.5.19. (A) CVs for CC with varied pH at poly (benzydamine) MCPE. B) Plot of Epa vs varied pH for CC

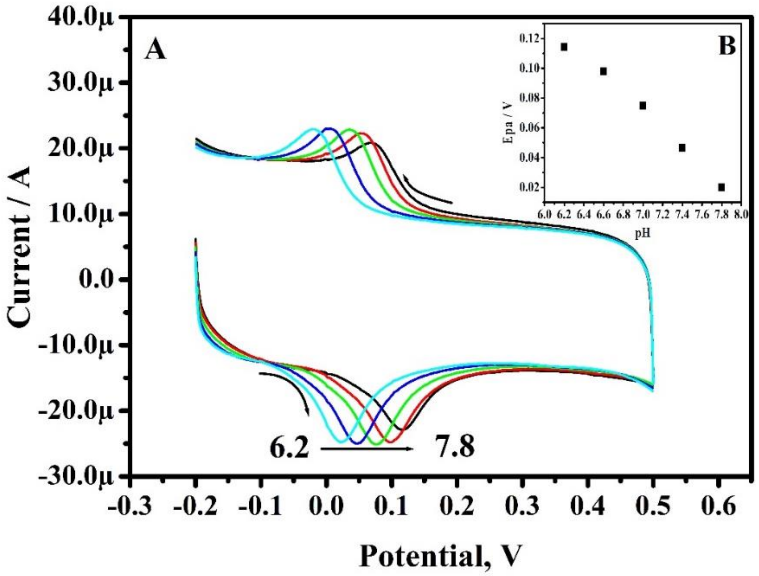


Fig.5.20. (A) CVs for HQ with varied pH at poly (benzydamine) MCPE. B) Plot of Epa vs varied pH for HQ

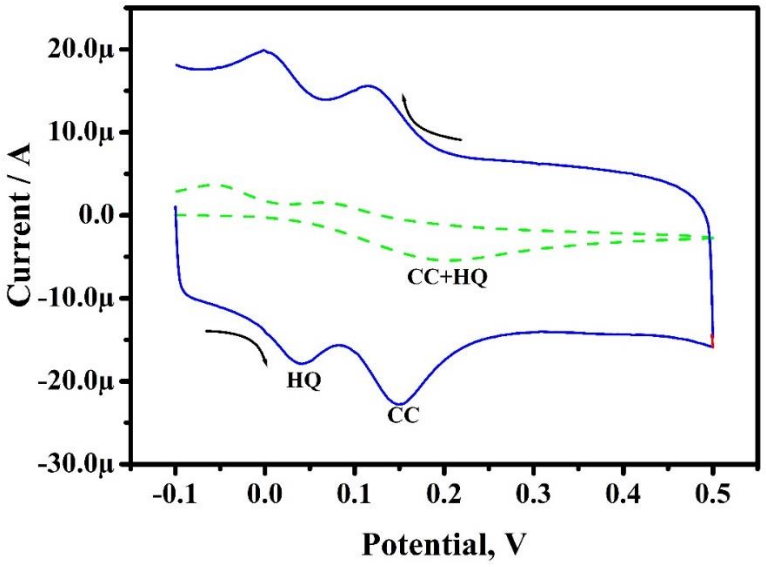


Fig.5.21. CVs for equimolar mixture(0.1mM) of analytes CC and HQ at BCPE (dotted line) and poly (benzydamine) MCPE (solid line)

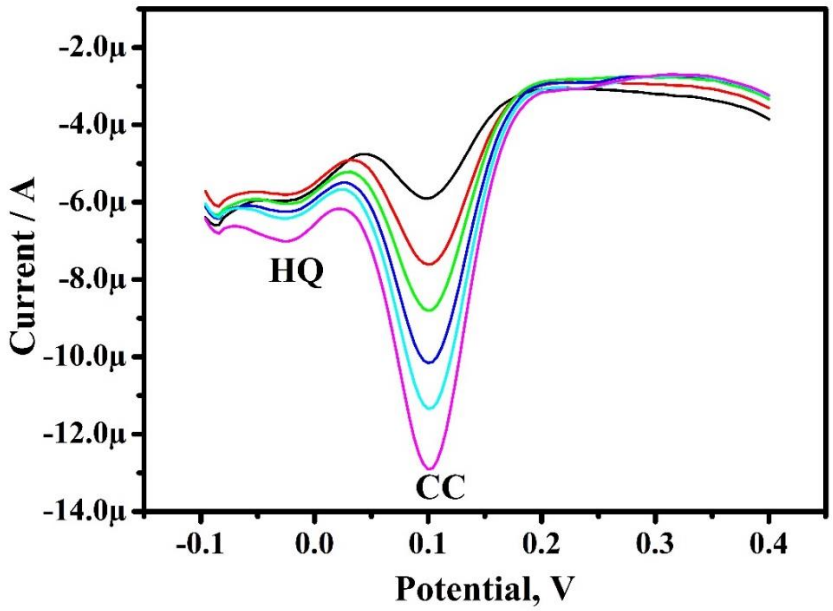


Fig.5.22A. DPVs for CC with varied concentration (10-60 µM) in PBS (0.2 M, pH 7.4) at sweep rate 50 mVs^{-1} at poly (benzydamine) MCPE

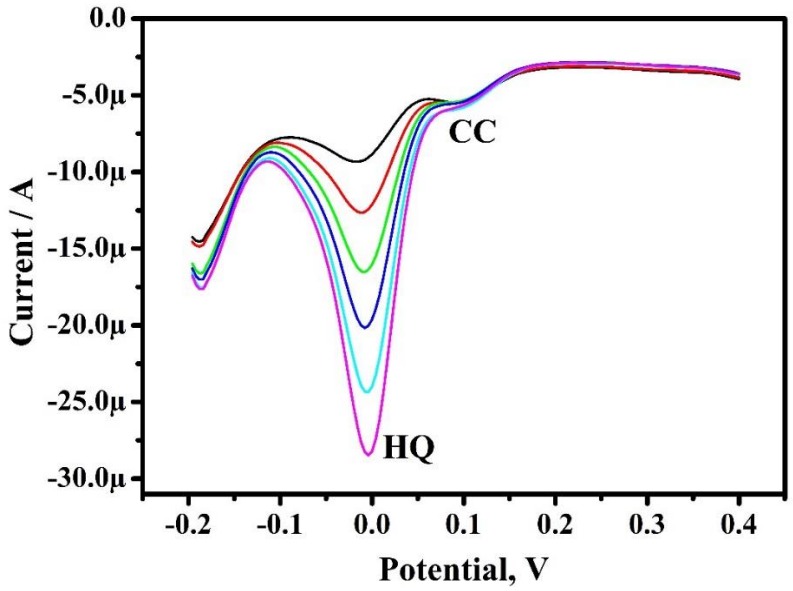


Fig.5.22B. DPVs for HQ with varied concentrations (10-60 µM) in PBS (0.2 M, pH 7.4) at sweep rate 50 mVs^{-1} at poly(benzydamine) MCPE

Table 5.2. Variation of the voltammetric parameters at different sweep rates for CC and HQ.

Scan rate (mVs^{-1})	ΔEp (mV)		Heterogeneous rate constant (k^0) s^{-1}	
	CC	HQ	CC	HQ
20	0.0235	0.0312	0.634494	0.634439
40	0.0266	0.0312	1.268944	1.268877
60	0.0334	0.0303	1.903268	1.903335
80	0.0373	0.0288	2.537577	2.537824
100	0.0404	0.0302	3.171859	3.172229
120	0.0501	0.0312	3.805809	3.806631
140	0.0551	0.0312	4.439856	4.44107
160	0.0588	0.0312	5.073907	5.075508
180	0.0648	0.0320	5.707754	5.709894
200	0.0698	0.0312	6.341586	6.344385

Table 5.3. The comparisons of the lower detection limit of the modified electrode with other reported methods

Sl.No	Electrode	Limit of Detection in µM		Method	References
		CC	HQ		
01	DL-methionine/MCPE	55.66	45.8	CV	[48]
02	PNR/MCPE	6.46	4.97	CV	[56]
03	Poly (malachite green)	31.1	18.1	CV	[65]
04	Silsesquioxane/MCPE	10.0	10.0	DPV	[66]
05	Poly (Benzydamine)MCPE	5.79	3.78	CV	This work

5.10. References

[1] M. Arago, C. Arino, A. Dago, J. M. Diaz-Cruz, M. Esteban, *Talanta* 160 **(2016)** 138

[2] A. A. Kumar, B. E. K. Swamy, P. S. Ganesh, T. S. Rani, G. V. Reddy, *J. Electroanal.Chem.*799 **(2017)** 505

[3] L. Zhao, J. Yu, S. Yue, L. Zhang, Z. Wang, P. Guo, Q. Liu, *J. Electroanal. Chem.* 808 **(2018)** 245

[4] V. K. Gupta, H. Karimi-Maleh, R. Sadegh, *Int. J. Electrochem. Sci.* 10 **(2015)** 303

[5] V. K. Gupta, A. K. Singh, L. K. Kumawat, *Sens. Actuators B Chem*. 195 **(2014)** 98

[6] V. K. Gupta, R. Jain, K. Radhapyari, N. Jadon, S. Agarwal, *Anal. Biochem.,* 408 **(2011)** 179

[7] R. Jain, V. K. Gupta, N. Jadon, K. Radhapyari, *Anal. Biochem.* 407 **(2010)** 79

[8] P. S. Ganesh, B. E. K. Swamy, *Anal. Bioanal. Electrochem.* 9 **(2017)** 547

[9] T. S. S. K. Naik, B. E. K. Swamy, *J. Electroanal. Chem.* 826 **(2018)** 23

[10] H. Khani, M. K. Rofouei, P. Arab, V. K. Gupta, Z.Vafaei *J. hazardous materials* 183 **(2010)** 402

[11] V.K. Gupta, A. K. Jain, G. Maheshwari, *Talanta* 72 **(2007)** 1469

[12] V.K. Gupta, A.K. Jain, G. Maheshwari, H. Lang, Z. Ishtaiwi, *Sens. Actuat. B.,* 117 **(2006)** 99

[13] V. K. Gupta, M. R. Ganjali, P. Norouzi, H. Khani, *Crit. Rev. Anal. Chem*., 41 **(2011)** 282

[14] P. S. Ganesha, B. E. K. Swamy, *J. Mol. Liq.*, 220 **(2016)** 208

[15] D. Song, J. Xia, F. Zhang, S.Bi, W. Xiang, Z. Wang, L. Xia, Y. Xia, Y. Li, L. Xia, *Sens. Actuat. B* 206 **(2015)** 111

[16] S. Cui, L. Li, Y. Ding, J. Zhang, *J. Electroanal. Chem.* 782 **(2016)** 225

[17] Y. H. Huang, J. H. Chen, X. Sun, Z. B. Su, H. T. Xing, S. R. Hu, W. Weng, H. X. Guo, W. B. Wu, Y.S. He, *Sens. Actuat. B.*, 212 **(2015)** 165

[18] Y. Zhang, S. Xiao, J. Xie, Z. Yang, P. Pang, Y. Gao, *Sens. Actuat. B.*, 204 **(2014)** 102

[19] W. Sun, Y. Wang, Y. Lu, A. Hu, F. Shi, Z. Sun, *Sens. Actuat. B.,* 188 **(2013)** 564

[20] P. S. Ganesh, B. E. K. Swamy, *Sci. Lett. J.* 5 **(2016)** 236

[21] D. Jiang, J. Pang, Q. You, T. Liu, Z. Chu, W. Jin, *Biosen. Bioelectron.* 124 **(2019)** 260

[22] M. Elancheziyan, D. Manoj, D. Saravanakumar, K. Thenmozhi, S. Senthilkumar, *Microchim. Acta* 184 **(2017)** 2925

[23] C. M. Kuskur, B. E. K. Swamy, H. Jayadevappa, *J. Electroanal. Chem.* 833 **(2019)** 512

[24] Ş. U. Karabiberoğlu, Ç. C. Koçak, Z. Dursun, *J. Electroanal. Chem.,*850 **(2019)** 113415

[25] Y. Ding, W. Liu, Q. Wu, X. Wang, *J. Electroanal. Chem.,* 575 **(2005)** 275

[26] Ç. C. Koçak and S. Koçak, *Electroanalysis*, 32 **(2020)** 358-

[27] C. M. Kuskur, B. E. K. Swamy, H. Jayadevappa, *J. Electroanal. Chem.* 804 **(2017)** 99

[28] J. A. G. Mesa, R. Mateos, *J. Agric. Food Chem.* 55 **(2007)** 3863

[29] Y. Sun, H. Cui, Y. Li, X. Lin, *Talanta* 53 **(2000)** 661

[30] M. F. Pistonesi, M. S. D. Nezio, M. E. Centurion, M. E. Palomeque, A. G. Lista, B. S. F. Band, *Talanta* 69 **(2006)** 1265

[31] G. Marrubini, E. Calleri, T. Coccini, A. F. Castoldi, L. Manzo, *Chromatographia*, 62 **(2005)** 25

[32] P. Nagaraja, R. A. Vasantha, K. R. Sunitha, *J. Pharma. Biomed. Anal.* 25 **(2001)** 417

[33] S. C. Moldoveanu, M. Kiser, *J. Chromatogr. A*, 1141 **(2007)** 90

[34] G. K. Chao, J. C. Suatoni, *J. Chromatogr. Sci.* 20 **(1982)** 436

[35] L. Wang, Y. Kuo, *Chromatographia*, 49 **(1999)** 208

[36] Rekha, B. E. K. Swamy, P. S. Ganesh, *Anal. Bioanal. Electrochem*. 9 **(2017)** 819

[37] T. Thomas, R. J. Mascarenhas, F. Cotta, K.S. Guha, B.E. K. Swamy, P. Martis, Z. Mekhalif, *Colloids and Surf. B* 101 **(2013)** 91

[38] P. F. Huang, L.Wang, J. Y. Bai, H. J. Wang, Y. Q. Zhao, and S. D. Fan, *Microchim Acta* 157 **(2007)** 41

[39] D. S. de Jesus, C. M. C.M. Couto, A. N. Araujo, M. C. B. S. M. Montenegro, *J. Pharma. Biomed. Anal*. 33 **(2003)** 983

[40] V. Sarveiya, J. F. Templeton, H. A. E. Benson, *Eur. J. Pharm. Sci.* 26 **(2005)** 39

[41] B. Silvestrini, P. S. Barcellona, A. Garau, and B. Catanese, *Toxicol. Appl. Pharmacol.,* 10 **(1967)** 148

[42] G. A. Baldock, R. R. Brodie, L. F. Chasseaud, T. Taylor, *J. Chromatogr.* 529 **(1990)** 113

[43] K. R. Mahanthesha, B. E. K. Swamy, *Anal. Bioanal. Electrochem*. 10 **(2018)** 321

[44] M. Kumar, B. E. K. Swamy, *Mater. Sci. Eng. C,* 58 **(2016)** 142

[45] Rekha, B. E. K, Swamy, P. S. Ganesh, *J Biosens. Bioelectron* 7 **(2016)** 3

[46] P. S. Ganesha, B. E. K. Swamy, *J. Electroanal. Chem.* 752 **(2015)** 17

[47] C. M. Kuskur, B. E. K. Swamy, K. Shivakumar, H. Jayadevappa, S. C. Sharma, *J. Electroanal. Chem.* 840 **(2019)** 52

[48] P. S. Ganesh, B. E. K. Swamy, K. V. Harisha, *Anal. Bioanal. Electrochem.* 9 **(2017)** 47

[49] B. N. Chandrashekar, B. E. K. Swamy, K. J. Gururaja, S. Chitravathi, M. *Chem. Sensors* 2 **(2012)** 5

[50] K. Chetankumar, B. E. K. Swamy, S. C. Sharma, *Microchem. J.* 156 **(2020)** 104979

[51] O. Gilbert, B. E. K. Swamy, U. Chandra, B. S. Sherigara, *Chem. Sensors* 4 **(2014)** 24

[52] U. Chandra, B. E. K. Swamy, O. Gilbert, B. S. Sherigara, *Electrochimica Acta* 55 **(2010)** 7166

[53] M. Kumar, B. E. K. Swamy and D. R. Rekha, *Sci. Lett. J.* 4 **(2015)** 211

[54] M. Kumar, B. E. K. Swamy, U. Chandra, A. W. Gebisa, *Int. J. Nanotechnol*. 14 **(2017)** 930

[55] K. Chetankumar, B. E. K. Swamy, T. S. S. K. Naik, *Chem. Data Collect.,* 28 **(2020)** 1000392

[56] T. S. S. K. Naik, B. E. K, Swamy, *J. Electroanal. Chem.* 804 **(2017)** 78

[57] K. Chetankumar, B. E. K. Swamy, *Sensors International* 1 **(2020)** 100001

[58] K. Chetankumar, B. E. K. Swamy, S. C. Sharma, *J. Electroanal. Chem.* 849 **(2019)** 113365

[59] M. Kumar, B. E. K. Swamy, S. Reddy, W. Zhao, S. Chethana, V. G. Kumar, *J. Electroanal. Chem.* 835 **(2019)** 96

[60] K. V. Harisha, B. E. K. Swamy, E. E. Ebenso, *J. Electroanal. Chem.* 823 **(2018)** 730

[61] L. Lin, J. Chen, H.Yao, Y. Chen, Y. Zheng, X. Lin, *Bioelectrochemistry* 73 **(2008)** 11

[62] M. Kumar, B. E. K. Swamy, M. H. M. Asif, C. C. Viswanath, *Appl. Surf. Sci.* 399 **(2017)** 411

[63] P. S. Ganesha, B. E. K. Swamy, O. E. Fayemi, E. M. Sherif, E. E. Ebenso, *Sens. Bio-Sens. Res.*20 **(2018)** 47

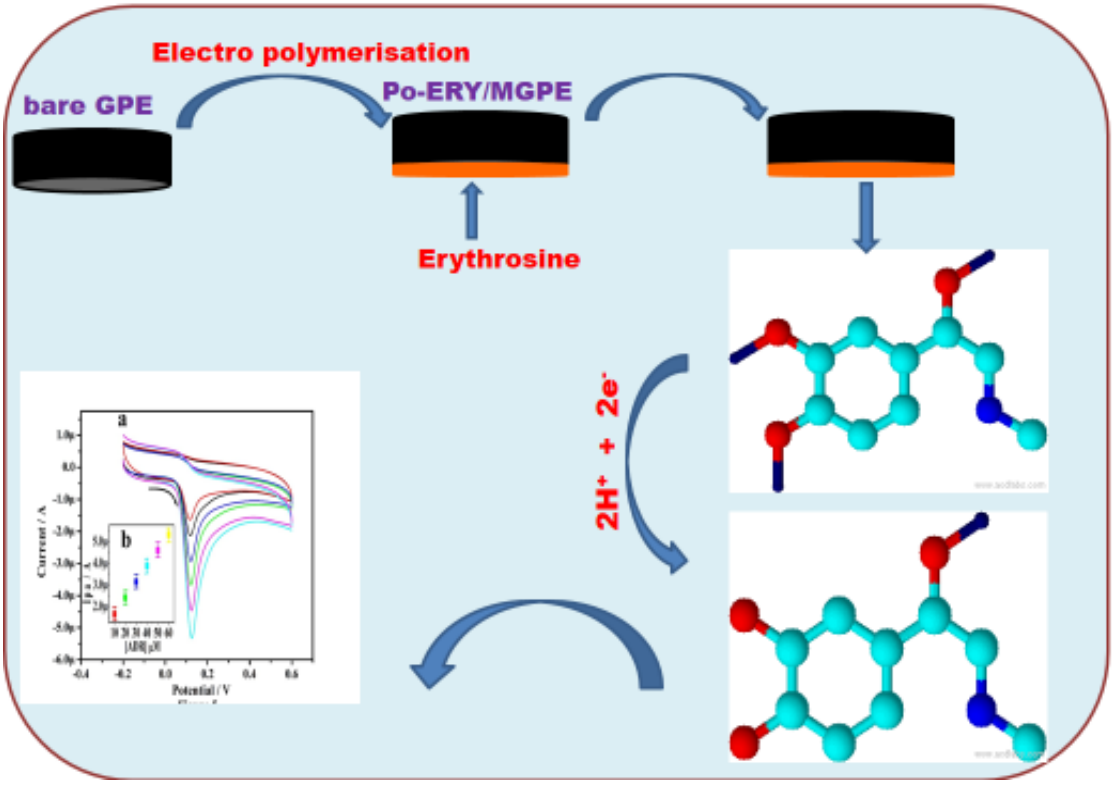
[64] P. S. Ganesh, B.E. K. Swamy, *J. Electroanal. Chem.* 756 **(2015)** 193

[65] Y. Umasankar, A. P. Perasamy, S. M. Chen, *Anal. Biochem.* 411 **(2011)** 71

[66] P. S. da Silva, B.C. Gasparini. H. A. Magosso, A. Spinelli, *J. Braz. Chem. Soc.* 24 **(2013)** 695

Chapter -6

A novel, extreme low-cost poly (Erythrosine) modified pencil graphite electrode for determination of Adrenaline



Communicated in Scientific Report (Revised manuscript submitted)

6.1 Introduction

In contemporary electro analytical chemistry, Graphite pencil electrodes (GPEs) are versatile analytical tools. GPE has gotten a lot of attention as a form of carbon electrode. GPEs has been employed in an expanding array of applications, primarily as sensors and biosensors, since the modern era, despite the fact that they were initially published in 1960 [1, 2]. It has good properties that are equivalent to glassy carbon and carbon paste electrode. GPE has extra advantages for the sensing of bioactive compounds by its lower costs, viability, mechanical robustness, high conductivity, renewability, easy disposability, low background current, wide potential range [3-8].

Adrenaline (ADR) is a neurotransmitter, a hormone, and a prescription drug. ADR is a well-known catecholamine secreted by the endocrine system [9, 10]. ADR content in blood is linked to a slew of physiological activities [11]. Aberrant ADR levels are linked to a various disease, including Alzheimer's disease, Sclerosis, hypoglycemia, stress, thyroid hormone disorders, and Parkin's disease [12-14]. It stiffens blood vessels, raising heart rate, dilates lungs, and is known to activate the sympathetic nervous system's flight or fight mechanism [15-16]. ADR is a drug used to treat alleviate bronchiolitis, cardiac surgery, heart attack, heart blockage, hypertension, asthma, and anaphylaxis [17- 19]. ADR and UA are biological substances that plays a vital function in human metabolism. They often reside in the central nervous system's extracellular fluid and body fluids. Therefore, both in life science and in pathological research, the individual or simultaneous determination of ADR inexistence of UA is a crucial issue [20].

To date, a lot of techniques have been used, including capillary electrophoresis [21], fluorimetry [22, 23], liquid chromatography [24], spectrophotometry [25], chemiluminescence [26] etc. These approaches are not only costly, and moreover time intensive and inconvenient. The electrochemical approach, on the other hand, has long been known as a simple, quick, low cost, high efficiency, and convenient method of analysis and it employs electrodes. The employ of bare electrodes, on the other hand, is reported to be plagued by low sensitivity and selectivity, making selective and simultaneous detection of compounds in a sample matrix problematic. The major solution to these problems related with bare electrodes has been electrode modification.

Dyes have indeed been widely employed for electrode modification by electro polymerization method in this regard [27].

In this work, Erythrosine (ERY) (Scheme 6.1), a xanthene food dye was electropolymerized onto the GPE surface using the CV method [28]. The fabricated novel, simple and less cost Po-ERY/MGPE sensor plays a critical role in detecting ADR. Various parameters were analyzed, including pH effect, scan rate effect, and ADR concentration.

6.2. Experimental Part

6.2.1 Chemicals and stock solutions

ADR and UA were provided by Himedia. ERY was procured from Sigma-Aldrich. Nice chemicals provided perchloric acid, $NaH_2PO_4.H_2O$, NaOH, and Na_2HPO_4. The Camlin lead pencil rods with dimensions of 0.5 mm and 6 cm in length were purchased from the local book depot. 25 x 10^{-4} M stock solutions of ERY, ADR, and UA were prepared in distilled water, 0.1 M perchloric acid, and 0.1 M NaOH, respectively. A supporting electrolyte, 0.2 M PBS (7.4 pH) was prepared from Na_2HPO_4 and $NAH_2PO_4.H_2O$. All the chemicals employed were analytical grade and were utilized without further refining.

6.2.2 Instruments of investigation

All voltammetric (CVs and DPVs) assessments were obtained using CH instrument type 660 (CHI-660c model). The electrode setup consists of three electrodes: a working electrode (bare GPE or Po-ERY/MGPE), a counter electrode (Platinum), and a reference electrode (saturated calomel electrode). All voltammetric data were recorded at room temperature.

6.3. Results and discussion

6.3.1 Fabrication of Po-ERY/MGPE

The Po-ERY/MGPE was produced by electropolymerized a bare GPE in 1mM aqueous ERY prepared with 0.1 M NaOH (supporting electrolyte) in an electrochemical cell using the CV method. Fig. 6.1a shows the results of CVs of the electropolymerization process at a potential gradient of -0.6 to 1.4V with a scan rate (υ)

of 100 mVs^{-1} over 10 cycles. As the number of cycles increased, the voltammogram reduced and eventually became relatively steady, reflecting the accumulation of a uniform thin layer of ERY on the surface of bare GPE [29]. The thickness of the adherent film, which can be varied by adjusting the number of sweep intervals during electropolymerization is strongly attributable to the modified electrode's catalytic performance [30]. As indicated in the inset Fig.6.1b, the maximal Ipa was attained at the 10 cycles, hence 10 cycles are being used as a standard for the electropolymerization on the bare GPE surface in all electrochemical analysis. The equation 6.1 was employed to compute an adequate estimated amount of surface coverage concentration or thickness of ERY on bare GPE surface, which was found to be 0.0124 M/cm^2 [27, 31].

$$Ip = n^2F^2A\Gamma\upsilon /4RT \text{ ---------- (6.1)}$$

Where, 'n' is number of electron transferred, 'Ip', 'A', 'Γ', and 'υ' are peak current, area of the electrode, surface coverage area (M/cm^2), and sweep speed respectively. F, R, and T are scientific terms.

6.3.2 Electrocatalytic behavior of ADR at Po-ERY/MGPE

The electrochemical performance of 10 µM ADR was explored using CV technique at the bare GPE (curve 'a') and Po-ERY/MGPE (curve 'b') in 0.2 M PBS (7.4 pH) at potential ranging from -0.2 to 0.6 V with scan rate of 50 mVs^{-1} shown in Fig.6.2. ADR voltammetry responses depict broad massive oxidation waves at about 136 mV upon that at bare GPE, implying a slow and poor electron transport kinetics due to the fouling of electrode surface by oxidation reaction. Conversely, as contracted to bare GPE, the projected PO-ERY/MGPE revealed a strong peak at 113 mV that is displayed favorably by 23mV. These findings show that the ADR electron transport kinetics are speedier at Po-ERY/MGPE [10, 32]. According to the established method, when the potential was initially swept from -0.2 V to 0.6V, a clear Ipa occur at 1.48 µA, and the electro behavior of ADR was completely irreversible, with no peak seen in the reverse sweep. The oxidation mechanism is attributed to two- electron oxidation of the hydroxy group, as detailed in scheme 6.2.

6.3.3 Significance of pH

The contribution of supporting electrolytes in the development of sensors is critical for the analytes. At modified electrodes, pH has been shown to have a considerable influence on electron transport performance. As a result, it is often crucial to investigate the effect of buffer solution pH on analyte perception at modified electrode. Fig.6.3a depict the achieved CVs for 10.0 µM ADR in 0.2 M PBS of pH-adjusted from 6.2 to 7.8. As the pH increased, the oxidation peak potential of ADR switched to a more negative potential with difference in peak current. The graph of Epa for ADR vs pH (inset Fig.6.3b) revealed a linear connection with regression equation Epa (mVs^{-1}) = 0.703 - 0.058 (pH), (R^2= 0.997) and slope of 0.058 V/pH, confirming that the oxidation of ADR comprises the same number of electrons and protons. [20, 33].

6.3.4 Impact of scan rate

The influence of scan rate from 50- 500mVs^{-1} on electrochemical analysis of 10.0 µM ADR in 0.2 M PBS (7.4 pH) was explored using the CV technique, as seen in Fig.6.4a. The oxidation peak current of ADR is shown to grow as the scan rate goes up. Adsorption at Po-ERY/MGPE for ADR controls the electrode phenomenon, as evidence by the good linearity of the Ipa vs scan rate (inset Fig.6. 4b) and Ipa vs square root of scan rate (inset Fig.6.4c) regression equations Ipa (µA) = 13.11 υ (Vs^{-1}) + 2.06 (R^2= 0.998) and Ipa (µA) = 39.48 $\upsilon^{1/2}$ (Vs^{-1}) – 5.96 (R^2= 0.994) [34].

6.3.5 Concentration study of ADR

Fig.6.5a presents CVs of ADR in 0.2 M PBS (7.4 pH) at 50mVs^{-1} with a progressive increase in ADR levels from 10 – 60 µM achieved at Po-ERY/MGPE. Ipa of ADR increases as the ADR levels rise (inset Fig.5b). With the regression equation of Ipa (µA) = 0.073 (µM) + 0.91(R^2= 0.999), it offers better linearity. Equations, 6.2 and 6.3 were utilized to define the LOD and LOQ [35]. According to the estimates, the LOD is 0.499 µM and LOQ is 1.66 µM. Table 6.1 compares the LOD of Po-ERY/MGPE for ADR with that of other published sensors.

$$LOD = 3S/M \quad \text{---------- (6.2)}$$

$$LOQ = 10S/M \quad \text{----------(6.3)}$$

6.3.6 Simultaneous electro analysis of ADR and UA

The goal of this analysis was to utilize the developed electrode to assess ADR in the presence of UA in a selective and sensitive manner. The CVs obtained at sweep speed of 50 mVs^{-1} at bare GPE (curve 'a') and Po-ERY/MGPE (curve 'b') in 0.2 M PBS (7.4 pH) for a mixture of ADR (0.1µM) and UA (0.1 µM) are illustrated in Fig.6. 6. At bare GPE, low current intensities with weak sensitivity and selectivity were measured. Po-ERY/MGPE also exhibited substantial current intensities with greater sensitivity and selectivity for ADR and UA oxidation at 0.111 and 0.254 V, respectively, under the same circumstances. As a result, the built in Po-ERY/MGPE serves as an excellent ADR sensor.

6.3.7 Interference study

The interference analysis was most significant in deciding the efficacy of the fabricated electrode and the DPV method was used for the study. The DPVs curve is depicted in Fig.6.7a for a range of ADR concentrations (50 - 250 µM) while preserving UA (50 µM) constant. Likewise, the concentration of UA (50 - 300 µM) was varied while the ADR concentration remained constant (Fig.6.8a). A linear plot of Ipa vs concentration of ADR (inset Fig.6.7b) and Ipa vs concentration of UA (inset Fig.6. 8b), producing the regression equation Ipa (nA) = 0.073 (µM) + 9.14 (R^2= 0.999) and Ipa (nA) = 0.020 (µM) + 3.76 (R^2=0.997), respectively. According to the above-mentioned experimental data, as the level of one analyte was increased, the Ipa climbed wonderfully, but the Ipa and Epa of constant analyte remained unchanged. Because the oxidation of ADR does not influence the variance of the other analytes, our data suggest that the accurate and precise estimation of ADR at Po-ERY/MGPE is achievable.

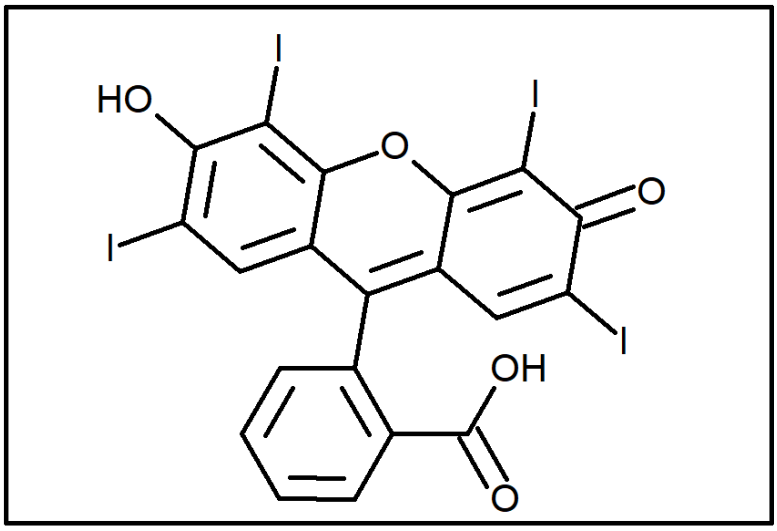
6.3.8 Real sample analysis

In addition to the studies, the Po-ERY/MGPE sensing potential towards ADR in an injection sample was assessed using the standard addition method. The injection sample was procured from Harson Laboratories, which had a defined concentration of

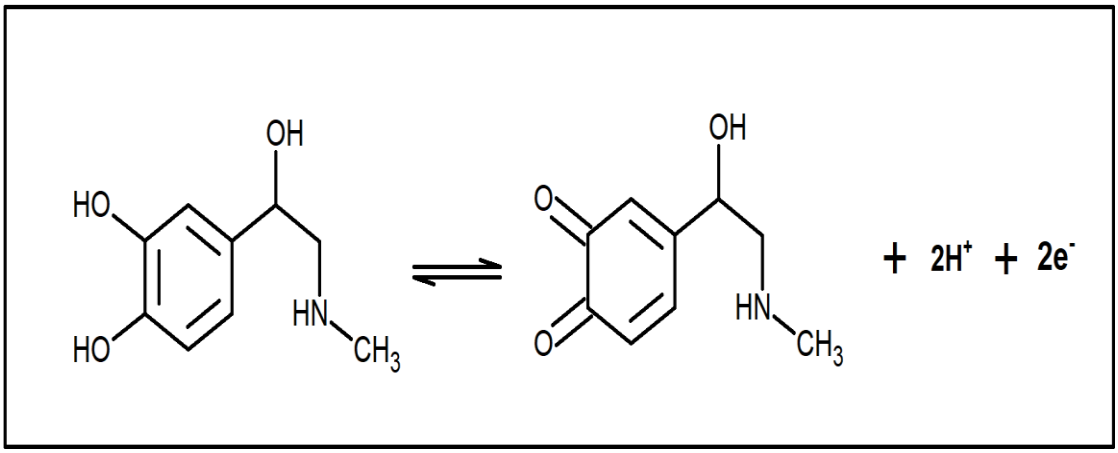
1.8 mg/mL ADR and was used after a sufficient dilution in 0.1 M perchloric acid. Table 6.2 displays, 4 consecutive ADR concentrations in the range of 10 to 40 µM, result in a strong recovery in the range of 97.0 to 99.9 percent. These results demonstrate Po-ERY/MGPE's sensing potential for ADR analysis in an injection sample.

6.4. Conclusion

In conclusion, we proposed a simple and efficient way for fabricating of Po-ERY/MGPE. The core attractive features of Po-ERY/MGPE include the prompt modification process, increased sensitivity, high stability, and low detection limits. The developed sensor was used for specific and simultaneous detection of ADR and UA. For the electro-oxidation of ADR and UA the designed sensor showed highly distinct and defined peaks. In real sample analysis, the developed sensor recovered ADR with high accuracy. As a result, Po-ERY/MGPE will have a high opportunity in sensor field.



Scheme 6.1: Structure of Erythrosine



Scheme 6.2: Oxidation of Adrenaline

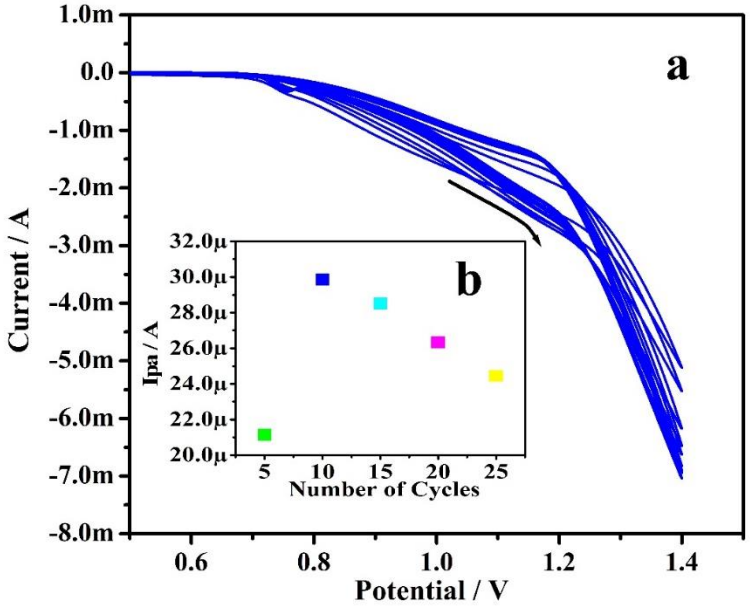


Fig. 6.1. a) CVs of fabricated Po-ERY/MGPE with NaOH (0.1 M) for 10 cycles at scan rate of 100 mVs^{-1}. b) Graph of Ipa Vs Number of voltammetric scans

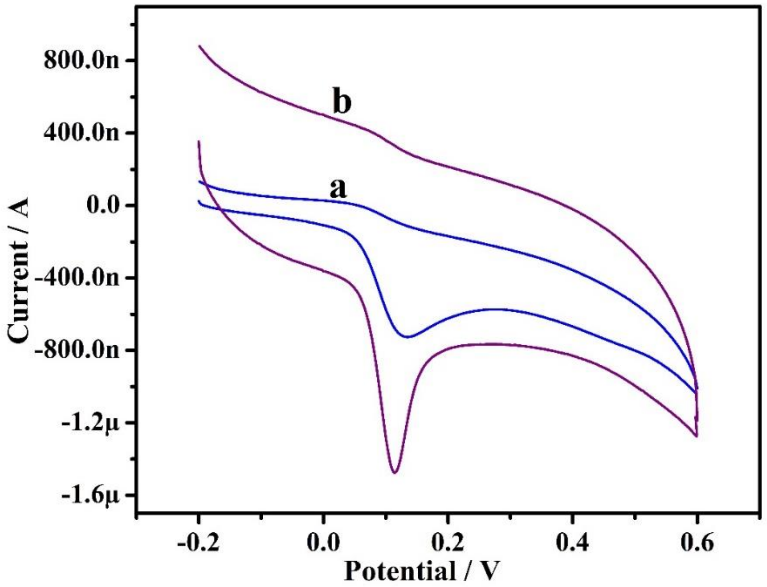


Fig. 6.2. CVs of ADR in 0.2 M PBS (7.4 pH) at BCPE (curve 'a') and Po-ERY/MGPE (curve 'b') at scan rate of 50 mVs^{-1}

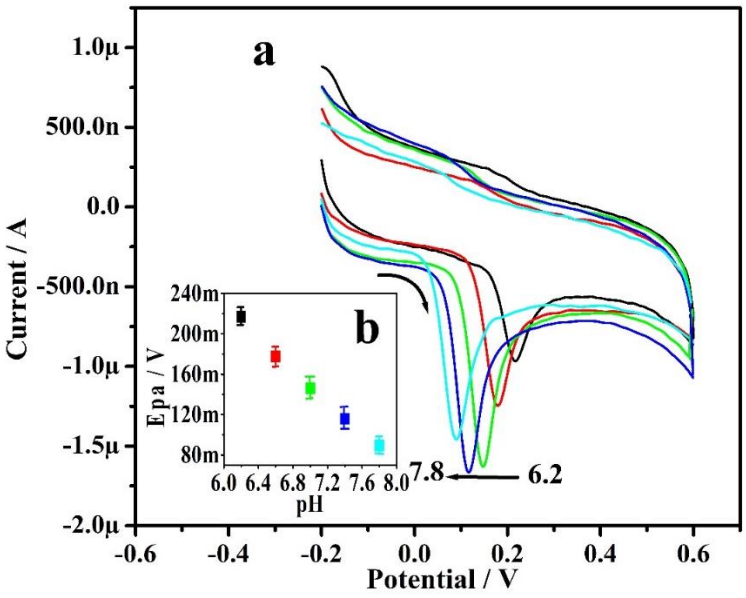


Fig. 6.3. a) CVs for ADR with pH series at Po-ERY/MGPE. b) Graph of Epa Vs altered pH for ADR.

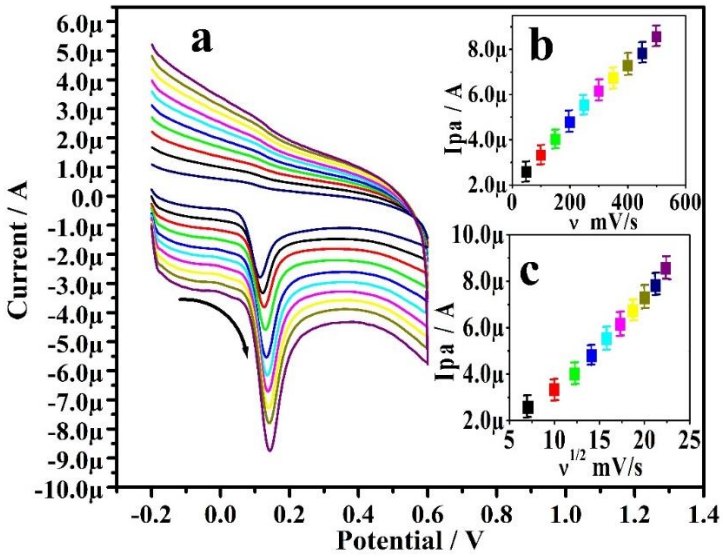


Fig. 6.4. a) CVs documented for ADR (10 µM) in 0.2 M PBS (7.4 pH) at Po-ERY/MGPE with various scan rate (50-500 mVs^{-1}). b) Graph of Ipa Vs scan rate of ADR (10 µM) in 0.2 M PBS (7.4 pH). c) Graph of Ipa Vs square root of scan rate of ADR (10 µM) in 0.2 M PBS (7.4 pH)

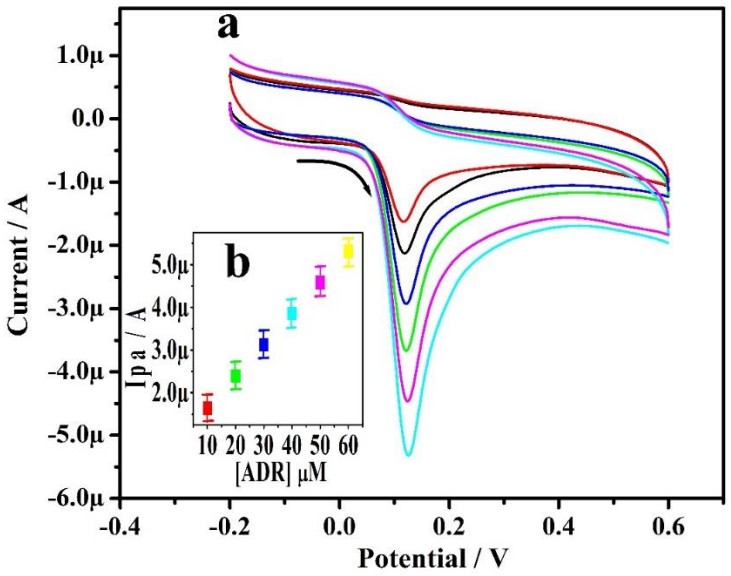


Fig. 6. 5. a) CVs documented for different concentration of ADR (10 – 60 µM) using 0.2 M PBS (7.4 pH) at Po-ERY/MGPE. b) Graph of Ipa Vs ADR concentrations

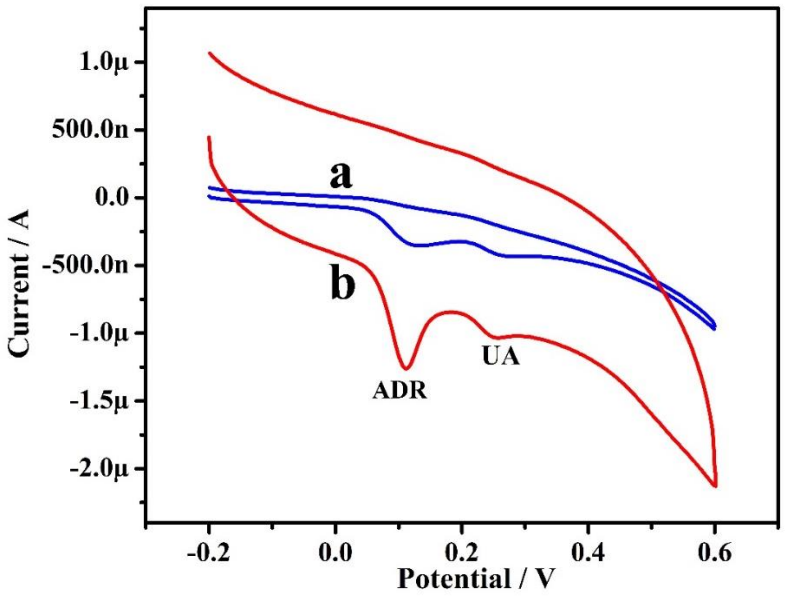


Fig. 6.6. Resulted CVs for simultaneous studies of ADR (0.1mM) and UA (0.1mM) at BCPE (curve 'a') and Po-ERY/MGPE (curve 'b')

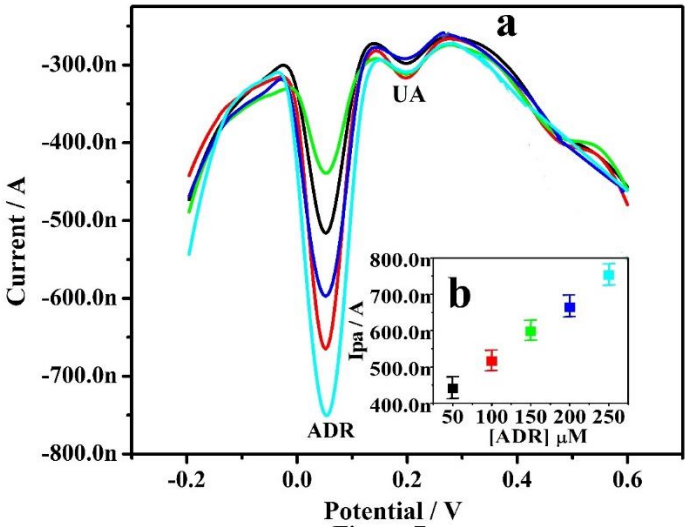


Fig. 6.7. a) DPVs of ADR at altered concentrations (50 – 250 µM) in 0.2 M PBS (7.4 pH) on Po-ERY/MGPE. b) Plot of Ipa vs concentration of ADR

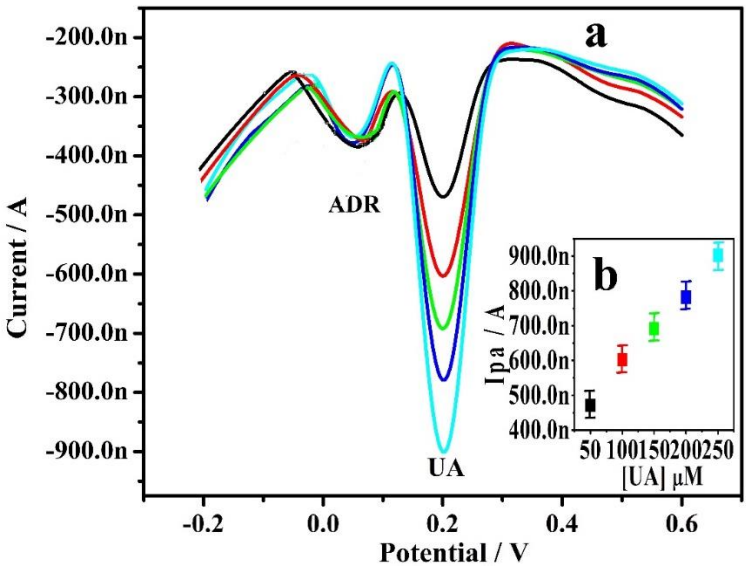


Fig. 6.8. a) DPVs of UA at altered concentrations (50- 300 µM) in 0.2 M PBS (7.4 pH) on Po-ERY/MGPE. b) Plot of Ipa vs concentration of UA

Table 6.1. LOD of the proposed electrode in comparison to earlier ADR sensors.

Sl. No	Electrode	LOD in µM	Method	References
1	PSAF-MCPE	0.61	CV	[14]
2	2-Hydroxybenzimidazole MCPE	3.0	CV	[20]
3	poly(vanillin) MCPE	5.4	CV	[34]
4	Gold nanoporous film modified gold electrode	19	CV	[36]
5	Au 4MpyAuNPs	4.5	CV	[37]
6	Au–Ag	5.05	CV	[38]
7	Nanoporous thin Au films	2.42	DPV	[39]
8	Carbon nanotube modified carbon film electrodes	0.9	DPV	[40]
9	Carbon paste electrode modified with iron phthalocyanine	0.5	DPV	[41]
10	Po-ERY/MGPE	0.499	CV	This work

Table 6.2. Result of recoveries of ADR at Po-ERY/MGPE in an injection sample.

Sample added (µM)	Found (µM)	Recovery (%)
10	9.7	97.0
20	19. 8	99.0
30	29.6	98.6
40	39.8	99.9

6.5. References

[1] Á. Torrinha, N. Jiyane, M. Sabela, K. Bisetty, M. C. B. S. M. Montenegro, A. N. Araújo, *Sci.Rep*. 10 **(2020)** 16535

[2] P. Prasertying, M. Yamkesorn, K. Chimsaard, N. Thepsuparungsikul, S. Chaneam, K. Kalcher, R. Chaisuksant, *J Sci-Adv Mater Dev*, 5 **(2020)** 330-336

[3] Z. Huang, Y. Zhang, J. Sun, S. Chen, Y. Chen, Y. Fang, *Sens. Actuators B Chem.* 321 **(2020)** 128550

[4] Z. Z. Tasi´c, M. B. P. Mihajlovi´c, A. T. Simonovi´c, M. B. Radovanovi´c, M. M. Antonijevic´, *Results Phys.* 22 **(2021)** 103911

[5] R. R. Sawkar, V. B. Patil, M. M. Shanbhag, N. P. Shetti, S. M. Tuwar, T. M. Aminabhavi, *Adv. Biomed. Eng.* 2 **(2021)** 100009

[6] G. Emir, S. Karakaya, S. Ayaz, D. G. Dilgin, Y.Dilgin, *Monatsh Chem.* 152 **(2021)** 1491

[7] Y. Huang, Y.Pang, X. Shen, R. Jiang, Y. Wang, *Talanta*, 236 **(2022)** 122859

[8] S. Deepa, B. E. K. Swamy, K. V. Pai, K. R. Mahanthesha, *Anal. Bioanal. Electrochem.* 11 **(2019)** 1240

[9] F. Alpat, K. Özdemir, S. K. Alpat, Voltammetric, *J. Sensors*, 2016 **(2016)** 9

[10] M. Taleb, R. Ivanov, S. Bereznev, S. H. Kazemi, I. Hussainova, *J. Electroanal. Chem.*, 823 **(2018)** 184

[11] W. Ren, H. Q. Luo, N. B. Li, *Biosens. Bioelectron.*, 21 **(2006)** 1086

[12] T. Tavana, M. A. Khalilzadeh, H. Karimi-Maleh, A.A. Ensafi, H. Beitollahi, D. Zareyee, *J. Mol. Liq.*, 168 **(2012)** 69

[13] N. Alizadeh, S. Ghasemi, A. Salimi, T. K. Sham, R. Hallaj, *Colloids Surf. B: Biointerfaces*, 195 **(2020)** 111228

[14] K. Chetankumar, B.E. K. Swamy, S.C. Sharma, *Microchem. J.*, 160 **(2021)** 105729

[15] D. Majer, M. Finšgar, *Biosen.*, 11 **(2021)** 285

[16] B. Mekassa, M. Tessema, B. Singh, Chandravanshi, P.G.L. Baker, F. N. Muya, *J. Electroanal. Chem.*, 807 **(2017)** 145

[17] Z. Jemelkova, J. Barek, J. Zima, *Anal. Lett.* 43 **(2010)** 1367

[18] S. S. Shankar, Rayammarakkar M. Shereema, R. B. Rakhi, *ACS Appl. Mater. Interfaces*, 10 **(2018)** 43343

[19] A. A. Ensafi, M. Taei, T. Khayamian, *Colloids Surf. B: Biointerfaces,* 79 **(2010)** 480

[20] H. D. Madhuchandra, B. E. K. Swamy, *Mater. Sci.Technol.,* 3 **(2020)** 464

[21] P. B. Mckibbin, J. Wong, D. D.Y. Chen, *J. Chromatogr. A*, 853 **(1999)** 535

[22] J. Yang, G. Zhang, X. Wu, F. Huang, C. Lin, X. Cao, L. Sun, Y. Ding, *Anal. Chim. Acta.,* 363 **(1998)** 105

[23] Y. Zhang, W. Ren, Y. Z. Fan, J. X. Dong, H. Zhang, H. Q. Luo, N. B. Li, *Anal. Chim. Acta.,* 1054 **(2019)** 167

[24] A. Mishra, A. Upadhyay, A. Patra, S. Chaudhury, P. Chattopadhyay, *Sci Pharm.* 77 **(2009)** 367

[25] T. Yadav, R.K. Sahu, V. Mukherjee, *J. Mol. Struct.,* 1176 **(2019)** 94

[26] N. T. Deftereos, A. C. Calokerinost, C. E. Efstathiou, *Analyst.,* 118 **(1993)** 627

[27] P. S. Ganesh, B. E. K. Swamy, O. E. Fayemi, E. M. Sherif, E. E. Ebenso, *Sens. Bio-Sens.,* 20 **(2018)** 47

[28] X. Zhao, W. Hu, Y. Wang, L. Zhu, L. Yang, Z. Sha, J. Zhang, *Carbon,* 127 **(2018)** 618

[29] Q. Qin, X. Bai, Z. Hu, *J. Electroanal. Chem.,* 782 **(2016)** 50

[30] N. Hareesha, J. G. Manjunatha, *Sci.Rep.,* 11 **(2021)** 12797

[31] S. Meenakshi, K. Pandian, S. C. B. Gopinath, *J. Taiwan Inst. Chem. Eng.,* 107 **(2020)** 15

[32] K. V. Harisha, B. E. K. Swamya, E. E. Ebenso, *J. Electroanal. Chem.,* 823 **(2018)** 730

[33] B. Demirkan, S. Bozkurt, K. Cellat, K. Arıkan, M. Yılmaz, A. Şavk, M. H. Çalımli, M. S. Nas, M. N. Atalar, M. H. Alma, F. Sen, *Sci.Rep.,* 10 **(2020)** 2946

[34] H. D. Madhuchandra, B. E. K. Swamy, *Mater. Sci.Technol.,* 2 **(2019)** 697

[35] M. M. Charithra, J. G. Manjunatha, *J. Electrochem. Sci. Eng.* 10 **(2020)** 29

[36] D. M. Fouad, W. A. El-Said, *J. Nanomat.* 2016 **(2016)** 1

[37] S. Ramirez, N. Silva, M. P. Oyarzun, J. Pavez, J. F. Silva, *J. Appl. Electrochem.* 799 **(2017)** 349

[38] E. Wierzbicka, G. D. Sulka, *J. Appl. Electrochem.* 762 **(2016)** 43

[39] E. Wierzbicka and G. D. Sulka, *Sens. Actuators B: Chem.,* 222 **(2016)** 270

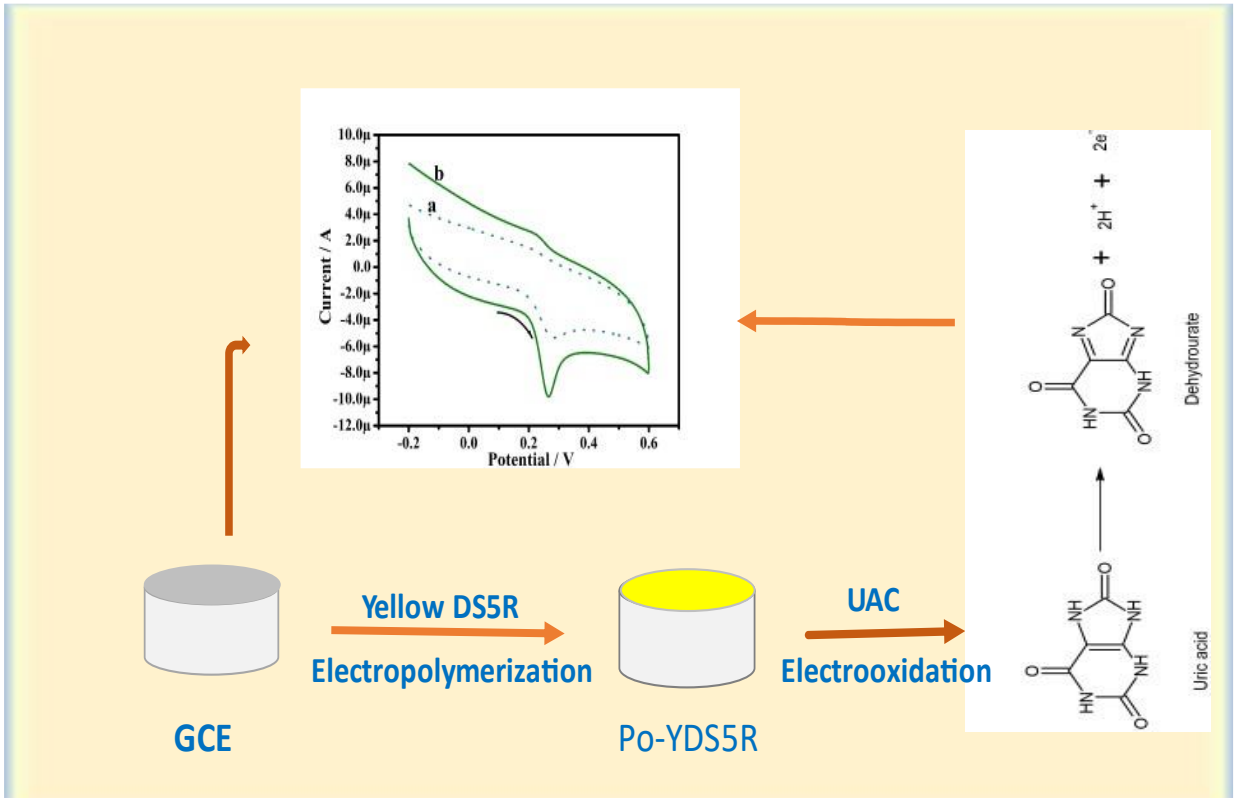
[40] M. E. Ghica and C. M. A. Brett, *Anal. Lett.,* 46 **(2013)** 1379

[41] S. Shahrokhian, M. Ghalkhani, and M. K. Amini, *Sens. Actuators B: Chem.*, 137 **(2009)** 669

Chapter -7

Part -A

An Affordable Yellow DS5R polymeric film modified glassy carbon electrode for voltametric assay of Uric acid



Communicated in Inorganic Chemistry Communications

7.1 Introduction

One of the electroanalytical approaches, voltammetric methods is indeed a crucial choice in drug analysis for detecting the active agent both qualitatively and quantitatively. The most effective voltammetric approaches offer great sensitivity, affordability, flexibility, timesaving, consistency, and environmentally friendly nature [1-4]. These strategies have evolved in recent years, demonstrating that they now constitute variable alternatives.

UAC is a by-product of the human body's purine nucleotide catabolism. The majority of UAC is dissolved in the blood and travels through the kidneys, which excrete in urine [5-7]. UAC is an extremely biologically important molecule found in body fluids, forming the ions and salt referred to as urates and acid urates, including ammonium acid urate [8]. Massive content of blood UAC can induce hyperuricemia, which results in the development of UAC crystals and irreversible bone, joint, and tissue damage, as well as renal and heart illness. The crystals create urinary stones in the kidneys and gouty arthritis in the joints, which are the most prevalent and common associated disorders [9-13]. Several lines of data revealed that increased blood UAC could be a substantial modifiable risk element [14]. As a response, UAC detection is crucial for reducing clinical consequences and preventing diseases that are linked to it. The frequent coexistence of UAC and DA body fluids in humans is well recognized. Due to their comparable oxidation potential, DA inferences the UAC detection [15, 16]. Hence, differently modified electrodes have been designed to fix these issues.

In voltammetric methods, choosing an optional working electrode material is of utmost importance. The glassy carbon electrode (GCE) is a practical conductive framework with a defined surface alignment, large operating window results that are specific and selective, good mechanical properties, and ease of surface modification [17-20]. However naked GCE does not demonstrate substantial and selective separation of biomolecules in the presence of interferences [21-23]. Therefore, to get around this problem, electrochemical researchers have discovered electrode modification with unique conducting layers. There are prior literature articles on the electropolymerization method's modification of GCE, which produced materials like poly (neutral red), poly (AHNSA), poly (ABSA), poly (celectein blue), poly (luminol) [24- 28].

Here, we advocated the electropolymerization of yellow DS5R (Scheme 7.1) polymeric film on the glassy carbon electrode surface (Po-YDS5R/GCE) for the detection of UAC. By examining all outcomes of the CV process, all experimental and voltammetric variables were checked. This method offers a precise, ecologically safe, easy, and inexpensive approach for evaluating UAC.

7.2. Experimental

7.2.1 Chemicals, reagents, and instrument

We purchased UAC, DA, NaOH, Na_2HPO_4, and NaH_2PO_4 from Nice Chemicals. Yellow DS5R was obtained from Astik Dyestuffs Pvt. Ltd Gujarat. All analytical grade compounds were employed directly after delivery without any refining. The electrochemical studies were conducted using voltammetric equipment of model CHI-660C (CHI-660 electrochemical workstation). Double-stilled water was used to prepare all the solutions. By mixing an appropriate amount of Na_2HPO_4 and NaH_2PO_4, PBS of 0.2M was made.

7.2.2 Configuring the GCE and Po-YDS5R/GCE

Alumina powder was used to polish the GCE before being cleaned with de-ionized water. The Po-YDS5R/GCE was successfully modified by electropolymerization of 1.0 mM aqueous solution of Yellow DS5R in 0.1M NaOH as supporting electrolyte on the GCE. As illustrated in Fig.7.1a, the potential range was sustained between -0.6 and 1.6V at sweep velocity (SV) of 100 mV/s for 10 cycles.

7.3. Results and Discussions

7.3.1 Optimization and electrochemical characterization of Po-YDS5R/GCE

The electrode coating of the polymeric material affects the electrochemical reactivity of the modified electrode. It was simple to adjust the coating thickness during electropolymerization by shifting the sweep cycle from 5 to 25. The voltammogram was first gradually increased by increasing the sweep cycles due to the development of the Yellow DS5R layer on the GCE surface, however after a few further sweeps, the electrocatalytic ability of the Po-YDS5R/GCE decreased as the Yellow DS5R thickness increased (inset Fig.7.1b) [29]. As a result, it was determined that 10 sweep cycles are ideal for obtaining the potent electrocatalytic response.

Fig.7.2 demonstrates the CVs for $K_4[Fe(CN)_6]$ system with 1M KCl (supporting electrolyte) at GCE (line a) and Po-YDS5R/GCE (line b) with SV of 100mV/s. The tiny peak current was detected on the GCE, while the Po-YDS5R/GCE hugely enhanced, indicating a significant increase in the speed of electron transfer [30]. The electroactive area of the GCE and Po-YDS5R/GCE was computed using Randels - Sevick expression (7.1) [31]. The discovered area is 0.0286 cm^2 for GCE and 0.0394 cm^2 for Po-YDS5R/GCE.

$$Ip = (2.69 \times 10^{-5})\ n^{3/2}\ D_0^{3/2}\ \upsilon^{1/2}\ A\ C \quad \text{----------(7.1)}$$

7.3.2 Voltammetric analysis of UAC at Po-YDS5R/GCE

Fig.7.3 depicts the CVs for electrochemical oxidation of 0.1mM UAC at GCE (blue line a) and Po-YDS5R/GCE (green line b) in buffer (0.2M, pH 7.4) with SV of 100 mV/s. The UAC displays a less sensible current at 0.280V with a broad peak at GCE. Like this, Po-YDS5R/GCE shows a high peak current at 0.266V and a minimal shift in the potential. The rise in peak current of UAC at Po-YDS5R/GCE is due to the interaction of UAC with the Po-YDS5R/GCE surface.

7.3.3 Sweep velocity and pH impacts

Fig.7.4a details the CVs execution for 0.1mM UAC in buffer (0.2M, pH 7.4) on Po-YDS5R/GCE at varied sweep velocity from 50-500 mV/s. The electrode oxidation peak currents and potentials are raised when rising the SV. Fig.7.4b and 7.4c show that there was good linearity for the graph of Ipa vs SV and Ipa vs square root of SV. The procured outcomes are fitted in the linear relations as Ipa (μA) = 0.047 υ (mV/s) + 5.70 (R^2 = 0.996) and Ipa (μA) = 1.14 $\upsilon^{1/2}$(mV/s) – 2.07 (R^2 = 0.996), reveals the nature of the kinetic property of UAC at Po-YDS5R/GCE surface was diffusion- controlled [32].

For optimization of pH, buffer solutions containing 0.1mM UAC were made in the pH of 6.2 to 7.8. Fig.7.5a was the electrochemical response at varied pH clarifying that all peak potentials shifted towards negative potential as pH increased, according to the Nernst formula [34]. The linearity graph of Epa vs pH (Fig.7.5b) was fitted with regression equation as, Epa (V) = -0.071 pH + 0.79 (R^2 = 0.999), confirms that the same quantity of protons and electrons are participating in the process (Scheme.7.2) [27].

7.3.4 Assay of UAC at varied concentration

Po-YDS5R/GCE was employed to assay UAC in a range of different UAC concentrations (10- 60 mM) in buffer (0.2M, pH 7.4). As seen in Fig.7.6a, CV curves gradually grew as the UAC concentration. When Ipa was plotted vs UAC concentrations (Fig.7.6b), the regression expression Ipa (μA) = 3.18(M) + 4.09 (R^2= 0.999) shows good linearity. The LOD (3S/M) and LOQ (10S/M) were detected to be 0.87 and 3.12 μM. The Po-YDS5R/GCE's achievement has been compared to other previously reported shown in Table 7.1.

7.3.5 Simultaneous study of UAC and DA

Fig.7.7 captures the electrochemical activity of 0.1mM UAC and 0.1mM DA mixture at GC (line a) and Po-YDS5R/GCE (line b) by the CV method. The use of Po-YDS5R/GCE results in two discrete Ipa peaks at E of 0.299V and 0.128V for UAC and DA with a ramp up in peak current. The electrolytic peak-to-peak splitting between UAC and DA was 0.171V. These findings imply that UAC can be selectively assayed in the vicinity of DA at Po-YDS5R/GCE.

7.3.6 Interferences analysis

This analysis was done by combining UAC and DA in buffer (0.2M, pH 7.4) at Po-YDS5R/GCE using the DPV technique. Fig.7.8 shows the electrochemical response of DA by boosting the concentration (10μM - 60μM) while holding the UAC concentration constant; only Ipa of DA increased. Fig.7.9 depicts the electrochemical response of UAC by boosting the concentration (10μM - 70μM) while DA concentration kept steady; only the Ipa of UAC was enhanced. The outcomes suggest that interference of DA did not affect on the electrolytic activity of UAC. Therefore, forecast method and Po-YDS5R/GCE are substantially free of interferences.

7.3.7 Stability and reproducibility

The stability of Po-YDS5R/GCE was tested by sweeping it for 20 cycles for 1mM $K_4[Fe(CN)_6]$. The Ipa signals recovered at about 97.6% of the initial value. As a consequence of these results, Po-YDS5R/GCE has a lifespan and exceptional stability. The reproducibility of Po-YDS5R/GCE was examined after 5 days kept at room

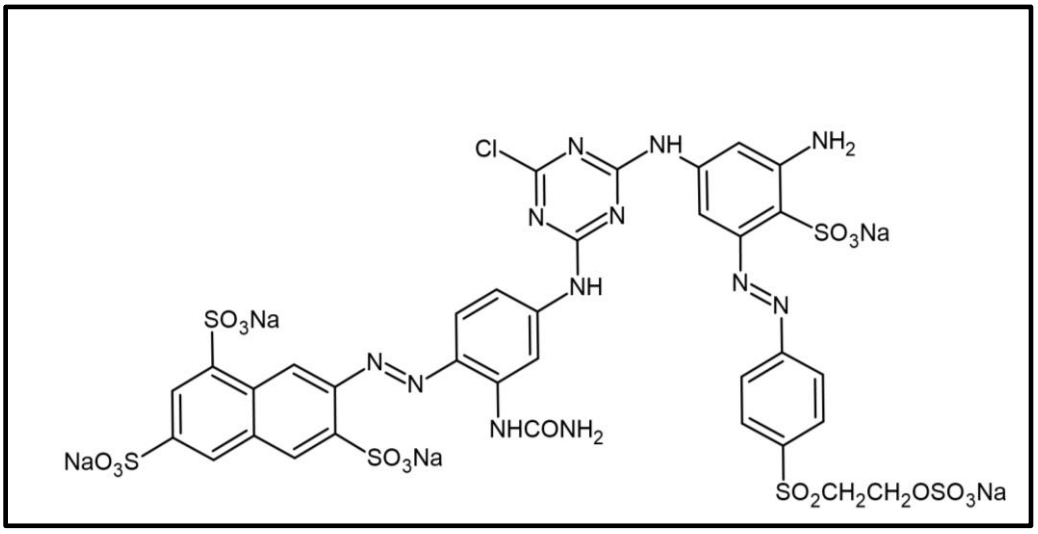
temperature. The Ipa signal recollected 97. 9% of the earlier start current response and did not affect on the peak potentials.

7.3.8 Analytical application

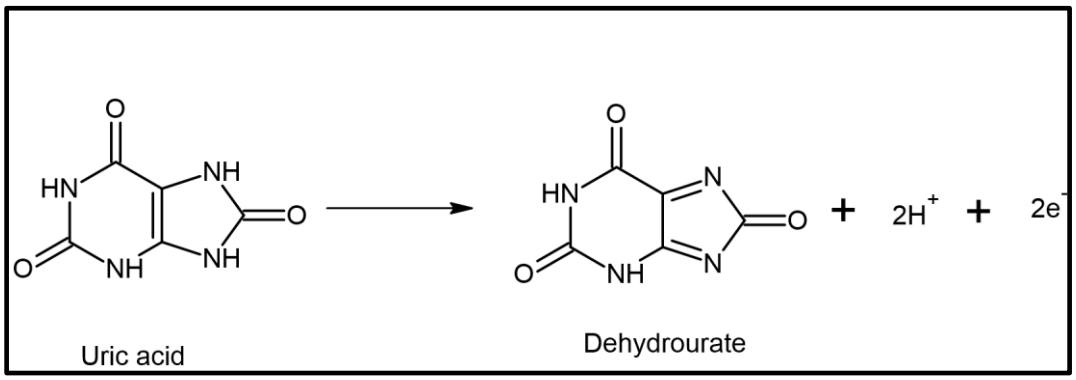
To verify the analytical applicability of Po-YDS5R/GCE for UAC assay, 10mM UAC was added to the urine sample. The recovery values of UAC were projected using the standard addition method (Table 7.2). The values obtained were revealed to be acceptable.

7.4. Conclusion

We have devised a simple technique for producing Po-YDS5R/GCE via the CV method. The resulting Po-YDS5R/GCE was employed for the assay of UAC. The key attractive properties of Po-YDS5R/GCE are the swift modification process, increased sensitivity, and selectivity, less- cost, and sustainability. The fabricated Po-YDS5R/GCE delivered better recovery in the urine sample. As a result, Po-YDS5R/GCE sensor will hold high potential in the area of biosensors.



Scheme. 7. 1 Structure of Yellow DS5R



Scheme. 7. 2 Electrooxidation of UAC

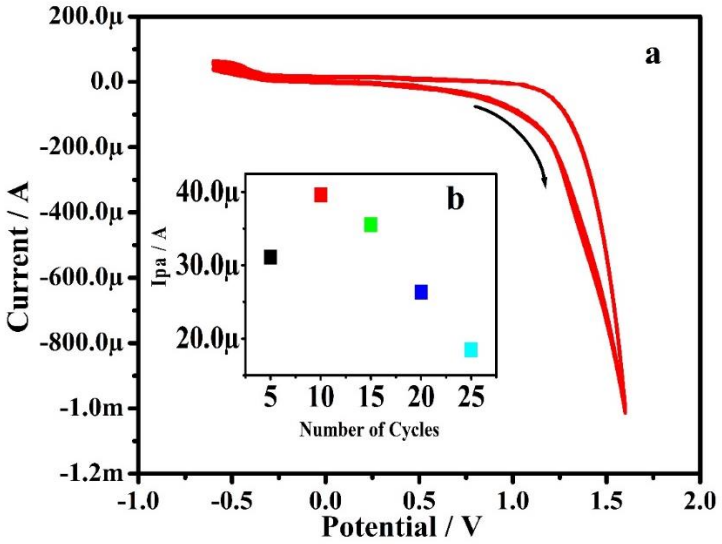


Fig. 7.1 a) CVs of fabrication of Po-YDS5R/GCE in 0.1 M NaOH at 10 sweep cycles b) Plot of Ipa vs number of cycles

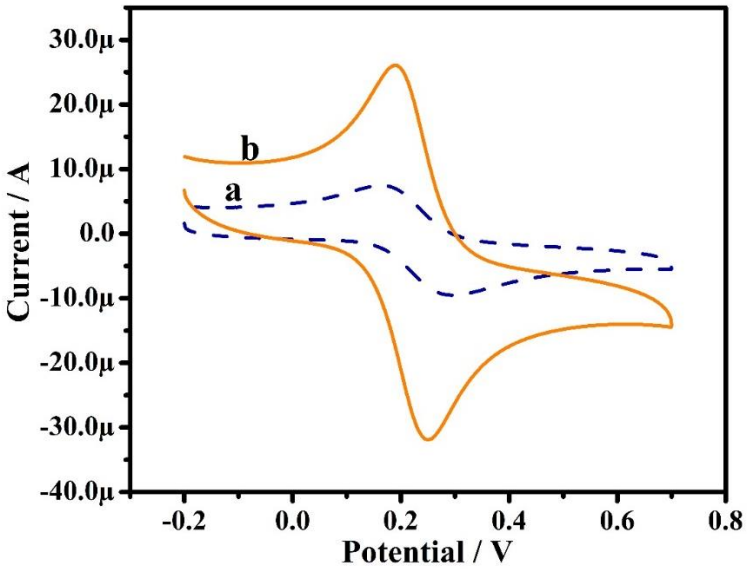


Fig. 7.2 CVs of 1mM $K_4[Fe(CN)_6]$ at GCE (line a) and Po-YDS5R/GCE (line b) at sweep velocity of 100mV/s

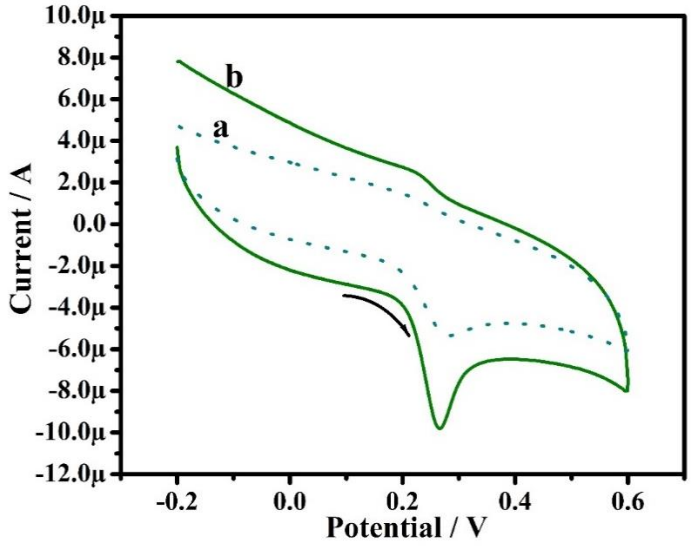


Fig. 7. 3 CVs of 0.1mM UAC at GCE (blue line a) and Po-YDS5R/GCE (green line b) at sweep velocity of 100mV/s

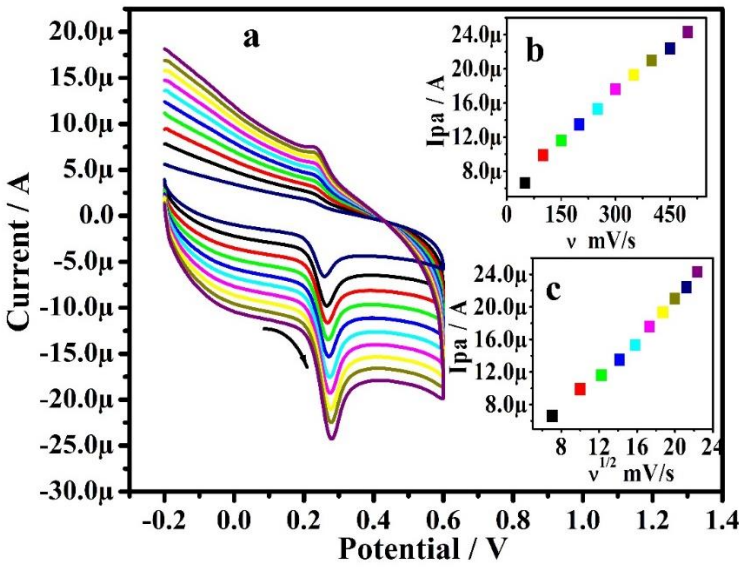


Fig. 7.4 a) CVs of 0.1 mm UAC at Po-YDS5R/GCE at varied sweep velocity(50-100mV/s) b) Plot of Ipa vs υ c) Plot of Ipa vs $\upsilon^{1/2}$

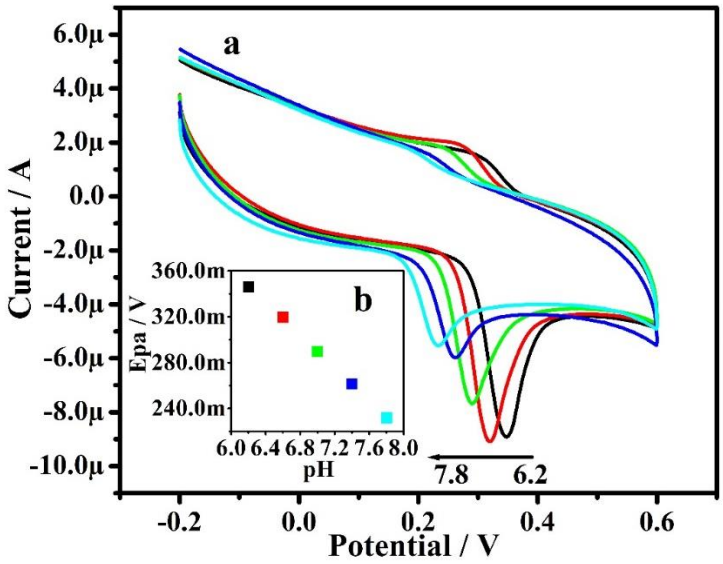


Fig. 7.5 a) CVs responses for impact of varied pH at Po-YDS5R/GCE b) Plot of Epa vs pH

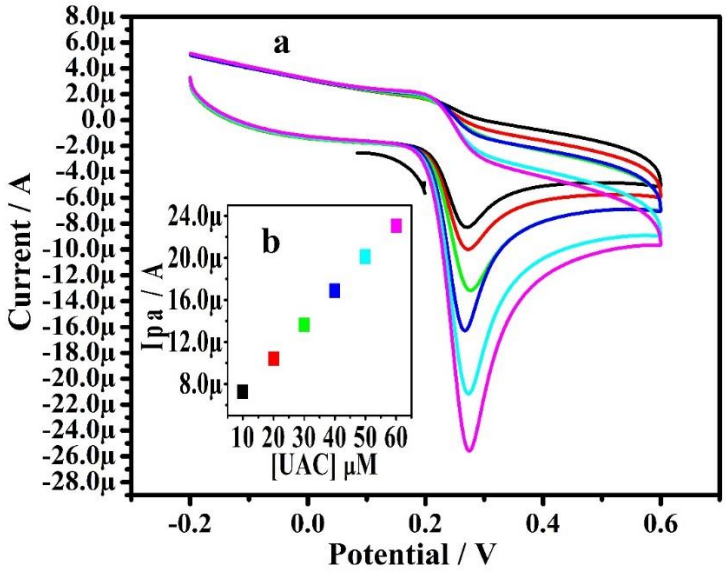


Fig. 7.6 a) CVs of Varied UAC concentrations (10- 60 µM) at Po-YDS5R b) Plot of Ipa vs UAC concentrations

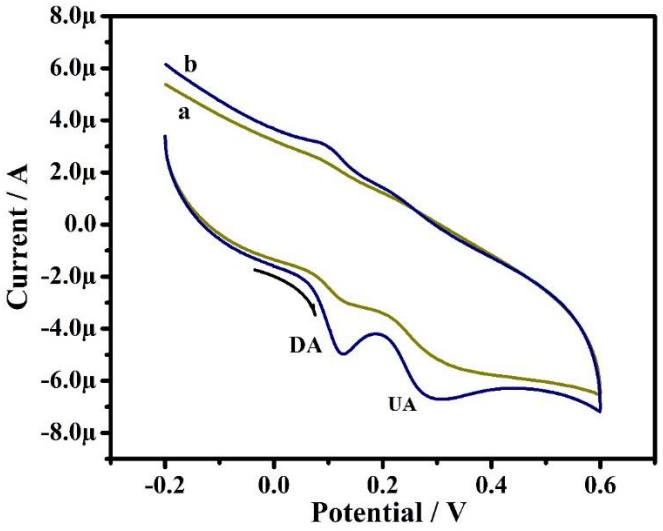


Fig. 7.7. CVs of simultaneous analysis of UAC and DA at GCE (line a) and at Po-YDS5R/GCE (line b)

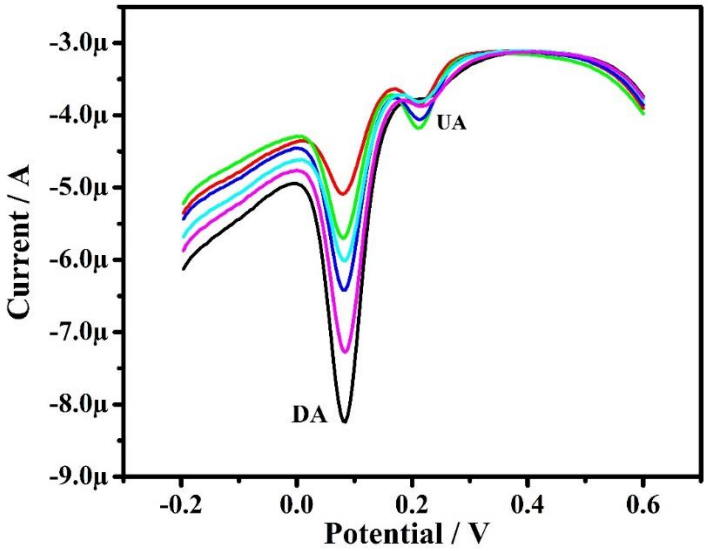


Fig. 7.8. DPVs response for varied concentration of DA (10 -60 µM) at Po-YDS5R/GCE

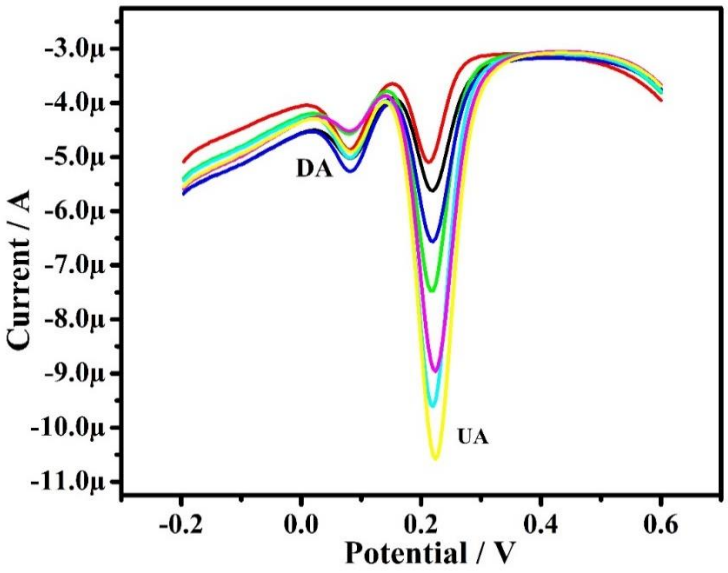


Fig. 7.9. DPVs response for varied concentration of UAC (10- 70 µM) at Po-YDS5R/GCE

Table 7.1 Comparative study of LOD of UAC at Po-YDS5R/GCE with previous report.

Sl.No	Electrode	LOD of UAC	Method	References
1	Poly (o -aminophenol)-MCPE	3.0 µM	CV	[33]
2	PPS/SAOS/MCPE	4.18 µM	CV	[34]
3	Pd/RGO/GCE	1.6 µM	DPV	[35]
4	Glu/GCE	1.1 µM	DPV	[36]
5	Au-Cu_2O/rGO/GCE	6.5 µM	DPV	[37]
6	Pdop@GR/MWCNTs	15.0 µM	DPV	[38]
7	PG/GCE	4.82 µM	CV	[39]
8	Po-YDS5R	0.87 µM	CV	Present paper

Table 7. 2 Determination of UAC in urine sample.

Sample	Added UAC (µM)	Estimated UAC (µM)	% Recovery
Urine	10	9.8	98.1±2
	20	19.6	96.2±4
	30	29.2	97.4±3

7.5 References

[1] A. Cetinkaya, B. D. Topal, E. B. Atici, S. A. Ozkan, *Electrochimica Acta,* 386 **(2021)** 138443

[2] E. Mehmeti, D. M. Stanković, A.Ortner, J. Zavašnik, K. Kalcher, *Food Anal. Methods,* 10 **(2017)** 3747

[3] A. Savk, B. Özdil, B. Demirkan, M. S. Nas, M. H. Calimli, M. H. Alma, Inamuddin, A. M. Asiri, F. Şen, *Mater. Sci, Eng. C,* 99 **(2019)** 248

[4] N. T. V. Hoan, N. N. Minh, N. T. H. Trang, L. T. T. Thuy, C. V. Hoang, T. X. Mau, H. X. A. Vu, P. T. K. Thu, N. H. Phong, D. Q. Khieu, *J. Nanomater*, 15 **(2020)** 9797509

[5] K. Shi, K. Shiu *Electroanalysis,* 16 **(2001)** 1319

[6] H. Kaur, B. Halliwell, *Chem. Biol. Interact*, 73 **(1990)** 235

[7] D. Aguayo, M. Emeterio, M. d. Valle, *Sensors*, 19 **(2019)** 3286

[8] C. Zhao, J. Xiao, T. Liu, H. Shi, Q. Li, Z. Ruan, *Int. J. Electrochem. Sci.,* 17 **(2022)** 2209

[9] Y. Changa, M. Lee, Y. Chien, *SLAS Technol,* 27 **(2022)** 54

[10] U. Chandra, B. E. K. Swamy, O. Gilbert, B. S. Sherigara, *Anal. Methods*,3 **(2011)** 2068

[11] T. Dhanasekaran, R. Manigandan, A. Padmanaban, R. Suresh, K. Giribabu, V. Narayanan, *Sci. Rep.* 9 **(2019)** 13250

[12] H. Vidya, B. E. K. Swamy, S. C. Sharma, G. K. Jayaprakash, S. A. Hariprasad, *Sci. Rep,* 11 **(2021)** 24040

[13] S. S. Shankar, B. E. K. Swamy, B. N. Chandrashekar, K. J. Gururaj, *Chem. Sensor,s* 2 **(2012)** 4

[14] D. I. Feig, M. Mazzali, D. Kang, T. Nakagawa, Karen Price, J. Kannelis, R. J. Johnson, *J. Am. So.c Nephro,l* 17 **(2006)** 69

[15] S. B. Tanuja, B. E. K. Swamy, V. K. Pai, *J Anal Bioanal Tech*, 7 **(2016)** 1

[16] Y. Yang, M. Li, Z. Zhu, *Talanta*, 20115 **(2019)** 295

[17] P. S. Ganesh, G. Shimoga, S. Lee, S. Kim, E. E. Ebenso, *J. Ana. Sci. Technol,* 12 **(2021)** 20

[18] S. A. Kumar, C. Tang, S. Chen, *Talanta,* 76 **(2008)** 997

[19] M. Ławrywianiec, J. Smajdor, B. Paczosa-Bator, R. Piech, *Anal. Methods***,** 9 **(2017)** 6662

[20] T. kokab, Afzal Shah, J. Nisar, A. M. Khan, S. B. Khan, A. H. Shah, *ACS Omega,* 5 **(2020)**10123

[21] V. Mani, Mani Govindasamy, S. Chen, R. Karthik, S. Huang, *Microchim. Acta,* 183 **(2016)** 2267

[22] S. B. Khoo, F. Chen, *Anal. Chem.,* 74 **(2002)** 5734

[23] O. E. Fayemi, A. S. Adekunle, B. E. K. Swamy, E. E. Ebenso, *J. Electroanal. Chem.,* 818 **(2018)** 236

[24] D. G. Dilgin, D. Gligor, H. İ.Gökçel, Z. Dursun, Y. Dilgi, *Microchim. Acta,* 173 **(2011)** 469

[25] M. Amare, S. Admassie, *J. Mater. Res. Technol.,* 5 **(2020)** 11484

[26] A. M. Abdel-Aziz, H. H. Hassan, I. H. A. Badr, *Anal. Chem.* 11 **(2020)** 7947

[27] P. S. Ganesh, G. Shimoga, S. Lee, S. Kim, E. E. Ebenso, *Chem. Select.* 6 **(2021)** 2379

[28] R. Jerome, A. K. Sundramoorthy, *J. Electrochem, Soc.,* 166 **(2019)** 3017

[29] N. Hareesha, J. G. Manjunatha, *Sci. Rep.,*11 **(2021)** 12797

[30] J. K. Shashikumara, B. Kalaburgi, B. E. K.Swamy, H. Nagabhushana, S. C. Sharma, P. Lalitha, *Sci. Rep.,* 11 **(2021)** 9372

[31] Amare, M. Teklay, W. *Cogent Chem.,* 5 **(2019)** 1576349

[32] N. Hareesha, J. G. Manjunatha, *J. Electroanal. Chem.,* 878 **(20201)** 14533

[33] E. Miland, A. J. M. Ordieres, P. T. Blanco, M. R. Smyth, C. O. Fagain, *Talanta,* 43 **(1996)** 765

[34] T. S. N. S. K. Naik, B. E. K. Swamy, *Anal. Bioanal. Electrochem*., 9 **(2017)** 424

[35] J. Wang, B. Yang, J. Zhong, B. Yan, K. Zhang, C. Zhai, Y. Shiraishi, Y. Du, P. Yang, *J. Colloid Interface Sci.,* 497 **(2017)** 172

[36] L. Zhang, X. Lin, *Analyst*, 126 **(2001)** 367

[37] T. K. Aparna, R. Sivasubramanian, M. A. Dar, *J. Alloys Compd.,* 741 **(2018)** 1130

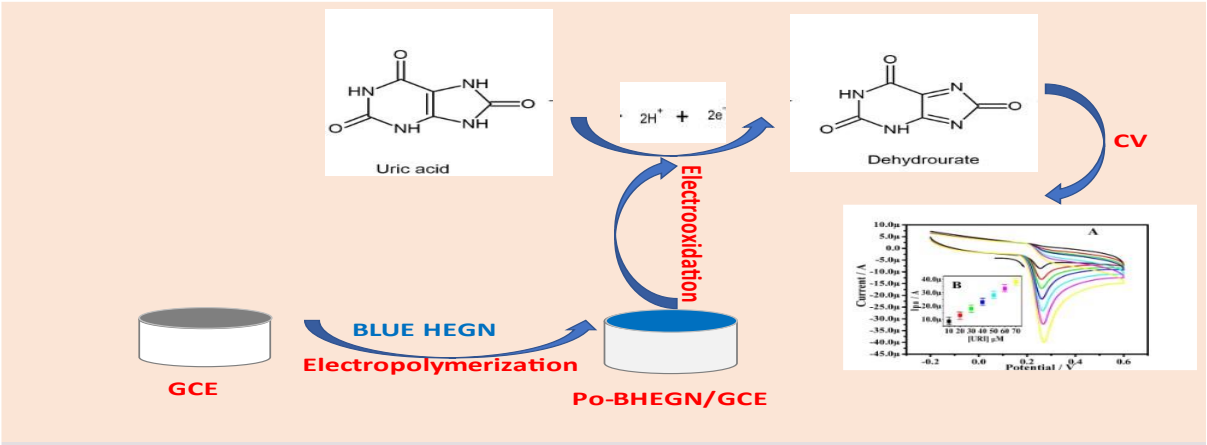
[38] C. Wang, J. Li, K. Shi, Q. Wang, X. Zhao, Z. Xiong, X. Zou, Y. Wang, *J. Electroanal. Chem.,* 770 **(2016)** 56

[39] S. Qi, B. Zhao, H. Tang, X. Jiang, *Electrochim. Acta*, 161 **(2015)** 395

Chapter -7

Part -B

Electroanalytical detection of Uric acid on Blue HEGN modified glassy carbon electrode by voltammetry



Published in
Analytical and Bioanalytical Electrochemistry 14(2022) 1114-1125

Anal. Bioanal. Electrochem., Vol. 14, No. 12, 2022, 1114-1125

Analytical & Bioanalytical Electrochemistry

2022 by CEE
www.abechem.com

Full Paper

Electroanalytical Detection of Uric Acid on Blue HEGN Modified Glassy Carbon Electrode by Voltammetry

S. D. Sukanya,[1] B. E. Kumara Swamy,[2,*] and J. K. Shashikumara[2]

7.6 Introduction

Electroanalytical approaches, which rely on the interplay of electrical energy and matter and have lately emerged as a notable analytical technique for detecting constituents contained in a sample. Various voltammetric methods with their simplicity and high sensitivity can easily predict the electro-active compounds. All voltammetric methods prioritize the selection of the best operating electrode material [1], [2]. The GCE is a valuable conductive material with a specific surface configuration, a broad operational window, selectivity and sensitivity in the findings, strong mechanical properties, and is easy to modify [3–6]. In the existence of interferences, the untreated GCE does not demonstrate the considerable and selective differentiation of electroactive [7–10]. Electrochemical researchers, on the other hand, have revealed that surface modification of an electrode is significant for boosting selectivity in detecting electrochemically active molecules. There are various publications in the literature on the electropolymerization of GCE, which led to invention of electrodes such as poly(o-aminophenol) [11], poly (neutral red)[12], poly (allura red) [13], poly (glutamic acid) [14], poly (PEDOT) [15] and poly (AHNSA) [16].

Uric acid (URI) is a waste product produced by the digestion of purine–containing foodstuff. URI is a significant by-product of purine metabolism. Typically, it stays within a stable range in healthy people [17–19]. The Kidneys dispose of around 70% of daily URI as a by-product of urine, while the balance is recirculated back into the bloodstream [20]. The typical blood URI content in healthy people is 3-7mg/dL, while the amount discharged in the urine is approximately 16-100mg/dL every 24 hours [21–23]. An excessive purine intake from food causes the body to release an excessive amount of URI. Excess URI concentrates and crystallizes, leading to various disorders. The deposition of URI crystallizes in human joints causing gout. Furthermore, chronic renal, and cardiovascular diseases, Lesch-Nyan syndrome, and hepatitis are all linked to high URI levels [24–26]. An incredibly low URI level also can lead to other disorders including Sclerosis[27]. In other words, Changes in URI level might cause physiological ailments. The quantitative amount of URI is critical for therapeutic purposes such as diagnosis and medication management. URI generally co-exists with DA in blood and urine[28, 29]. This behavior poses a unique challenge in distinguishing URI from DA. As a result, the design of a sensor with excellent sensitivity and

selectivity for quantifying URI level is extremely desirable.

In this paper, we presented the electropolymerization of Blue HEGN dye (Scheme 7.3) on the surface of GCE (Po- BHEGN/GCE) using CV techniques. There were no published reports on Blue HEGN's modification on GCE for the detection of URI. As a result, in this paper, we constructed simple and less-cost Po-BHEGN/GCE and characterized its electrochemical properties. At pH 7.4, Po-BHEGN/GCE exhibited an adequate response in the interference- free detection of URI in the existence of DA by DPV methods. The findings of this work have substantial implications for electroanalytical chemistry and sensors.

7.7 Experimental Part

7.7.1 Apparatus, chemicals, and reagents

The voltammetric measurements were taken with a model CHI-660C (CH instrument -660 electrochemical workstation). The three-electrode cell configuration features a glassy carbon working electrode, saturated calomel reference electrode, and a Pt counter electrode. Nice chemicals supplied URI, DA, Na_2HPO_4, NaH_2PO_4, and NaOH. Astik Dyestuffs Pvt. Ltd, Gujarath provided Blue HEGN dye. All the chemicals used in the experiment were utilized unprocessed. All the solutions are made with Double distilled water. 0.2M PBS was made by mixing enough Na_2HPO_4 and NaH_2PO_4.

7.7.2 Setup of GCE and Po-BHEGN/GCE

The GCE, which was used as a working electrode through all experiments was manually polished with alumina (0.05µm) powder. Eventually rinsed with de-ionized water after polishing. The Po-BHEGN/GCE was fabricated by electropolymerization of 0.1mM Blue HEGN dye in 0.1M NaOH as a supporting electrolyte on the GCE surface using CV techniques. According to Fig.7.10A, a potential zone of -0.6 to + 1.7 V was used for the electropolymerization with a sweep rate (SR) of 100 mV/s at 10 cycles.

7.8 Results and Discussion

7.8.1 Characterization and electroactive surface area of Po-BHEGN/GCE

The thickness of the polymer layer has a considerable impact on the electrocatytic properties of Po-BHEGN/GCE. The Po-BHEGN/GCE layer thickness

can be strongly suppressed by altering the number of sweep segments from 5 to 25. From Fig. 7.10B, we found an elevated oxidation peak current of URI from 5-10 segments; however, from 10-25 segments, the URI oxidation peak current steadily declined, which is most likely owing to adequate coverage of Po- BHEGN layer on the available GCE surface area. Ten sweep segments give appropriate coverage on the Po-BHEGN/GCE. As a consequence, 10 sweep segments were recognized as the best for this experiment.

CV was used to investigate the electrolytic interaction between Po-BHEGN/GCE and URI, with $K_4[Fe\ (CN)_6]$ (1.0mM) as a redox system containing supporting electrolyte KCl (1.0 M) at SR of 100 mV/s. The CV of Fig. 7.11 reveals that Po-BHEGN/GCE (curve B) has a much-amplified Ipa (28.76 μA) in contrast to GCE (curve A), which has a lower Ipa value (9.50 μA). This rise in Ipa of Po-BHEGN/GCE is attributed to the fact that there are more electroactive sites as a result of the growth of the thin layer of Blue HEGN on the electrode's exterior and hence facilitates speed up of electron transfer. The electroactive surface area of GCE and Po-BHEGN/GCE is calculated using the Randels-Sevick equation 7.1 [30–32]. The electroactive surface area of Po-BHEGN/GCE (0. 0412 cm^2) is nearly twice that of GCE (0.0248 cm^2), indicating that it is solid evidence for the productive and constructive modification of GCE by a polymer layer of Blue HEGN.

7.8.2 Voltammetric response of URI at Po-BHEGN/GCE

The cyclic voltammograms for 0.1mM URI in 0.2M PBS (7.4 pH) at SR of 100mV/s are illustrated in Fig. 7.12. The CV for GCE (dashed curve A) has quite a low oxidation peak current response and is noticed at 0.28 V with broad voltammogram. The CV produced for Po-BHEGN/GCE (solid curve B) displays a high oxidation peak current with a keen voltammogram at 0.25 V. Therefore, the Po-BHEGN/GCE is more vulnerable to URI oxidation than CGE.

7.8.3 Effect of sweep rate

The analysis of CVs of URI at various sweep rates revealed information on the influence of peak current on sweep rate and even the type of electrode process occurring at the electrode surface. The CVs (Fig. 7.13A) produced for the analysis of 0.1mM URI

in 0.2M PBS (7.4 pH) on Po-BHEGN/GCE indicate that the Ipa is proportional to sweep rate, demonstrating electron transport between the Po-BHEGN/GCE and URI. The Ipa is seen to move to the positive side as the sweep rate grows from 50-500 mV/s. The graphs of Ipa VS υ (Fig. 7.13B) and Ipa VS $\upsilon^{1/2}$ (Fig. 7.13C) suggested that the process is diffusion controlled [33]. Since they are linear with correlation coefficients 0.994 and 0.998 respectively, and linear regression expressions Ipa (μA) = 0.16 υ (m V/s) + 27.02 and Ipa (μA) = 4.97 $\upsilon^{1/2}$ (mV/s) – 6.93, respectively.

7.8.4 pH study

The buffer's pH plays an essential role in modulating the mechanism of electrochemical performance of URI at Po-BHEGN/GCE. The CVs produced on 0.1 mM URI by elevating the pH from 6.2 to 7.8 appears in Fig. 7.14A, and it clears that when pH rises, the peak potential switches to the negative potential. The linearity between Epa and pH in Fig. 7.14B is given by the linear regression expression Epa (mV/s) = -0.06 pH + 0.75 (R^2= 0.999), which describes the same number of electrons and protons engaged in the oxidation process (Scheme 7.4)[34].

7.8.5 Detection limit and quantification studies of URI at Po-BHEGN/GCE

Fig. 7.15A presents CVs of URI at Po-BHEGN/GCE in the concentration range of 10μM - 70μM. Where the peak current grows as the URI concentration grows. Linearity is evident in the graph of Ipa VS URI concentrations (Ipa (μA) = 0.49 [URI] (μM) + 3.35 (R^2=0.999)) (Fig. 7.15B). LOD and LOQ (LOD = 3S/ M, LOQ = 10S/M, where S and M stands for standard deviation and slope of the graph) were determined from the collected data to be 0.94 and 3.15 μM, respectively. Table 7.3 compares the LOD of URI at Po-BHEGN/GCE to other works published in the literature.

7.8.6 Simultaneous analysis of URI and DA

The CV techniques were adopted to explore 0.1 μM URI and DA mixture in 0.2 M PBS (7.4, pH) on the Po-BHEGN/GCE surface (curve B) and GCE (curve A) (Fig. 7.16). The curve B depicts well-defined electrooxidation peaks caused by URI and DA with an increase in Ipa at peak potentials of 0.271 and 0.126 V, correspondingly in the exclusion of baseline current. Furthermore, for GCE, the unique peak split up for URI and DA is not certain. As a result, Po-BHEGN/GCE is a sensitive sensor for novel and

selective evaluation of URI in occurrence with DA.

7.8.7 Interference studies

Interference studies were performed by combining URI and DA in 0.2 M PBS (7.4 pH) at Po-BHEGN/GCE using DPV techniques. Since URI and DA have unique actions in living beings, the action of URI and DA in the composite form was investigated to determine whether the electrocatalytic potential of the proposed Po-BHEGN/GCE towards URI is comparable. The levels of one specimen are altered while the levels of another specimen are kept static and vise-versa. Fig. 7.17 demonstrates that only the DA peak current was enhanced by raising the DA levels from 10 µM – 70 µM while preserving the URI level constant. Fig. 7.18 shows that only the URI peak current was raised by varying the URI levels from 10 µM- 60µM while maintaining the DA levels constant. These results imply that the interference of DA did not affect on the electrochemical behaviors of URI. In conclusion, the suggested model and Po-BHEGN/GCE are virtually free of interference.

7.8.8 Reproducibility, repeatability, and stability

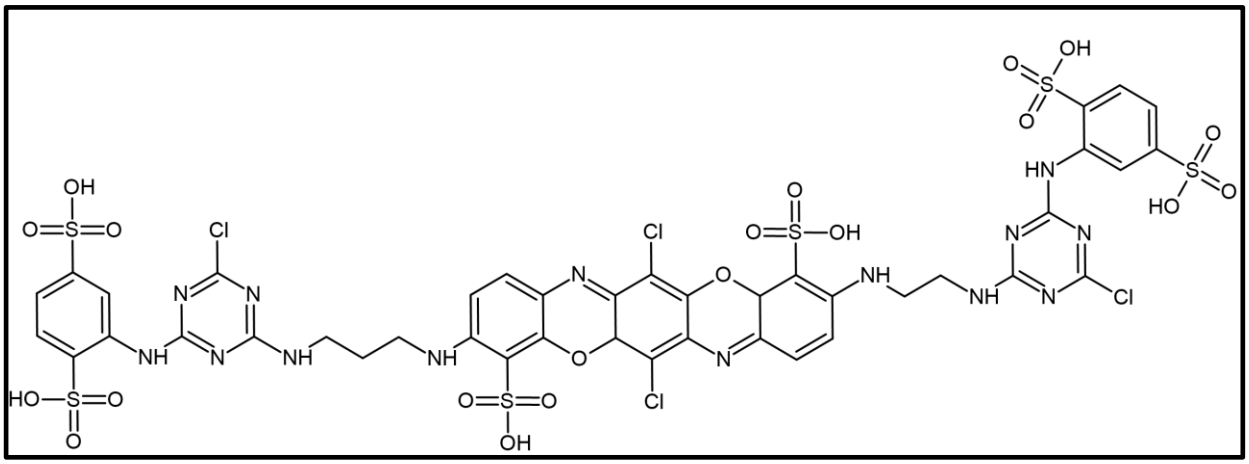
When the reproducibility of the Po-BHEGN/GCE was verified after 6 days, it was discovered that the peak current signal recovered 97.4% of the initial start-up current response and had no effect on the peak potential. The repeatability of the Po-BHEGN/GCE was checked by performing four consecutive CV cycles, and the results show excellent repeatability with an RSD value of 3.96. Sweeping 20 consecutive cycles to test the stability of the Po-BHEGN/GCE revealed that 96.4% of the initial current is preserved even after 20 cycles.

7.8.9 Analysis of URI in Urine sample

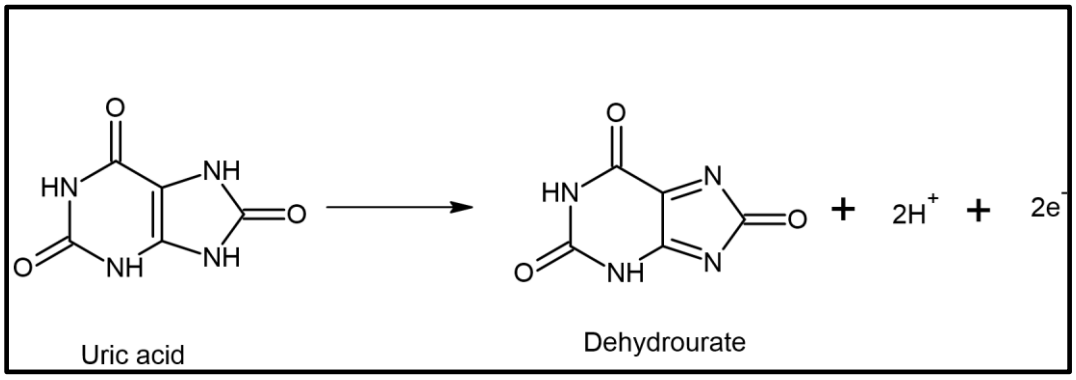
The Po-BHEGN/GCE was used to measure URI in a urine sample in 0.2M PBS 97.4 pH). When a known amount of URI (10µM) was injected into real samples, the standard addition approaches provided quantitative recovery ranging from 97.7±2 to 99.1±2 % (Table 7.4). These appealing characteristics of the Po-BHEGN/GCE point to a possible application for assaying URI in physiological situations.

7.9 Conclusion

In this work, Po-BHEGN/GCE an effective electroanalytical tool was fabricated by simply modifying a GCE with Blue HEGN dye by electropolymerization method via CV techniques. The major purpose of this study was to determine whether a Po-BHEGN/GCE could be used to quickly screen URI. A linear response between 10-70µM for URI with a detection limit of 0.94 µM was obtained. In simultaneous studies, the Po-BHEGN/GCE demonstrated excellent performance in the assay of URI in occurrence with DA. The interference study suggested that Po-BHEGN/GCE is free of any interferences. The developed Po-BHEGN/GCE fared better recovery in the urine sample. These attractive features concluded that the Po-BHEGN/GCE is the more promising and potent sensor in the study of other biologically active molecules and pharmaceutical samples.



Scheme 7.3 Structure of Blue HEGN



Scheme 7.4 Electrochemical Oxidation of URI

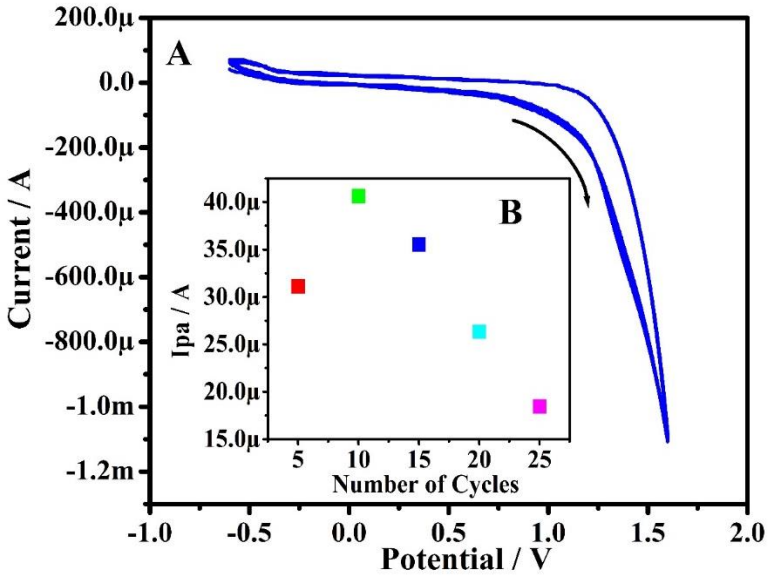


Fig. 7.10 A) CVs of construction of Po-BHEGN/GCE in 0.1M NaOH at 10 sweep rates B) Graph of Ipa VS Number of cycles

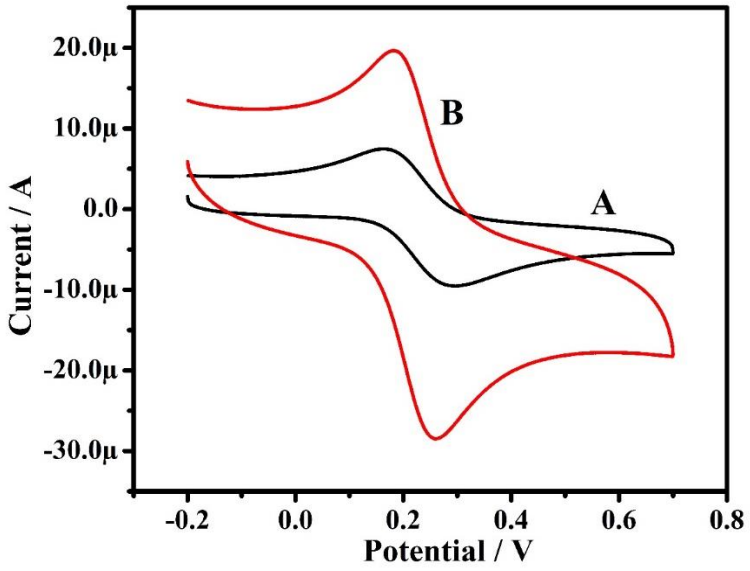


Fig. 7.11. CVs of 1mM of $K_4[Fe(CN)_6]$ in KCl (1.0 M) at GCE (curve A) and Po-BHEGN/GCE (curve B) at sweep rate 100 mV/s

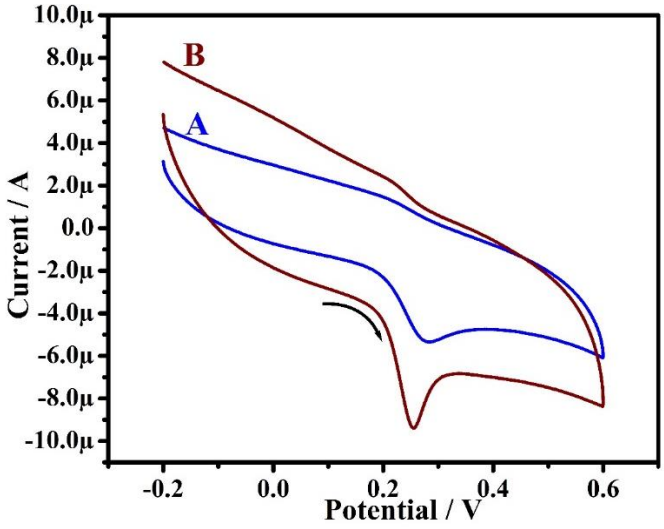


Fig. 7.12. CVs of 0.1mM URI in 0.2 M PBS (7.4 pH) at GCE (dashed curve A) and Po-BHEGN/GCE (solid curve B) at sweep rate 100mV/s

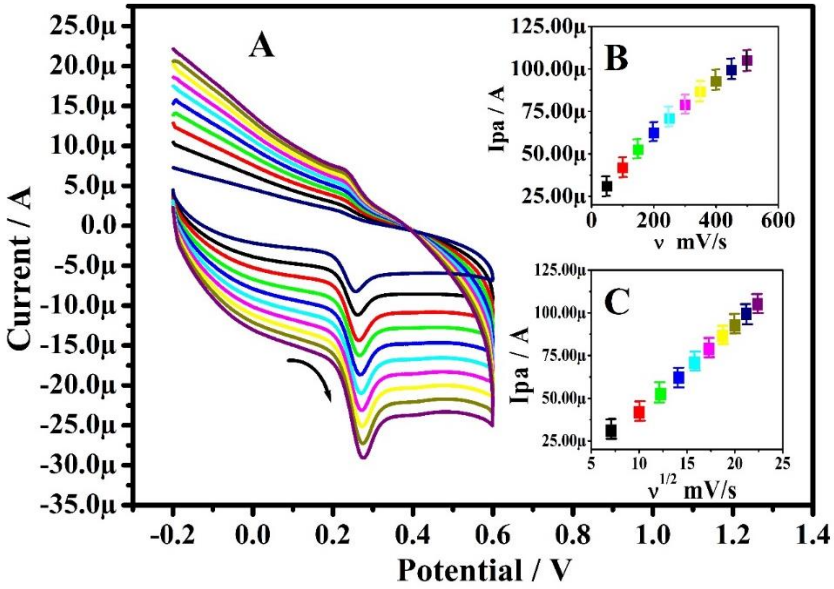


Fig. 7.13. A) CVs of 0.1mM URI at Po-BHEGN/GCE at sweep rate from 50-500 mV/s B) Graph of Ipa VS υ mV/s C) Graph of Ipa Vs $\upsilon^{1/2}$ mV/s

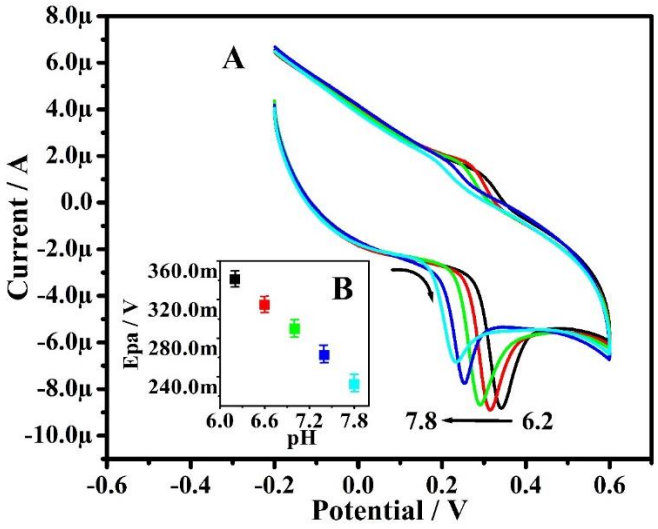


Fig. 7.14. A) CVs obtained for varied pH at Po-BHEGN/GCE B) Graph of Epa VS pH

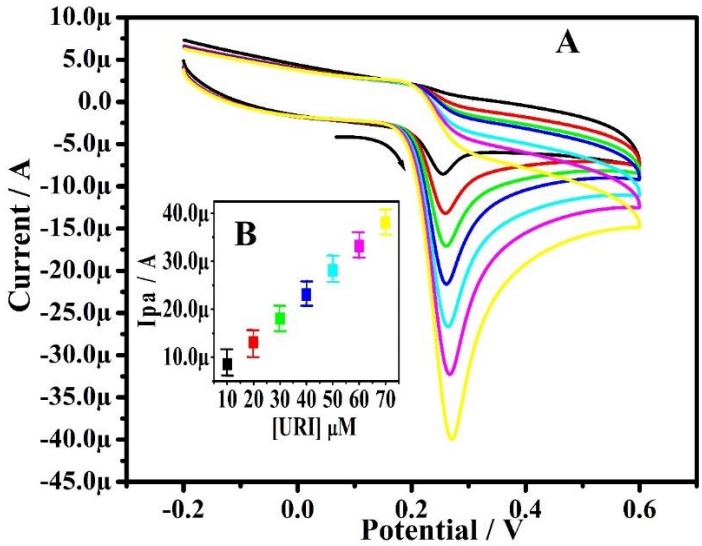


Fig. 7.15. A) CVs of increased URI concentrations (10- 70µM) at Po-BHEGN/GCE B) Graph of Ipa Vs URI concentration

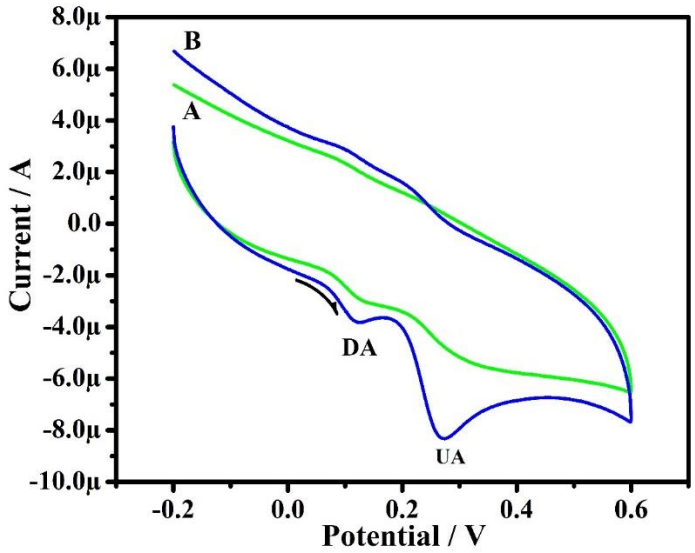


Fig. 7.16. CVs of simultaneous studies of URI and DA at GCE (curve A) and Po-BHEGN/GCE (curve B) at sweep rate 100mV/s

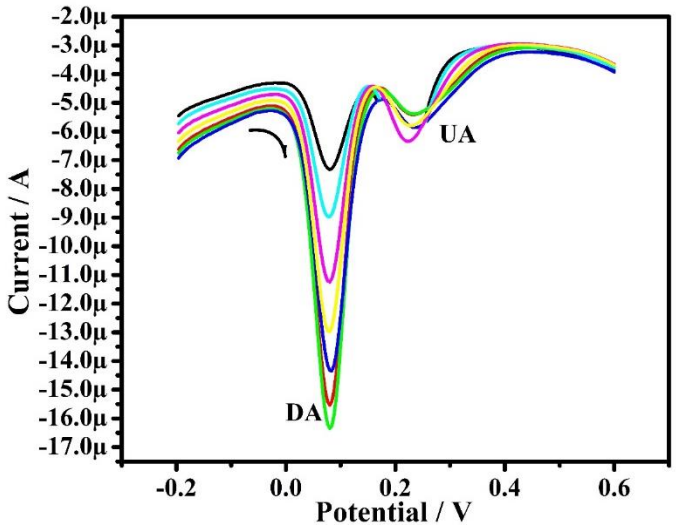


Fig. 7.17. DPVs of varied concentration of DA (10- 70µM) at static concentration of URI

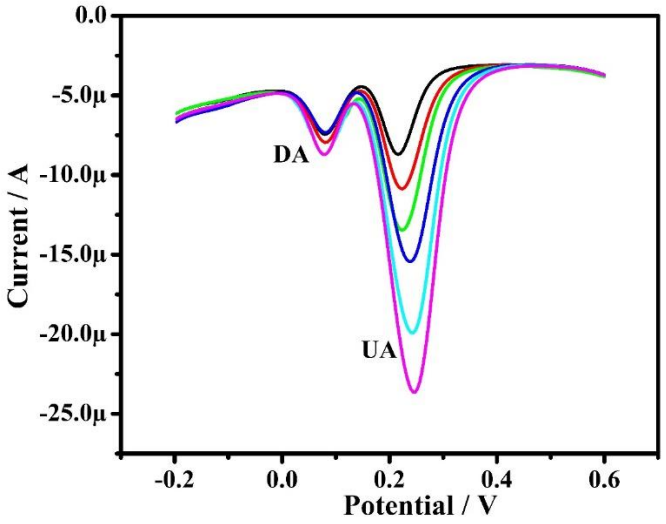


Fig. 7.18. DPVs of varied concentration of URI (10- 60 µM) at static concentration of DA

Table 7.3. Comparison of LOD of URI at Po-BHEGN/GCE with previously reported sensors

Sl.No	Electrode	LOD of URI µM	Method	References
1	Poly (o-aminophenol)-MCPE	3.0	CV	[11]
2	Pd/RGO/GCE	1.6	DPV	[35]
3	Glu/GCE	1.1	DPV	[36]
4	Au-Cu2O/rGo/GCE	6.5	DPV	[37]
5	Pdop@GR/MWCNTs	15.0	DPV	[38]
6	PG/GCE	4.82	CV	[39]
7	PPS/SAOS/MCPE	4.18	CV	[40]
8	Po-BHEGN/GCE	0.94	CV	Present work

Table 7.4. Detection of URI in urine sample

Sample	Added URI µM	Estimated URI µM	% Recovery
Urine	10	9.7	97.7± 2
	20	19.6	96.4± 3
	30	29.5	99.1± 2

7.10 References

[1] H. Yao, Y. Sun, X. Lin, Y. Tang, A. Liu, G. Li, W. Li, S. Zhang, *Anal. Sci.*, 23 **(2007)** 677

[2] M. Shabani-Nooshabadi, M. Roostaee, *J. Mol. Liq.*, 220 **(2016)** 329

[3] P. S. Ganesh, G. Shimoga, S. H. Lee, S. Y. Kim, E. E. Ebenso, *ChemistrySelect*, 6 **(2021)** 2379

[4] M. Ławrywianiec, J. Smajdor, B. Paczosa-Bator, and R. Piech, *Analytical Methods*, 9 **(2017)** 6662

[5] T. Kokab, A. Shah, J. Nisar, A. M. Khan, S. B. Khan, A. H. Shah, *ACS Omega*, 5 **(2020)** 10123

[6] A. M. Abdel-Aziz, H. H. Hassan, I. H. A. Badr, *Anal. Chem.*, 92 **(2020)** 7947

[7] R. Jerome, A. K. Sundramoorthy, *J. Electrochem. Soc.*, 166 **(2019)** 3017

[8] S. A. Kumar, C. F. Tang, S. M. Chen, *Talanta*, 76 **(2008)** 997

[9] V. Mani, M. Govindasamy, S. M. Chen, R. Karthik, and S. T. Huang, *Microchimica Acta*, 183 **(2016)** 2267

[10] S. B. Khoo, F. Chen, *Ana.l Chem.*, 74 **(2002)** 5734

[11] E. Miland, A. Mirandaordieres, P. Tunonblanco, M. Smyth, And C. Fagain, *Talanta*, 43 **(1996)** 785

[12] D. G. Dilgin, D. Gligor, H. I. Gökçel, Z. Dursun, and Y. Dilgin, *Microchimica Acta*, 173 **(2011)** 469

[13] P. S. Ganesh, G. Shimoga, S. H. Lee, S. Y. Kim, E. E. Ebenso, *J. Anal. Sci. Technol.*, 12 **(2021)** 20

[14] A. A. Kumar, T. S. Rani, P. S. Ganesh, B. E. K. Swamy, *Anal. Bioanal. Electrochem.*,9 **(2017)** 328

[15] A. Hammoud, D. Chhin, D. K. Nguyen, M. Sawan, *Biosens. Bioelectron.*, 180 **(2021)** 113089

[16] O. E. Fayemi, A. S. Adekunle, B. E. K. Swamy, E. E. Ebenso, *J. Electroanal. Chem.*, 818 **(2018)** 236

[17] T. S. Thanh, P. T. Qui, N. T. T. Tu, T. T. T. Toan, T. T. B. Hoa, L. V. T. Son, D. M. Nguyen, T. N. Tugen, D. Q. Khieu., *J. Nanomater*, 2021 9914062

[18] H. Kaur, B. Halliwell, *Chem. Biol. Interact.*, 73 **(1990)** 235

[19] N. T. V. Hoan, N. N. Minch, N. T. H. Trang, L. T. T. Thung, C. V. Hoang, T. X. Mau, H. X. A. Vu, P. T. K. Thu, N. H. Phong, D. Q. Khieu, *J. Nanomater.*,

2020 **(2020)** 9797509

[20] Y. J. Chang, M. C. Lee, Y. C. Chien, *SLAS Techno.l*, 27 **(2022)** 54

[21] D. Plausinaitis, L. Sinkevicius, U. Samukaite-Bubniene, V. Ratautaite, A. Ramanavicius, *Talanta*, 220 **(2020)** 121414

[22] I. Grabowska, M. Chudy, A. Dybko, Z. Brzozka, *Sens. Actuators. B. Chem.*, 130 **(2008)** 508

[23] M. Pucetaite, M. Velicka, J. Pilipavicius, A. Beganskiene, J. Ceponkus, V. Sablinskas, *J. Raman Spec.*, 47 **(2016)** 681

[24] B. Demirkan, S. Bozkurt, K. Cellat, K. Arikan, M. Yilmaz, A. Sark, M. H. Calimli, M. S. Nas, M. N. Atalar, M.H. Alma, *Sci. Rep.*, 10 **(2020)** 2946

[25] D. Ortiz-Aguayo, M. Bonet-San-Emeterio, M. del Valle, *Sensors*, 19 **(2019)** 3286

[26] C. R. Raj, F. Kitamura, T. Ohsaka, *Analyst*, 127 **(2002)** 1155

[27] B. Han, M. Pan, X. Liu, J. Liu, T. Cui, Q. Chen, *Materials*, 12 **(2019)** 214

[28] Y. Yang, M. Li, Z. Zhu, *Talanta*, 201 **(2019)** 295

[29] S. B. Tanuja, B. E. K. Swamy, P. K, Vasanthkumar, *J. Anal. Bioanal. Tech.*, 7 **(2016)** 1000297

[30] T. Dhanasekaran, R. Manigandan, A. Padmanaban, R. Suresh, K. Giribabu, V. Narayanan, *Sci. Rep.*, 9 **(2019)** 13250

[31] B. Mekassa, M. Tessema, B. S. Chandravanshi, P. G. L. Baker, F. N. Muya, *J. Electroanal. Chem.*, 807 **(2017)** 145

[32] S. Deepa, B. E. K. Swamy, K. V. Pai, K. R. Mahanthesha, *Anal. Bioanal. Electrochem.*,11 **(2019)** 1240

[33] D. Majer, M. Finšgar, *Biosensors* ,11 **(2021)** 285

[34] R. Sainz, M. del Pozo, M. Vilas-Varela, J. Castro-Esteban, M. P. Corral, L. Vázquez, E. Blanco, D. Peña, J. A. Martín-Gago, G. J. Ellis, M. D. Petit-Domínguez, C. Quintana, E. Casero, *Sc.i Rep.*, 10 **(2020)** 14614

[35] J. Wang, B. Yang, J. Zhong, B. Yan, K. Zhang, C. Zhai, Y. Shiraishi, Y. Du, P.Yang, *J Colloid Interface Sci*, 497 **(2017)** 172

[36] L. Zhang, X. Lin, *Analyst*, 126 **(2001)** 367

[37] T. K. Aparna, R. Sivasubramanian, M. A. Dar, *J. Alloys Compd.*, 741 **(2018)** 1130

[38] C. Wang, J. Li, K. Shi, Q. Wang, X. Zhao, Z. Xiong, X. Zou, Y. Wang, *J. Electroanal.*

Chem., 770 **(2016)** 56

[39] S. Qi, B. Zhao, H. Tang, and X. Jiang, *Electrochim. Acta*, 161 **(2015)** 395

[40] T. S. N. S. K. Naik, B. E. K. Swamy, *Anal. Bioanal. Electrochem.*, 9 **(2017)** 424

scientific reports

Check for updates

OPEN

Poly (Orange CD) sensor for paracetamol in presence of folic acid and dopamine

S. D. Sukanya[1], B. E. Kumara Swamy[1]✉, J. K. Shashikumara[1], S. C. Sharma[2,3]✉ & S. A. Hariprasad[4]

In the present work, Orange CD was chosen as an intriguing modifier for the electropolymerization on the surface of CPE by the CV technique. A novel, sensitive, and cost-effective poly (Orange CD) MCPE (PoOCD/MCPE) sensor was utilized for the selective detection of paracetamol (PA) in 0.2 M phosphate buffer solution (PBS) of pH 7.4. The oxidation peak current of PA was vastly enhanced at the sensor. The scan rate study is suggested that electro-oxidation of PA was adsorption-controlled. The pH study testifies the redox pathways transport with the same quantity of electrons and protons. The detection limit of PA is found to be 2.64 µM. DPV results show that substantial peak separation between PA, folic acid (FA), and dopamine (DA) could be facilitating their individual and simultaneous determination on the sensor. The decorated sensor demonstrates high sensitivity, stability, reproducibility, repeatability and has been successfully exploited for the detection of PA in a tablet with promising results.

Paracetamol (PA) is one of the most extensively used analgesics and antipyretic drugs in clinical practice[1,2]. It is a very effective agent recommended for mild to moderate pain alleviation such as flu-induced fever, migraine, arthritis, and extenuates pain (headache, toothache, joint, muscular, chronic, postoperative)[3,4]. PA relieves pain by inhibiting prostaglandin synthesis in the central nervous system, and it also relieves fever by sedating the hypothalamus heat-regulating center[5]. PA is easily degraded by glucuronidation and sulfation into inactive metabolites, which are excreted in the urine, with just 5% of PA remaining unaltered[6]. In general, PA is known to have an excellent safety profile at approved therapeutic doses. But, its toxic metabolite accumulation in case of overdosing and chronic use lead to harmful side effects such as liver problem, kidney damages, trembling, nervousness, seizures, insomnia and nausea and even death[7–10]. Therefore, developing a simple, fast response, economical, sensitive, accurate, and reliable detection method for the assessment of PA is highly demanded in the medical field. There are lots of methods like capillary electrophoresis[11], titrimetry[12], SEC, LC–MS, HPLC[13–15], chemiluminescence[16], spectrofluorimetric[17], and spectrophotometry[18–20] which have been availed for the assessment of PA. Among all these methods, the electrochemical method stands out with its simplicity, sensitivity, selectivity, modest and fast response.

Folic acid (FA) is a water-soluble vitamin B_9 and also known as folacin that helps the growth of healthy new cells especially during pregnancy and controls the generation of ferrohaeme. FA is involved in a variety of biological tasks related to cell metabolism, including DNA replication, repair, and methylation, as well as the production of nucleotides, vitamins, and amino acids. Deficiency of FA causes anemia, leucopoenia, devolution of mentality, neurosis and also increases the chances of heart attack and stroke[21–24]. Dopamine (DA) is the neurotransmitter involved in the functioning of the central nervous system. DA is also utilized as an injectable medicine that stimulates the sympathetic nervous system, causing effects such as increased blood pressure and heart rate. Deficiency of DA may cause disorders like Parkinson's disease, Schizophrenia, Alzheimer's disease, and HIV infection[25–28]. When used for a long time, nonsteroidal anti-inflammatory agent like PA can prevent FA from being absorbed by the human being. The simultaneous measurement of PA and FA is particularly relevant since PA enhances the need for FA. The usage of PA protects dopaminergic neurons against oxidative stress damage produced by acute exposure to increased amounts of DA, according to in vitro studies. Furthermore, prolonged PA use in in vivo model has been shown to dramatically lower DA levels. Selective or simultaneous detection of PA, FA, and DA have been achieved by voltammetric method due to their electroactive natures[29–32]. In the

[1]Department of P.G. Studies and Research in Industrial Chemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shivamogga, Karnataka 577451, India. [2]National Assessment and Accreditation Council (Work Carried Out as Honorary Professor), Jain University, Bangalore, Karnataka 560 069, India. [3]School of Energy Science and Engineering, Indian Institute of Technology Guwahati, Guwahati, India. [4]Jain University, Bangalore, Karnataka 560 069, India. ✉email: bek@kuvempu.ac.in; scsharma.bangalore@gmail.com

Inorganic Chemistry Communications 140 (2022) 109394

Contents lists available at ScienceDirect

Inorganic Chemistry Communications

journal homepage: www.elsevier.com/locate/inoche

Short communication

Poly (yellow PX4R) carbon paste electrode sensor for paracetamol: A voltammetric study

Sukanya [a], B.E. Kumara Swamy [b,*], J.K. Shashikumara [b], S.C. Sharma [c,d,e]

[a] Department of P.G. Studies and Research in Analytical Chemistry, Alva's College, Moodubidire, Dakshina Kannada, Karnataka 574227, India
[b] Department of P.G. Studies and Research in Industrial Chemistry, Kuvempu University JnanaSahyadri, Shankaraghatta, Shivmoga, Karnataka 577451, India
[c] National Assessment and Accreditation Council (NAAC), Naagarabhaavi, Bengaluru 560072, Karnataka, India
[d] Jain University, Bengaluru 560069, Karnataka, India
[e] Schoolof Energy Science and Engineering, Indian Institute of Technology Guwahati, Guwahati, India

ARTICLE INFO

Keywords:
Paracetamol
Yellow (PX4R)
Carbon paste electrode
Cyclic voltammetry

ABSTRACT

A simple, novel and less cost yellow (PX4R) modified carbon paste electrode (Po-YPX4R-MCPE) was successfully fabricated for the detection of paracetamol (PAR) in PBS (0.2 M, 7.4 pH). This reported senor showed excellent electrocatalytic activity, increased sensitivity, and fast electron transfer rate towards the oxidation of PAR in comparison to BCPE. The surface morphologies of BCPE and Po-YPX4R-MCPE were analyzed using scanning electron microscopy (SEM). The influence of pH, scan speed, and impact of concentration was studied at the sensor. The pH study reveals that the same amount of electrons and protons are involved in the redox reaction of PAR, and the scan speed study depicts that the electro-oxidation was adsorption controlled. In an optimal operating condition, Po-YPX4R-MCPE exhibits a fine linear increase for PAR oxidation in the concentration range of working dynamic range 10 to 60 μM with an estimated LOD of 0.084 μM. The DPV data indicate that there is a significant peak divergence of 0.097 V for PAR and 0.292 V for dopamine (DA) which could make it easier to determine them alone and simultaneously on the sensor. The established sensor also contributed to good stability and reproducibility. The described method has been employed for the determination of PAR in pharmaceutical tablets. Good recovery values indicate the efficacy and applicability of the sensor in detecting PAR.

1. Introduction

PAR is a significant antipyretic and analgesic drug of the first choice worldwide [1,2]. PAR is a key component in most cold and flu reversal medications. PAR is administered to treat mild to moderate pain, including headaches, muscle aches, rheumatic pain, joint pain, backaches, toothaches, relief of fever, osteoarthritis, and cancer pain [3,4]. At the proper therapeutic dose, PAR is easily metabolized. Overdosing on PAR causes toxic metabolite buildup, which leads to acute liver necrosis and increased morbidity and mortality [5]. Frequently intake of PAR by pregnant women is linked to a higher risk of wheezing and eczema in their baby in early life [6]. Owing to in vitro studies, the use of PAR protects dopaminergic neurons against oxidative stress caused by acute exposure to high levels of dopamine (DA). Conversely, in an in vivo model, chronic PAR usage has been found to significantly decrease DA levels [7,8]. PAR monitoring becomes critical to control and avoid these issues [9]. PAR research is important in the life sciences and medicine, hence quick, easy, and accurate method for the determination of PAR in physiological pH circumstances is of greater interest. Numerous methods including chemiluminescence [10], chromatography [11], LC-MS, HPLC [12–14], spectrophotometric [15–17], spectrofluorimetric [18] etc. availed for the quantification of PAR. Since PAR has an electrochemically oxidizable phenolic hydroxyl group, it will be easier to quantify it using an electrochemical approach [19].

Electrochemical sensors are the most well-known promising devices in modern days because of their high sensitivity and selective properties, fast response, ease of automation, eco-friendly and has been widely employed in analytical strategies for drug molecule testing, clinical diagnosis, agriculture, and food sector quality control, and environmental monitoring [20–23]. In this area, the kind of working electrode material chosen has a significant impact on the practical application of the fabricated sensor. From the literature, it is noteworthy that, carbon paste electrodes (CPEs) are popular electrochemical sensors that have a broad range of applications in CV studies. CPEs have many advantages

* Corresponding author.
E-mail address: bek@kuvempu.ac.in (B.E. Kumara Swamy).

https://doi.org/10.1016/j.inoche.2022.109394
Received 24 December 2021; Received in revised form 12 March 2022; Accepted 17 March 2022
Available online 26 March 2022

Sensors International 1 (2020) 100045

Contents lists available at ScienceDirect

Sensors International

journal homepage: www.keaipublishing.com/en/journals/sensors-international

Voltammetric investigation of uric acid in existence of dopamine at Poly(benzydamine) modified carbon paste electrode

Sukanya, B.E. Kumara Swamy *, J.K. Shashikumara

Department of P.G. Studies and Research in Industrial Chemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shimoga, Karnataka, 577451, India

ARTICLE INFO

Keywords:
Uric acid
Dopamine
Benzydamine
Cyclic voltammetry
Electropolymeri
z
ation
Carbon paste electrode

ABSTRACT

Electropolymerization of Benzydamine was achieved on carbon paste electrode surface by cyclic voltammetric technique. The poly (benzydamine) MCPE exhibited an excellent electrocatalytic behavior of uric acid (UA). The critical examination was conducted on the electrochemical measurements at poly (benzydamine) MCPE for analysis of UA and dopamine (DA) by CV and DPV techniques. From the scan rate study, it was found that the oxidation of UA was diffusion controlled. Uric acid exhibits good detection limit (11.33 nM) at modified electrode in DPV technique. Hence, the investigated sensor poly (benzydamine) MCPE shows excellent selectivity and sensitivity towards resolution of UA in existence of DA.

1. Introduction

UA is a most important biological molecule present in body fluids [1]. It is a major nitrogenous compound in the urine. UA is the final product of a many exogenous purines and endogenous purine metabolism. The diet as well as animal proteins alters exogenous pool remarkably. Endogenous production of uric acid predominantly occurs in the intestines, liver, kidneys, muscles, and vascular endothelium [2–4]. In healthy person, the normal UA concentration in urine is around 2 mM and in plasma depending on the age and gender, an average value of UA in healthy 19 year adult blood ranges from 2.6 to 7.2 mg/dL (male 3.5 – 7.2 mg/dL, female 2.6 – 6 mg/dL) [5] The abnormality of it in human body leads to symptoms of several physiological diseases such as, hyperuricaemia, gout, Lesch–Nyan disease and heavy hepatitis [6]. Pneumonia and leukemia are also accompanied with increased urate levels [7]. UA is also a sign for renal failure as well as toxicity [8]. Therefore, accurate determination of UA concentration in human body fluids has become important for the treatment of diseases. In general, irreversible oxidation of electroactive UA in aqueous solution can be utilized for this purpose with the major product being allantoin [9] (Scheme 1).

DA, a catecholamine is well known as a neurotransmitter widely distributed in mammalian central nervous system [10]. High concentration level of DA is found in caudate nucleus of brain and very low concentration of it is observed in extra cellular fluids (0.01–1 μM) [11]. DA is used as a medication for Parkinson's disease, Schizophrenia and Alzheimer's disease [12]. It has been observed that the dopamine is an electrochemical active, which gives dopamine-o-quinone as oxidation product [13] (Scheme 2). It is well known that UA and DA commonly co-exist in human body fluids [14]. Several methods like fluorescence [15–17], colorimeter method [18,19], HPLC [20], capillary electrophoresis [21], gas chromatography [22] have been employed for the selective or simultaneous investigation of UA and DA. But, because of the high sensitivity and selectivity, modest and fast response, the electrochemical methods have attained a great influence [23]. However, resolution of UA in existence of DA is always challenging, as they have similar oxidation potential [24,25]. The variously modified electrodes have been fabricated to overcome this problem. But, in sensor field, CPEs are a good choice for electrochemical investigation, because of their easy preparation, wider potential window, fast response, renewability of the electrode surface and lower residual currents than glassy carbon electrodes. The CPEs can be modified to enhance their properties by different modifiers [26,27]. Now a days, the electropolymer film coated electrode plays

* Corresponding author.
E-mail address: bek@kuvempu.ac.in (B.E. Kumara Swamy).

Production and hosting by Elsevier

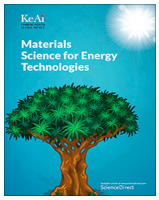
https://doi.org/10.1016/j.sintl.2020.100045
Received 30 July 2020; Received in revised form 18 September 2020; Accepted 18 September 2020
Available online 22 September 2020

Materials Science for Energy Technologies 3 (2020) 640–647

Contents lists available at ScienceDirect

Materials Science for Energy Technologies

journal homepage: www.keaipublishing.com/en/journals/materials-science-for-energy-technologies



Poly (benzydamine) sensor for electrochemical resolution of catechol and hydroquinone

Sukanya, B.E. Kumara Swamy *, J.K. Shashikumara

Department of P.G. Studies and Research in Industrial Chemistry, Kuvempu University, JnanaSahyadri, Shankaraghatta, Shimoga, Karnataka 577451, India

ARTICLE INFO

Article history:
Received 8 June 2020
Revised 25 June 2020
Accepted 27 June 2020
Available online 23 July 2020

Keywords:
Catechol
Hydroquinone
Benzydamine
Electropolymerisation
Carbon paste electrode

ABSTRACT

The carbon paste electrode was modified with poly (benzydamine) by cyclic voltammetric technique (CV) and its electrocatalytic activity towards the oxidation of catechol (CC) and hydroquinone (HQ) was studied. The detailed investigation on the various parameters influencing the activity was also carried out. From the obtained result, it was concluded that the process was adsorption - controlled and the involvement of same number of electron and proton. The simulations resolution of CC and HQ along with excellent selectivity and sensitivity were also exhibited by the studied MCPE. The detection limits 5.79 and 3.78 μM were found for CC and HQ respectively.

1. Introduction

In analytical chemistry, the simultaneous and interference less analysis of organic isomers is a fascinating aspect for many researchers [1,2]. The electrochemical techniques such as voltammetric method have drawn much more attention due to their fast response, high selectivity and separation, low cost and simple operation [3–7]. Recently, in the electroanalysis of biologically important oxidizable compounds, the MCPE has attracted ample recognition due to its low cost, high sensitivity, simple preparation, reproducibility with low detection limit and its eco-friendly nature [8–13]. The two positional isomers of a dihydroxybenzene moiety namely, CC and HQ [14,15] are widely used in cosmetics, dyes, photostabilizers, plasticizers, pesticide, oil refineries, antioxidants, leather, coal tar, and pharmaceutical industries [16–19]. Both CC and HQ are difficult to degrade and are found to be extremely harmful to humans and animals and are listed as the possible human carcinogens by the International Agency for Research on Cancer [20–22]. Hence, US Environmental Protection Agency (EPA) and European Union (EU) have considered these isomers as the serious environmental pollutants [23,24]. Moreover, Both CC and HQ usually co-exist due to their structural and property resemblance [25,26]. These factors necessitate the development of simple and efficient qualitative and quantitative analytical methods for these phenolic isomers [27]. There are many research reports in this regard, such as pH based flow injection analysis [28], Electrochemiluminescence [29], Synchronous fluorescence [30], fluorescence [31], Spectrophotometry [32], mass spectrometry [33], HPLC [34,35]. All these techniques are complicated, time consuming and expensive. Owing to the same physico- chemical properties of these isomers, they show overlapped and broad voltammograms at BCPE [36]. Electropolymerization leading to the modification of carbon paste electrode would be interesting because of their simplicity, chemical stability, good sensitivity, strong adherence of polymer film; promote electron transfer rates and broad potential window [37,38]. This paper describes the preparation of poly (benzydamine) MCPE for the electrochemical sensing of CC and HQ in binary mixtures using CV and DPV techniques. Benzydamine (Scheme.1), a tertiary amine is a basic drug with analgesic, anti-inflammatory, antipyretic and local anesthetic effects [39–41], used both topically and systemically for the treatment of primary or normoreactive types of inflammation [42]. The poly (benzydamine) MCPE revealed excellent electrocatalytic activity, sensitivity, stability, selectivity and reproducibility, low detection limit, wide linear ranges towards the resolution of CC and HQ.

* Corresponding author.
E-mail address: bek@kuvempu.ac.in (B.E. Kumara Swamy).

Production and hosting by Elsevier

https://doi.org/10.1016/j.mset.2020.06.009

Anal. Bioanal. Electrochem., Vol. 14, No. 12, 2022, 1114-1125

Analytical & Bioanalytical Electrochemistry

2022 by CEE
www.abechem.com

Full Paper

Electroanalytical Detection of Uric Acid on Blue HEGN Modified Glassy Carbon Electrode by Voltammetry

S. D. Sukanya,[1] B. E. Kumara Swamy,[2,*] and J. K. Shashikumara[2]

[1]*Department of P. G. Studies and Research in Analytical Chemistry, Alva's College, Moodubidire, Dakshina Kannada, Karnataka, 574227, India*

[2]*Department of P. G. Studies and Research in Industrial Chemistry, Kuvempu University, JnanaShyadri, Shankaraghatta, Shivmoga, Karnataka, 577451, India*

*Corresponding Author, Tel.: +91-8282-256225

E-Mail: kumaraswamy21@yahoo.com

Received: 31 October 2022 / Received in revised form: 22 December 2022 / Accepted: 22 December 2022 / Published online: 31 December 2022

Abstract- An electroanalytical technique was advanced for the detection of uric acid (URI) relying on its oxidation behaviour. Using cyclic voltammetry (CV) techniques, the electrochemical performance and detection of URI were easily accomplished on poly (Blue HEGN) modified glassy carbon electrode (Po-BHEGN/GCE). The role of pH on anodic peak current and potential was examined. Phosphate buffer of 7.4 pH was opted for subsequent data analysis. Sweep rate studies were carried out and showed that electrode reaction was a diffusion-controlled process. A linear calibration curve was established in the URI concentration levels from 10-70 µM. The LOD and LOQ were estimated to be 0.94 and 2.91 µM, respectively. A simultaneous study of URI and dopamine (DA) revealed that well-separated peak at Po-BHEGN/GCE compare to GCE. To sum up, a straightforward and inexpensive sensor Po-BHEGN/GCE is built for the sensitive and focused detection of URI in samples.

Keywords- Uric acid; Blue HEGN; Glassy carbon electrode; Electroplolymerization; Cyclic Voltammetry

KUVEMPU UNIVERSITY

VOLTAMMETRIC STUDIES OF SOME BIOLOGICALLY IMPORTANT ORGANIC COMPOUNDS AT DIFFERENT MODIFIED ELECTRODES

Thesis to be submitted to the Faculty of Science
Kuvempu University

For the Award of the Degree of

Doctor of Philosophy

In

Industrial Chemistry

By

Mrs. SUKANYA M.Sc.,

Guide

Dr. B.E. KUMARA SWAMY M.Sc., Ph.D.,
Professor
Department of Post Graduate Studies and Research
in Industrial Chemistry
Kuvempu University,
Jnana Sahyadri, Shankaraghatta - 577451,
Shivamogga, Karnataka, India

2023

Summary of the Thesis

The goal of the thesis is to design an electrochemical sensor by altering the electrode surface (carbon paste electrode, glassy carbon electrode, and pencil electrode) to examine the electrochemical behaviour of some biologically important organic compounds employing cyclic voltammetric techniques. The biologically important compounds like paracetamol, uric acid, adrenaline, catechol, and hydroquinone were chosen for electrochemical investigations. The following aspects like number of electrons involved in the electrochemical reaction, impact of sweep rate, the surface area of the electrode, concentration, pH condition of electroactive species, simultaneous analysis and real sample were observed.

The work carried out in this thesis is divided and described into seven chapters.

Chapter–1

Introduction and Overview of Cyclic Voltammetry

This chapter involves the introduction about voltammetry and its techniques, fundamental principles, theoretical aspects, and applications of cyclic voltammetry. Moreover, the solvents, supporting electrolytes and electrode interaction can be seen in this section. A brief review of cyclic voltametric investigations of some biologically important compounds has been presented. Also, objective and scope of the present thesis were discussed in this chapter.

Chapter-2

Experimental

This chapter describes the basic experimental setup which is very much essential for voltammetric techniques. Also, procedure for the preparation of bare carbon paste electrode and its modification was explained in detail.

Chapter-3

Poly (Orange CD) sensor for paracetamol in presence of folic acid and dopamine

In the present work, Orange CD was chosen as an intriguing modifier for the electropolymerization on the surface of CPE by the CV technique. A novel, sensitive, and cost-effective poly (Orange CD) MCPE (PoOCD/MCPE) sensor was utilized for the selective detection of paracetamol (PA) in 0.2 M phosphate buffer solution (PBS) of pH 7.4. The oxidation peak current of PA was vastly enhanced at the sensor. The scan rate study is suggested that electro-oxidation of PA was adsorption-controlled. The pH study testifies the redox pathways transport with the same quantity of electrons and protons. The detection limit of PA is found to be 2.64 µM. DPV results show that substantial peak separation between PA, folic acid (FA), and dopamine (DA) could be facilitating their individual and simultaneous determination on the sensor. The decorated sensor demonstrates high sensitivity, stability, reproducibility, repeatability and has been successfully exploited for the detection of PA in a tablet with promising results.

Chapter-4

Poly (yellow PX4R) carbon paste electrode sensor for paracetamol: A voltammetric study

A simple, novel and less cost yellow (PX4R) modified carbon paste electrode (Po-YPX4R-MCPE) was successfully fabricated for the detection of paracetamol (PAR) in PBS (0.2 M, 7.4 pH). This reported senor showed excellent electrocatalytic activity, increased sensitivity, and fast electron transfer rate towards the oxidation of PAR in comparison to BCPE. The surface morphologies of BCPE and Po-YPX4R-MCPE were analyzed using scanning electron microscopy (SEM). The influence of pH, scan speed, and impact of concentration was studied at the sensor. The pH study reveals that the same amount of electrons and protons are involved in the redox reaction of PAR, and the scan speed study depicts that the electro-oxidation was adsorption controlled. In an optimal operating condition, Po-YPX4R-MCPE exhibits a fine linear increase for PAR oxidation in the concentration range of working dynamic range 10 to 60 µM with an estimated LOD of 0.084 µM. The DPV data indicate that there is a significant peak divergence of 0.097 V for PAR and 0.292 V for dopamine (DA) which could make it easier to determine them alone and simultaneously on the sensor. The established sensor also contributed to good stability and reproducibility. The described method has been employed for the determination of PAR in pharmaceutical tablets. Good recovery values indicate the efficacy and applicability of the sensor in detecting PAR.

Chapter-5

This chapter is divided into two parts such as Part-A and Part-B

Part-A

Voltammetric investigation of uric acid in existence of dopamine at Poly(benzydamine) modified carbon paste electrode

Electropolymerization of Benzydamine was achieved on carbon paste electrode surface by cyclic voltammetric technique. The poly (benzydamine) MCPE exhibited an excellent electrocatalytic behavior of uric acid (UA). The critical examination was conducted on the electrochemical measurements at poly (benzydamine) MCPE for analysis of UA and dopamine (DA) by CV and DPV techniques. From the scan rate study, it was found that the oxidation of UA was diffusion controlled. Uric acid exhibits good detection limit (11.33 nM) at modified electrode in DPV technique. Hence, the investigated sensor poly (benzydamine) MCPE shows excellent selectivity and sensitivity towards resolution of UA in existence of DA.

Part-B

Poly (benzydamine) sensor for electrochemical resolution of catechol and hydroquinone

The carbon paste electrode was modified with poly (benzydamine) by cyclic voltammetric technique (CV) and its electrocatalytic activity towards the oxidation of catechol (CC) and hydroquinone (HQ) was studied. The detailed investigation on the various parameters influencing the activity was also carried out. From the obtained result, it was concluded that the process was adsorption - controlled and the involvement of same number of electron and proton. The simulations resolution of CC and HQ along with excellent selectivity and sensitivity were also exhibited by the studied MCPE. The detection limits 5.79 and 3.78 l μM were found for CC and HQ, respectively.

Chapter-6

A novel, extreme low-cost poly (Erythrosine) modified pencil graphite electrode for determination of Adrenaline

A simple, novel, and less cost yellow (Erythrosine) modified pencil graphite electrode (Po-ERY/MGPE) was successfully fabricated via electropolymerization method using cyclic voltammetric (CV) techniques. The fabricated Po-ERY/MGPE opted as a sensor for the detection Adrenaline (ADR) in 0.2M PBS (7.4 pH). This reported senor displayed excellent electrocatalytic activity, increased sensitivity, high stability, superior electron transfer kinetics in the oxidation of ADR once relative to BGPE. The significance of pH, sweep speed, and impact of concentration was assessed at the sensor. As per the pH and sweep speed study, redox routes carry the same number of electrons and protons, and electro-oxidation of ADR was adsorption controlled, respectively. The LOD of ADR was found to be 0.499 µM. The DPV data indicate that there is a significant peak divergence among ADR and Uric acid (UA) which could make it easier to determine them alone and simultaneously on the sensor. The described method has been employed for the determination of ADR in injection sample. Good recovery values indicate the efficacy and applicability of the sensor in detecting ADR.

Chapter-7

This chapter is divided into two parts such as Part-A and Part-B

Part-A

An Affordable Yellow DS5R polymeric film modified glassy carbon electrode for voltametric assay of Uric acid

This work focuses on the development of a novel and affordable yellow DS5R polymeric film on the glassy carbon electrode surface (Po-YDS5R/GCE) for the detection of Uric acid (UAC). Po-YDS5R/GCE was effectively developed using cyclic voltammetry techniques (CV). The sweep velocity study revealed that the overall electrode reaction was diffusion controlled. The static linear range of 10 - 60 µM of UAC was used to compute the LOQ and LOD, and the results yield 3.12 and 0.87µM, respectively. The interference of dopamine on the electrooxidation of UAC was tested at the sensor. It was discovered that the modified sensor allowed for the selective detection of UAC. These impressive outcomes showed that a simple and less-cost Po-YDS5R/GCE sensor has the potential to be a significant improvement over current electrochemical sensor technology.

Part-B

Electroanalytical detection of Uric acid on Blue HEGN modified glassy carbon electrode by voltammetry

An electroanalytical technique was advanced for the detection of uric acid (URI) relying on its oxidation behaviour. Using cyclic voltammetry (CV) techniques, the electrochemical performance and detection of URI were easily accomplished on poly (Blue HEGN) modified glassy carbon electrode (Po-BHEGN/GCE). The role of pH on anodic peak current and potential was examined. Phosphate buffer of pH 7.4 was selected for subsequent data analysis. Sweep rate studies were carried out and showed that electrode reaction was a diffusion-controlled process. A linear calibration curve was established in the URI concentration levels from 10- 70 µM. The LOD and LOQ were determined to be 0.94 and 3.15µM, respectively. A simultaneous study of URI and dopamine (DA) revealed that well-separated peak at Po-BHEGN/GCE compared to GCE. To sum up, a straightforward and inexpensive sensor Po-BHEGN/GCE is built for the sensitive and focused detection of URI in samples.