MICROCHIP-BASED ELECTROPHORESIS AND ELECTROCHEMICAL SENSORS FOR MULTI-RESIDUE PESTICIDE ANALYSIS

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Microchip-based Electrophoresis and Electrochemical Sensors for Multi-residue Pesticide Analysis

by

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Dedicated to My Mother, Father, Brother and Sister

and

My Friends Avtar, Gaurav, Rishabh, Devansh, Sumit, Aquib Komal and Dr. Sachidanand

"Little by little, through Patience and repeated Effort, the Mind will become stilled in the Self"

– Bhagavad Gita

CERTIFICATE

This is to certify that the thesis entitled **'Microchip-based Electrophoresis and Electrochemical sensors for Multi-residue Pesticide Analysis'** being submitted by **Mr. Rishi Raj** to the Indian Institute of Technology Delhi for the award of **Doctor of Philosophy** is a record of Bonafide research work carried out by him. **Mr. Rishi Raj** has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to our knowledge has reached the requisite standard.

The results presented in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.

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Abstract

The issue of pesticide residue pollution is now recognized as a pressing problem due to its substantial impact on both human health and environmental sustainability. As the global population grows, the demand for agriculture increases. Consequently, the use of pesticides has significantly risen, resulting in the introduction of hazardous, mobile, and bio accumulative substances into the environment. Traditional detection techniques, such as Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC), are effective but have drawbacks such as being sensitive to heat, limited capacity to detect specific pesticides with low volatility, requiring time-consuming sample preparation, and relying heavily on organic solvents. Capillary Electrophoresis (CE) has become a promising alternative because to its high sensitivity, minimum reagent consumption, and streamlined operational processes. Nevertheless, the application of CE is impeded by its poor detection sensitivity caused by the small quantities of samples.

This thesis addresses these problems by developing a portable device that combines a microfabricated chip with a pulse amperometric detecting mechanism, all based on CE technology. The development process entails the integration of a Data Acquisition (DAQ) system with LabVIEW for the purpose of collecting and controlling data, as well as the fabrication of the microchip through the use of photolithography. The effectiveness of the microchip is confirmed through electrochemical tests employing cyclic voltammetry and chronoamperometry.

The work progresses by developing a disposable chip made of Polymethyl Methacrylate (PMMA) with screen-printed carbon electrodes and laser-engraved microchannels. This portable device, created using the Arduino platform, has been verified for its ability to separate and identify pesticides, including Chlorpyrifos, Imidacloprid, and Fipronil, in soil

samples. The working electrode is enhanced with the addition of Prussian Blue to improve sensitivity and selectivity.

The results illustrate the effective segregation and measurement of pesticides utilizing the inhouse developed portable instruments, which serves as a cost-efficient and effective solution for on-site uses. The device's adaptability and portability, along with the creative production techniques, signify significant progress in the field of pesticide detection and environmental monitoring. This research establishes a strong basis for future advancements in electrophoresis technology and its utilization in protecting the environment and human well-being.

सार

कीटनाशक अवशेष प्रदूषण की समस्या अब एक गंभीर मुद्दा मानी जाती है क्योंकि इसका मानव स्वास्थ्य और पर्यावरणीय स्थिरता पर महत्वपूर्ण प्रभाव पड़ता है। वैश्विक जनसंख्या के बढ़ने के साथ कृषि की मांग भी बढ़ रही है। परिणामस्वरूप, कीटनाशकों का उपयोग तेजी से बढ़ा है, जिससे पर्यावरण में खतरनाक, मोबाइल और जैव-संचयी पदार्थों का प्रवेश हुआ है। परंपरागत पहचान तकनीकें, जैसे गैस क्रोमैटोग्राफी (GC) और हाई-परफॉर्मेंस लिक्विड क्रोमैटोग्राफी (HPLC), प्रभावी तो हैं लेकिन इनमें कुछ सीमाएँ हैं। ये तकनीकें गर्मी के प्रति संवेदनशील होती हैं, कम वाष्पशील कीटनाशकों की पहचान में सीमित होती हैं, नमूनों की तैयारी में समय लेती हैं और भारी मात्रा में कार्बनिक विलायकों पर निर्भर करती हैं। कैपिलरी इलेक्ट्रोफोरोसिस (CE) एक संभावित विकल्प के रूप में उभर रहा है, क्योंकि इसमें उच्च संवेदनशीलता, न्यूनतम रसायनों की खपत और सरल संचालन प्रक्रियाएँ होती हैं। हालांकि, CE की उपयोगिता इसकी कम पहचान संवेदनशीलता के कारण सीमित हो जाती है, क्योंकि यह छोटे सैंपल ही संभाल सकता है।

यह शोध इन समस्याओं का समाधान प्रदान करता है और एक पोर्टेबल डिवाइस विकसित करता है जो माइक्रोफैब्रिकेटेड चिप को पल्स एम्पेरोमेट्रिक डिटेक्शन मैकेनिज्म के साथ जोड़ता है, जो CE तकनीक पर आधारित है। यह विकास प्रक्रिया डेटा एक्विज़िशन (DAQ) सिस्टम को LabVIEW के साथ जोड़कर डेटा संग्रह और नियंत्रण को सक्षम बनाती है और फोटोलीथोग्राफी का उपयोग करके माइक्रोचिप का निर्माण करती है। माइक्रोचिप की प्रभावशीलता को साइक्लिक वोल्टामेट्री और क्रोनोएम्पेरोमेट्री जैसे इलेक्ट्रोकेमिकल परीक्षणों के माध्यम से प्रमाणित किया गया है।

अगले चरण में, पॉलीमेथाइल मेथाक्रायलेट (PMMA) से बनी एक डिस्पोजेबल चिप विकसित की गई, जिसमें स्क्रीन-प्रिंटेड कार्बन इलेक्ट्रोड और लेजर-नक़्क़ाशी वाले माइक्रोचैनल शामिल हैं। इस पोर्टेबल डिवाइस को Arduino प्लेटफॉर्म का उपयोग करके बनाया गया है और इसे मिट्टी के सैंपल

में क्लोरपायरीफोस, इमिडाक्लोप्रिड और फिप्रोनिल जैसे कीटनाशकों को अलग और पहचानने में सक्षम साबित किया गया है। वर्किंग इलेक्ट्रोड की संवेदनशीलता और चयनात्मकता को बढ़ाने के लिए इसमें प्रशियन ब्लू जोड़ा गया है।

परिणामों से पता चलता है कि विकसित पोर्टेबल उपकरण का उपयोग करके कीटनाशकों को प्रभावी ढंग से अलग और मापा जा सकता है, जो ऑन-साइट उपयोग के लिए एक किफायती और प्रभावी समाधान प्रदान करता है। इस उपकरण की पोर्टेबिलिटी और बहुमुखी प्रतिभा, साथ ही इसके अभिनव निर्माण तकनीकें, कीटनाशक पहचान और पर्यावरण निगरानी के क्षेत्र में एक महत्वपूर्ण प्रगति को दर्शाती हैं। यह शोध इलेक्ट्रोफोरोसिस प्रौद्योगिकी और पर्यावरण व मानव स्वास्थ्य की सुरक्षा में इसके उपयोग के लिए एक मजबूत आधार प्रदान करता है।

Contents

List of Figures

List of Table

List of Abbreviations

Abbreviations	Full form
Au	Gold
CE	Capillary electrophoresis
CE-Ad	Capillary electrophoresis amperometric detection
CE-pAD	Capillary electrophoresis Pulse amperometric detection
CNT	Carbon Nanotube
Cr	Chromium
CV	Cyclic voltammetry
DPV	Differential Pulse voltammetry
DIW	Deionized water
HPLC	High performance liquid Chromatography
GC	Gas Chromatography
GCMS	Gas chromatography mass Spectrometer
GO	Graphitic oxide
IPA	Iso Propyl alcohol
LCMS	Liquid chromatography mass spectroscopy
LOC	Lab on Chip
LOD	Limit of detection
OPPs	Organophosphates
µTAS	Micro total analysis
PB	Prussian blue
PBS	Phosphate buffer saline
PCR	Polymerase Chain Reaction
PDMS	Polydimethylsiloxane
PMMA	Polymethyl methacrylate
PEO	Polyethylene oxide
POC	Point of care
rGO	Reduced graphitic oxide
RSD	Relative standard deviation
SDS	Sodium Dodecyl Sulphate
SPCE	Screen printed carbon electrode
SPT	Screen printing technique