

Harnessing Dean Flow Effects to Enhance the Performance of a Microfluidic Electrochemical Sensor for Lead Detection

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ABSTRACT

Laboratory-based water quality analysis techniques are well established and effective but they have important limitations, namely complexity, high cost, and the need for highly trained technicians to run the equipment. Additionally, standard laboratory methods cannot be deployed in remote sensing applications. Microfluidic sensors are smaller, less expensive, and potentially easier to deploy, but commercially available microfluidic water quality sensors continue to have shortcomings. The complexity of fabrication processes, which frequently involve multiple intricate steps and specialized equipment, impedes efforts toward miniaturization, integration, and scalability. Compounding this complexity, the reliance on expensive resources such as photolithography, deposition systems, and cleanroom facilities during sensor fabrication, increases the overall cost of sensor development. Furthermore, certain sensors' lack of fluid handling and continuous detection capabilities limits their practical utility in real-time monitoring applications. The main goal of this research project was to contribute to the advancement and optimization of microfluidic electrochemical sensors for water quality analysis by simplifying the fabrication process and harnessing secondary mixing effects to improve the output signal of the device.

To meet the goals, three objectives were designed: (1) the complete design, construction, and characterization of microfluidic electrochemical sensors using inexpensive materials and a laser-cutting technique; (2) the examination of the signal obtained from straight-channel and curved-channel sensors using square wave voltammetry measurement with potassium ferricyanide sample solution; and (3) extending the results to experimental validation of microfluidic electrochemical sensors for the detection of lead, an important drinking water contaminant, using square wave anodic stripping voltammetry in deionized water and treated water samples from a water treatment plant in Southern Ontario.

Microfluidic electrochemical sensors were successfully designed, constructed, and characterized using cost-effective polyethylene terephthalate and pressure-sensitive adhesive. Precise sensor layer fabrication was achieved using a laser-cutting technique.

Graphene-carbon paste was chosen for electrode fabrication due to its advantageous properties.

The effects of flow rate and microfluidic channel curvature on sensor performance were examined through experimental research backed by thorough statistical analysis. The results highlighted the key role curved channels play in enhancing sensor response. Analyte migration toward the electrode surface was facilitated by the Dean vorticity formation within the curved channels due to convective transport. The main outcome was improving sensor performance by employing the convective-diffusive mass transfer around the electrodes caused by the secondary flows.

Square wave voltammetry experiments presented curved-channel sensors' superior lead detection performance over their straight-channel counterparts. Then, using deionized and real water samples, calibration curves built for different lead concentrations confirmed the sensors' accuracy and reliability over a wide concentration range.

Furthermore, the developed sensor was tested employing square wave anodic stripping voltammetry. The LOD and sensitivity of the straight-channel sensor was 180 $\mu\text{g/L}$ and 0.105 A/M (0.0005 $\mu\text{A}/(\mu\text{g/L})$), respectively, while the curved-channel sensor achieved a detection limit of 2.26 $\mu\text{g/L}$ and a sensitivity of 0.0332 $\mu\text{A}/(\mu\text{g/L})$ for lead detection. The fabricated microfluidic electrochemical sensor offers a low-cost, easy-to-fabricate, and modifier-free option for heavy metal detection, particularly lead, in drinking water.

DEDICATION

To my beloved wife, Maryam,

Your love and unwavering support have guided me through the late nights and early mornings, keeping me grounded and sane. You are my partner and best friend, and I am endlessly grateful for your presence in my life.

To my dear children, Melia and Ania

I regret every moment I've missed with you, but your resilience and love remind me of what truly matters. Every sacrifice I make is for your happiness and future.

To my parents,

Your unwavering support, guidance, and love have shaped me into who I am today. I owe you everything.

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CHAPTER 1.

INTRODUCTION

1.1. Introduction to Microfluidic Sensors

Microfluidics involves controlling and manipulating small volumes of fluids (typically in the microliter or nanoliter range) within microscale channels or devices. Microfluidic systems offer precise fluid handling, reduced reagent consumption, faster reaction times, and the ability to integrate multiple functions into a compact platform. This miniaturization is especially helpful for point-of-care or on-site testing applications where mobility is essential. Compared to traditional techniques, microfluidic sensors can provide better sample containment and a lower risk of contamination. Microfluidic devices reduce the chance of sample loss or cross-contamination by precisely controlling fluid flow and reaction chambers, guaranteeing the accuracy of analytical results. Improvement in electrochemical detection can result from the control of fluid flow and reaction conditions in microfluidic systems. Microfluidic sensors can improve analytical performance by optimizing mass transfer and reaction kinetics, even for difficult analytes or complex sample matrices.

1.1.1. Fluid Flow and Mixing in Microfluidic Sensors

Microfluidic systems are characterized by narrow channels and low fluid velocities, which results in laminar flow (low Reynolds number, Eq. 1-1).

$$Re = \frac{\rho U D}{\mu} \quad (1-1)$$

where ρ is the fluid density, μ shows the fluid dynamic viscosity, and U represents the fluid velocity either in the axial or lateral direction.

Laminar flow can restrict mass transport orthogonal to the direction of the flow. This can become a limitation in applications involving the detection of analytes where effective mixing and analyte transfer towards sensing electrodes are necessary for sensitive and precise readings.

One possible way to address the gap in fluid control and fluid-electrode interactions is to replace the straight microchannel in the fluidic portion of the electrochemical sensor with a curved microchannel to induce greater mixing.

Dean vortices are a secondary flow type in curved microchannels (see Fig 1.1). These vortices are usually in the form of two or multiple lateral counter-rotating vortices^{1,2}. The fluid elements close to the channel center experience a higher centripetal force and travel to the outer side of the curve. The stagnant elements near the walls travel inwards, resulting in two symmetric vortices³. These conditions are known as Dean instability.

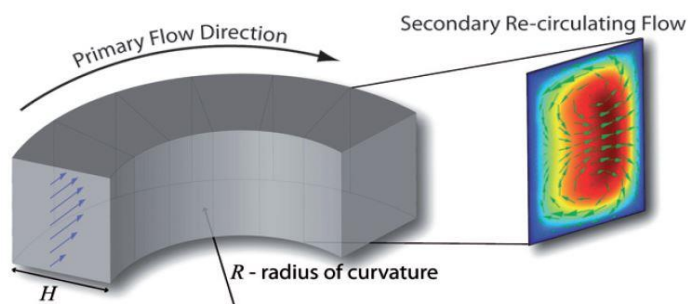


Fig. 1.1 Schematic showing the Dean flow in curvilinear microchannels³. Left: Primary flow (main direction) and right: secondary re-circulating flow (vortices induced by curvature). Adapted with permission from RSC.

The pressure and velocity gradient imbalance caused by Dean instability results in vortices/secondary flow defined by a non-dimensional number called the Dean number. In fluid mechanics, the dimensionless Dean number provides insights into the complex dynamics of curved flow systems. It represents the balance between two fundamental forces: centrifugal forces, which originate from the flow path's curvature, and inertia forces, which come from the fluid's resistance to changes in velocity. The Dean number compares which of these forces is more prevalent in a particular flow scenario. Lower Dean numbers, for example, indicate more inertia and, thus, more stable flow regimes; higher values, on the other hand, indicate centrifugal forces have a greater

effect and may result in instability in the fluid flow. Dean number is often used to specify the strength of secondary flows and can be defined as follows:

$$De = Re \sqrt{\frac{D}{2R}} \quad (1-2)$$

where R represents the microchannel's radius of curvature, D is the channel hydraulic diameter (which is defined as four times the cross-sectional area of flow divided by the wetted perimeter), and Re denotes the Reynolds number in Eq. 1-1.

1.2. Introduction to Water Quality Analysis with Electrochemical and Microfluidic Electrochemical Sensors

1.2.1. Electrochemical Detection Techniques

An electrochemical sensor is a tool that measures and finds the concentration of analytes in a sample by applying the concepts of electrochemistry. Electrochemical methods offer a more practical and cost-effective solution for routine monitoring than conventional analytical techniques because they are straightforward, accessible, and easy to use. Electrochemical methods typically require simpler instrumentation, often consisting of devices that can be easily operated. This accessibility reduces the reliance on specialized personnel and expensive laboratory equipment, consequently lowering the overall analysis cost.

Typically, an electrochemical sensor consists of a counter electrode (CE), reference electrode (RE), and working electrode (WE). An electric potential is applied between electrodes, and the resulting current or potential is measured. Different electrochemical sensors exist, such as potentiometric, amperometric, and impedimetric sensors.

Potentiometry: Potentiometry measures the potential difference between two electrodes, typically a reference electrode and a working electrode. It's commonly used for pH measurements and other ion concentration determinations. Potentiometric sensors have been extensively used as practical sensors since the early 1930s. They can be classified into three categories: ion-selective electrodes (ISEs), coated-wire electrodes (CWEs), and field-effect transistors (FETs). These sensors are

widely used to analyze organic ions and anionic or cationic species in various industries, such as the oil industry, drug manufacturing, and environmental monitoring ⁴.

Cyclic Voltammetry (CV): In cyclic voltammetry, the potential difference between two electrodes is swept linearly with time, and the resulting current is measured. This technique provides information about reduction-oxidation (redox) reactions (a type of chemical reaction where electrons are transferred between reactants) and the electrochemical properties of materials.

Square Wave Voltammetry (SWV): A square wave potential waveform is applied to the working electrode. This waveform consists of a series of potential steps, typically a forward step followed by a reverse step, superimposed on a staircase potential. During each potential step, the current response is measured. The resulting current-voltage curve provides information about the electrochemical behavior of the analyte, including its concentration and redox properties. SWV offers advantages such as high sensitivity, low background current, and the ability to discriminate against capacitive currents, making it a popular method for trace analysis and characterization of electroactive species.

Differential Pulse Voltammetry (DPV): A potential waveform that consists of a series of potential steps is applied to the working electrode. However, unlike in traditional voltammetry techniques, where the potential is continuously scanned, in DPV, each potential step is preceded by a short period of relaxation time during which no current measurement is made. This relaxation time allows the system to return to equilibrium before applying the potential step, resulting in a differential current measurement. The difference in current between the beginning and end of each potential step is measured, hence the term “differential.” This technique enhances the signal-to-noise ratio and improves sensitivity and resolution in analyzing electroactive species, making it particularly useful for trace analysis and detecting redox-active compounds.

Chronoamperometry: This method involves applying a constant potential to an electrochemical cell and measuring the resulting current as a function of time. It's often used to study electrode kinetics and diffusion-controlled processes.

Chronopotentiometry: Here, a constant current is applied to the electrochemical cell, and the resulting potential is measured as a function of time. This technique helps study electrode processes and determine reaction mechanisms.

Electrochemical Quartz Crystal Microbalance (EQCM): This technique combines the principles of electrochemistry and mass measurement by monitoring the frequency change of a quartz crystal resonator due to mass changes occurring at the electrode surface. It's used to study processes such as electrodeposition, adsorption, and corrosion.

Electrochemical Impedance Spectroscopy (EIS): EIS is a technique for investigating the electrical properties of electrochemical systems over a range of frequencies. It provides information about charge transfer resistance, double-layer capacitance, and mass transport phenomena.

Anodic Stripping Voltammetry (ASV): In ASV the analyte ions are first deposited onto the working electrode surface at a specific applied potential. This deposition step allows for the preconcentration of the analyte ions onto the electrode surface. After the deposition step, the potential at the working electrode is scanned in the positive direction. During this scan, the deposited analyte ions result in the generation of a current peak. The magnitude of this current peak is proportional to the concentration of the analyte ions in the solution.

1.2.2. Microfluidic Electrochemical Sensors

Microfluidic-based electrochemical sensors (MESs) integrate microfluidic channels with electrodes, creating a platform where the sample is transported and manipulated in controlled microenvironments. The electrodes serve as the sensing elements, detecting the target analyte through electrochemical reactions.

Conventional electrochemical sensors, while effective, often face limitations in size, response time, and sample volume requirements. In contrast, modern electrochemical sensors, such as MES, offer compactness, rapid response times, and the ability to handle small sample volumes. Their capability to integrate with miniaturized electronics makes them ideal for portable applications in

healthcare, environmental monitoring, and beyond. As research increasingly focuses on these advantages, MES have emerged as promising solutions to overcome the shortcomings of conventional counterparts, driving innovation in sensing technology toward greater efficiency, affordability, and accessibility. Their lightweight and compact design makes it simple to transport them to point-of-care settings, remote locations, or places without laboratory infrastructure, allowing for on-the-spot analysis without requiring the transportation of samples. This on-site capability is beneficial when prompt decisions are necessary. Compared to laboratory instruments, portable devices may be less expensive initially and over time. Still, they are superior at quick screening and qualitative analysis, giving quick feedback and facilitating the early detection of contaminants.

1.3. Introduction to Lead as a Toxic Heavy Metal in the Environment

Lead (chemical symbol Pb) is a heavy metal that is found naturally in the crust of the Earth. Its malleability, low melting point, and other physical characteristics have led to its widespread use by humans for various purposes, such as batteries, paints, pipes, and welding materials, among others⁵. Lead is highly poisonous, and exposure to it can have detrimental consequences on one's health. Lead can impact the neurological system, kidneys, and blood circulation, particularly in children, infants, and fetuses. It is distributed in the brain, liver, kidneys, and bones, potentially accumulating in teeth and bones, reflecting cumulative human exposure. Additionally, lead may adversely affect brain and intellectual development in children, triggering apoptosis in organ tissues and, in some instances, causing irreversible neurological damage. In 2016, lead was implicated in approximately 540,000 deaths globally. Lead exposure is estimated to contribute to 0.6% of the global burden of disease, measured in disability-adjusted life years (DALY), with the highest burden observed in developing regions ⁶.

Lead has several natural and anthropogenic sources. Lead is naturally present in the Earth's crust. It can be found in various minerals, including galena (lead sulfide) and cerussite (lead carbonate). Lead can be present in soil due to the weathering of rocks containing lead minerals⁷. Water can become contaminated with lead due to several factors, such as the extraction and smelting of lead-

containing ores, lead emissions from industrial processes like metal processing and battery production, and the combustion of fossil fuels, especially coal, which can release lead into the atmosphere^{8,9}. The contamination of drinking water by lead is primarily caused by corrosion of water distribution infrastructure and domestic plumbing components that contain lead. Lead can also be present in consumer goods like cosmetics and glazes for ceramics. Old plumbing fixtures and equipment continue to be contamination sources despite regulations¹⁰. The maximum acceptable concentration (MAC) for lead in drinking water established by Health Canada is 5 µg/L¹¹. Even at this level, lead exposure has been demonstrated to adversely affect children, leading to compromised development and the emergence of learning and behavioral issues¹².

Developing accurate lead detection techniques is the primary goal of our research because of the dangers that lead contamination in drinking water poses, especially to children and other susceptible populations. Considering Health Canada's strict regulations and the increasing public consciousness regarding non-specific lead sources, our goal is to protect public health by guaranteeing the safety of drinking water via novel, efficient, and low-cost detection methods.

1.4. Lead Detection Methods

1.4.1. Conventional Lead Detection Methods

Various methods can be employed to detect and quantify lead and other metals in water. Some traditional methods include Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Atomic Absorption Spectrometry (AAS), atomic fluorescence spectroscopy (AFS), Neutron Activation Analysis (NAA), X-ray fluorescence (XRF), and various colorimetric methods¹³⁻¹⁷. All of these instruments are expensive to purchase, operate, and maintain but offer high sensitivity and accuracy. A subset of these analytical instruments are described in the following paragraphs.

ICP-MS is often considered the best choice due to its exceptional sensitivity, low detection limits, and high selectivity. It can measure most elements in the periodic table with detection limits at or below the parts per trillion (ppt) range. This makes it ideal for detecting trace amounts of lead in drinking water, far below the Canadian government's regulation of 5 ppb for lead in drinking

water¹¹. The ICP-MS instrument uses plasma to ionize the elements in a sample and then measures the ions using a mass spectrometer.

AAS is a technique that measures the concentration of elements in a sample by absorbing light at specific wavelengths. It is commonly used to detect Pb and other heavy metals in various samples. In flame AAS, the sample is aspirated into a flame where the atoms are vaporized and absorb light from a hollow cathode lamp specific to the element of interest^{18,19}.

XRF is an analytical technique that uses X-rays to excite atoms in a sample, causing them to emit characteristic X-rays that can be detected and analyzed to determine the elemental composition. XRF is used to analyze Pb and other heavy metals in environmental, geological, and industrial samples²⁰.

NAA is a highly sensitive analytical technique that involves irradiating a sample with neutrons, which induces radioactivity in the sample. The radioactive decay of the induced isotopes is then measured, allowing the identification and quantification of the elements²¹.

All of these conventional laboratory analytical methods suffer from complexity and the need for specialized training and expertise. Additionally, the need to collect samples and transport them to the lab can result in long delays between sample collection and analysis.

1.4.2. Electrochemical Pb Sensors

The number of articles published on electrochemical methods for Pb²⁺ detection has significantly increased in the last few years compared to more conventional analytical techniques like spectroscopy, colorimetry, and fluorescence²².

Zhou et al compared electrochemical sensing methods for detecting Pb²². Cyclic voltammetry, square wave anodic stripping voltammetry (SWASV), differential pulse voltammetry, anodic stripping voltammetry (DPASV), and EIS are more widely recognized. Analyzing their comparison table, we found that SWASV and DPASV techniques have been used more frequently for Pb detection than other methods. Additionally, we noticed that the SWASV technique offers a

lower limit of detection (LOD) than the DPASV technique. We will compare the LOD for various methods in the upcoming sections and Chapter 4.

Modern electrochemical Pb sensors, like Screen Printed Electrodes (SPEs), are essential instruments for identifying and measuring lead ions in various samples because of quick reaction times, portability, affordability, and simplicity of use. With the help of these sensors, environmental sample lead contamination can be promptly monitored, allowing for prompt exposure risk mitigation. In the following subsections, we introduce some of these sensors based on material and the techniques used to make these sensors. We'll explore the uses of microfluidic electrochemical sensors and their application in heavy metal detection detection in Section 1.5.

1.4.3. Screen-Printed Electrodes

SPEs are electrode systems manufactured using a screen-printing process. Due to their adaptability for mass production, these electrodes are widely used in various electrochemical applications.

In contrast, conventional solid electrodes, like glassy carbon (GC), necessitate meticulous polishing and cleaning before each measurement and rely on external reference and counter electrodes. SPEs have become one of the most widely utilized platforms for in situ electrochemical methods with their disposable, cost-effective, reproducible, and easily customizable nature.²³ This versatility extends to diverse sensing applications, including gases, food quality, biomolecules, and drugs of abuse, among others^{24,25}. Significantly, SPEs have been instrumental in the expansive industry of biological glucose sensing, acting as crucial components in point-of-care devices. Their disposable characteristic facilitates cost-effective daily usage, providing individuals with diabetes the ability to track their blood glucose levels precisely without the necessity for frequent trips to clinics, hospitals, or pharmacies^{26,27}. The SPE surface can be modified for detecting the Pb in drinking water²⁸.

1.4.4. Rotating Disk Electrochemical Sensor for Pb Detection

The development of a rotary disc voltammetric sensor system for semi-continuous and on-site measurements of Pb aims to introduce a novel analytical method using a rotary disc voltammetric (RDV) sensor for the semi-continuous and on-site measurements of trace levels of the lead²⁹. Figure 1.2 shows the concept through a schematic illustration. The analysis includes a deposition step to concentrate the target metal ions onto the electrode surface by reducing them to atoms, followed by a stripping step to convert the metal to its ionic form. SWASV is efficiently employed to measure heavy metals in the sub-nanomolar range. In addition, the RDV sensor showed a correlation coefficient of 0.998 for the Pb(II) concentration range of 10 nM– 10 μ M at the deposition time of 180 s, and its low detection limit was 6.19 nM (1.3 μ g/L).

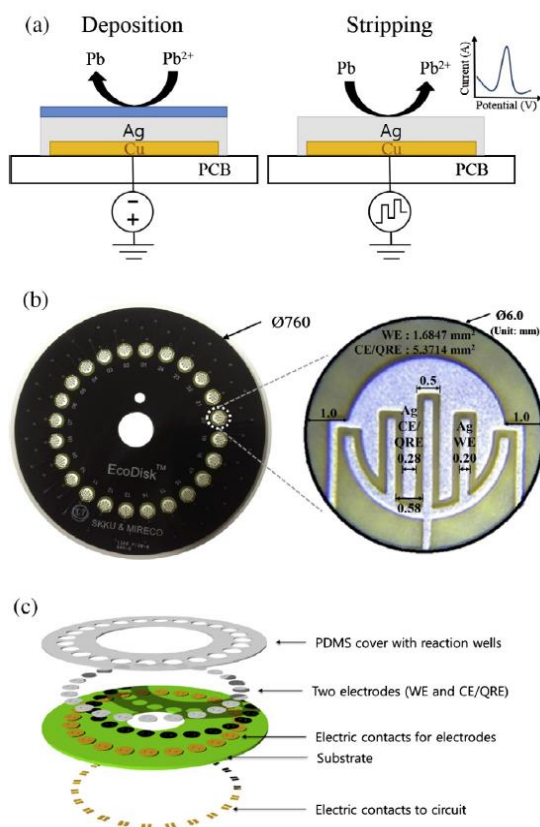


Fig. 1.2 Rotary disc voltammetric sensor (a) a schematic diagram illustrating the SWASV of Pb(II) on the RDV sensor, (b) a scheme describing the RDV sensor, and (c) the fabrication process of the RDV sensor utilizing multilayer lamination²⁹. Adapted with permission from Elsevier.

Based on the review paper by Zhou et al., most of the research on electrochemical sensing of lead ion has indeed focused on modifying the electrode surface to improve the performance²². The review highlights that chemically modified electrodes are an effective approach to enhancing the electrochemical properties of bare electrodes for Pb detection. According to this review paper, various surface modifications have been explored, including Polymers like polypyrrole and polyaniline that can be electropolymerized onto electrodes to improve conductivity and reduce impedance. Combining them with nanomaterials can further enhance performance. They mentioned that metal-organic frameworks (MOFs) have high surface area and porosity, though their poor conductivity can be addressed by compositing them with conductive materials like carbon nanotubes. Nonmetallic oxides, such as mesoporous silica and reduced graphene oxide, exhibit good catalytic and adsorption properties for Pb, which can be improved by combining with other functional materials. They also reported that Organic groups like amide, amine, and hydroxyl can enhance the adsorption and chelation of Pb on the electrode surface. Moreover, Carbon nanomaterials in various dimensions can modify electrodes due to their unique electronic properties and high surface area. Biological materials like DNA-based probes can specifically interact with Pb.

There are some limitations to using the SPES and rotating disk electrodes for Pb detection. For example, SPEs are not suitable for injecting samples and handling fluids during electrochemical measurement. The construction of electrodes and cells in rotating disk electrodes is complicated, and the theoretical treatment requires the determination of the solution flow velocity profiles as functions of rotation rate, viscosities, and densities, which is not easy. Regarding the detection of lead in drinking water, there seems to be a lack of research on sensor fabrication methods that use bare electrodes instead of surface-modified electrodes, which use chemicals and make the fabrication process complex. Given the above-mentioned techniques' limitations and complexity, researchers focus on microfluidic electrochemical sensors.

1.4.5. Microfluidic Electrochemical Sensors for Pb Detection

MESs are analytical devices that integrate microfluidics and electrochemical sensing techniques for detecting and analyzing chemical or biological substances. These sensors combine the

advantages of microfluidics, which deals with manipulating small fluid volumes in microscale channels, and electrochemical sensing, which relies on measuring electrical signals generated during chemical reactions.

3D printed Microfluidic Electrochemical Sensors for Lead Detection

Numerous studies have reported integrating microfluidic systems with electrochemical setups to develop sensors for detecting heavy metal ions. For instance, Hong et al. developed a 3D-printed microfluidic device for cadmium and lead determination in water using a microporous SPE modified with Mn_2O_3 (Fig. 1.3) ³⁰. They claimed the willow-type velocity profile induced by sample injection along the tangential direction has the longest flow path in the microfluidic cell, which facilitates the deposition of more reactants on the electrode surface. Figure 1.3a shows the configuration of the 3D-printed microfluidic device, measuring 34 mm in length, 24 mm in width, and 8 mm in height. Fig. 1.3b highlights the microfluidic device's transparent and smooth surface, facilitating easy observation of internal processes. Subsequently, the SPE (36×14 mm) was integrated into the device. All three electrodes, including Ag (CE), Ag/AgCl (RE), and working electrodes, were placed within the cavity. Fig. 1.3c showcases the screen-printed electrode made from a flexible material, emphasizing its ease of bending. This characteristic expands its potential applications, particularly in flexible electronics. The Navier–Stokes equations and the convection-diffusion equation were simulated using COMSOL Multiphysics, with the computational domain and boundary conditions detailed in Fig. 1.3d and Fig. 1.3e. The limits of detection in this work were estimated to be $0.5\mu\text{g/L}$ for Cd(II) and $0.2\mu\text{g/L}$ for Pb(II).

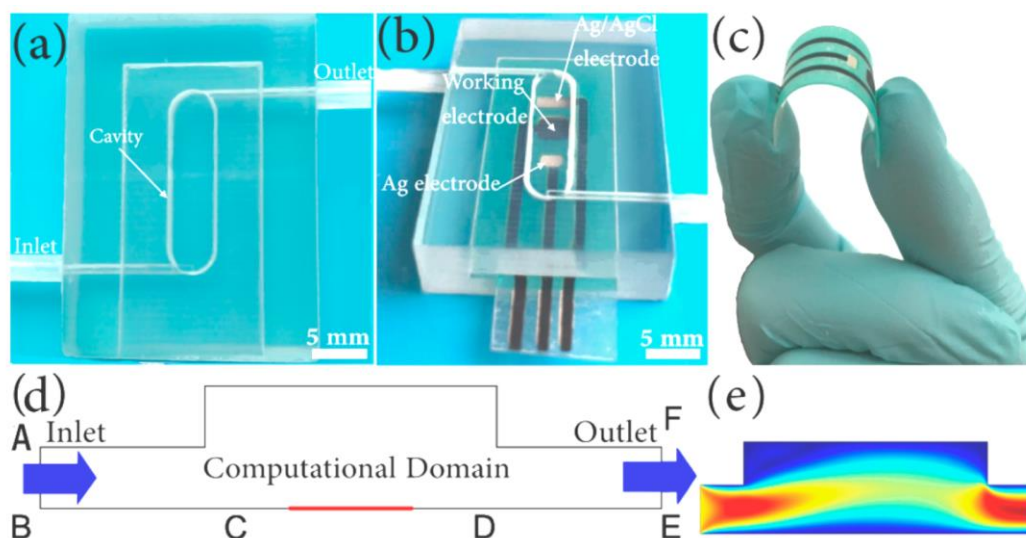


Fig. 1.3 3-D printed microfluidic electrochemical sensor; (a) and (b) optical images of the devices with and without the SPE. A photograph in (c) illustrates the flexibility of the SPE, showing its easy bending capability. In (d), the computational domain of the microfluidic cell with the working electrode is depicted. (e) displays the velocity profile of the microfluidic cell from a side view³⁰. Used with permission from ACS.

Zhao et al.³¹ developed a homemade electrochemical cell integrated with a 3D-printed flow cell for the multiplexed detection of As(III), Cd(II), and Pb(II) ions. The electrochemical detection was achieved using the square wave ASV technique. The study optimized various experimental parameters for the stripping analysis of heavy metal ions (HMIs) and reported the developed sensor platform's high selectivity, accuracy, and potential applicability, even in complex matrices such as simulated river water. The paper also discusses screen-printed electrodes, 3D printing, and nanocomposite modification to enhance the sensing of HMIs. The research provides a promising method for rapidly and effectively detecting heavy metals in water.

The study's findings have implications for developing on-site and near-real-time monitoring systems for heavy metal ion detection in water. A peristaltic pump was employed to transport samples, combined with acetate buffer (pH 5.0, 0.2 M), through the tube and flow cell at a flow rate of 120 $\mu\text{L/s}$. The electrochemical workstation connected to the SPE facilitated the deposition and stripping steps. Calibration plots were established using injected standards composed of acetate buffer (0.2 M, pH 5.0) with varying concentrations of Cd(II), Pb(II), and As (III). The

SWASV detection occurred once the WEs baseline signal in the fluid channel had stabilized. They reported that the flow injection analysis procedure aligned with the SWASV detection process, involving three steps. In the initial step, the peristaltic pump delivered the water sample to the flow cell via the channel, applying a deposition potential of -1.2 V for 200 s on Cd(II) and Pb(II) WEs and -0.9 V for 250 s, on As (III) WE (where metal ions were electrochemically reduced to metal). During the second step, pumping was manually paused for 10 s, and potential scanning (frequency: 25 Hz; increment potential: 5 mV; amplitude: 25 mV) occurred on WEs in a quiescent sample solution within a range of -0.55 to 0.05 V for As (III) and -1.25 to -0.65 V for Pb(II) and Cd(II). In the final step, the surfaces of WEs were activated/regenerated by applying a potential of -0.4 V for Cd(II) and Pb(II) and +0.6 V for As (III) to eliminate residual metals. This prepared the electrodes for a new cycle of stripping voltammetry analysis. The limits of detection were estimated to be 2.4 µg/L for As(III), 1.2 µg/L for Pb(II) and 0.8 µg/L for Cd(II).

PDMS-Based Microfluidic Electrochemical Sensors for Lead Detection

The integration of the screen-printed electrode within the polydimethylsiloxane (PDMS) fluidic chip was successfully achieved in the paper by Nguyen et al. (Fig. 1.4) ³². The electrochemical methods for detecting trace metal ions focus on using polyaniline (PANi) doped with Sodium Dodecyl Sulfate (SDS) for mercury detection. The PANi-SDS-modified electrode was integrated into a poly(dimethylsiloxane) microfluidic chip as an electrochemical sensor for mercury detection. The SPEs were fabricated using thick-film technology with a printing machine. The working and counter carbon-based screen-printed electrodes were prepared from carbon ink, and the Ag/AgCl-based screen-printed pseudo-reference electrode was printed from silver/silver chloride ink. The working surface of the electrodes was polished and activated in sulfuric acid before modification. A PDMS microfluidic chip was fabricated using a replica molding process. The chip included a channel cap and a bottom substrate, with the SPEs fixed on the bottom substrate. The PDMS channel cap was bonded to the bottom substrate using a reversible bonding method. The fabrication process involved specific steps to ensure the bonding and functionality of the microfluidic chip. The mercury detection limit was 2.4 nM in their experiments.

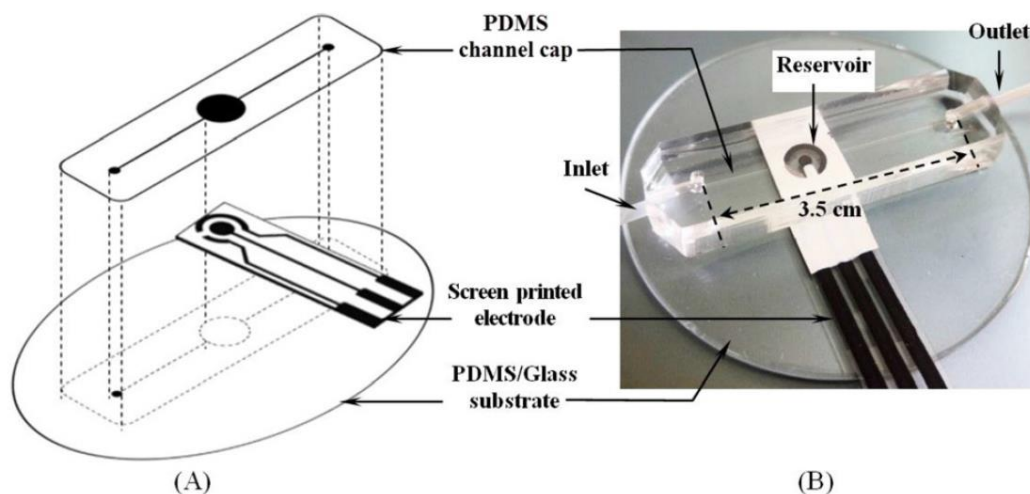


Fig. 1.4 (A) The fabrication of the microfluidic chip. (B) The PDMS microfluidic chip system with electrochemical components³². Used with permission from Wiley.

Chen et al. reported a two-step photolithography fabrication method (See Fig. 1.5) for creating an on-chip integrated two-metal electrode system comprising a silver reference electrode and two gold electrodes serving as working and counter electrodes ³³. Integrated with a microfluidic channel, this detector offers advantages such as ease of use, low analyte consumption, and rapid sensing, which are particularly suitable for the in situ determination of target metal ions. The three-electrode system was utilized for measuring Hg^{2+} ions, with both gold working and silver reference electrodes characterized using electrochemical techniques. Results demonstrate high sensitivity and reproducibility in Hg^{2+} determination, with a detection range from 10 to 1000 ppb and a low detection limit of 3 ppb. The device, realized through microfabrication techniques, offers a disposable, cost-effective solution with minimal reactant consumption, making it conducive to in situ sample analysis. Its compact size and ease of integration suggest promising applications in environmental monitoring, particularly for real-time or online analysis of mercuric pollutants.

In the Han et al.³⁴ study, a versatile microfluidic device featuring gold nanostructures fabricated within microchannels via electrochemical deposition was developed for on-chip electrochemical analysis. The developed microfluidic devices comprised three electrodes fabricated with single-walled carbon nanotubes (SWCNTs), gold nanoparticles, and a microfluidic channel. These nanostructures served as electrocatalytic materials for glucose detection and as suitable sensing

surfaces for ultratrace arsenic (III) detection. The continuous streaming solution in the microfluidic devices enhanced mass transport to the electrodes, eliminating fouling effects and resulting in increased currents and high sensitivities. The device exhibited strong linear dependence ($R^2 = 0.995$) over a concentration range of 0.1–9 mM with a sensitivity of $1.10 \pm 0.07 \text{ mA cm}^{-2} \text{ mM}^{-1}$ for glucose detection. Similarly, ultra-trace arsenic (III) analysis showed good linearity ($R^2 = 0.998$) up to 30 ppb concentration with a remarkable sensitivity of $4.49 \pm 1.01 \text{ mA cm}^{-2} \text{ ppb}^{-1}$ in just 60 seconds of accumulation time.

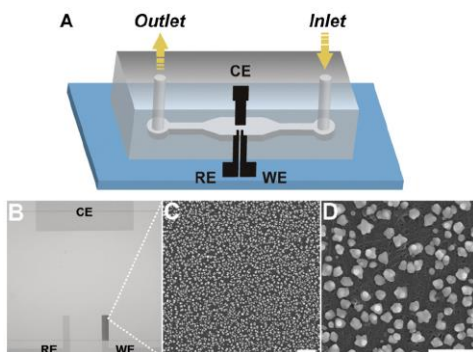


Fig. 1.5 MES for HM detection; A) Illustration depicting a SWCNT-based microfluidic device, with annotations indicating the working electrode (WE), reference electrode (RE), and counter electrode (CE). B) Optical microscope image displaying the device post- electrochemical deposition of gold particles in a solution containing 0.05 M H₂SO₄ and one mM HAuCl₄ at -0.8 V for 10 seconds, with a flow rate of $0.5 \mu\text{L min}^{-1}$. C) and D) Scanning electron microscope (SEM) images showing the dark region on the working electrode. The scale bars in (C) and (D) represent one μm and 500 nm, respectively³⁴. Used with permission from Elsevier.

An article by Zhou et al.³⁵ presents a method for fabricating a compact and dependable Ag/AgCl reference electrode integrated into microfluidic chips, featuring nanofluidic channels serving as a salt bridge. Demonstrating remarkable stability with minimal millivolt variations, this reference electrode is seamlessly incorporated into a portable electrochemical chip, allowing direct connection to a USB-port microelectrochemical station and computer for data analysis. The chip's low production cost facilitates its disposable nature, rendering it suitable for real-world analysis, exemplified by the quantitative analysis of heavy metal ions (Cd^{2+} and Pb^{2+}) in seawater. This microelectrochemical chip offers several advantages, including stable performance, cost-effectiveness, scalability for mass production, and the ability to accommodate multiple

independent electrochemical cells. Its use of laminar flow in microchannels enhances reproducibility and reliability, making it particularly appealing for microfluidics, environmental science, clinical diagnostics, and food research applications.

Thermoplastic-based Microfluidic Electrochemical Sensors for Lead Detection

Zou et al. proposed a solid-state disposable sensor that provides a rapid and effective detection for in situ ASV measurement of various heavy metal ions in non-deaerated solutions without generating toxic waste ³⁶. The paper presents a disposable heavy metal ion sensor with a microfabricated on-chip bismuth electrode for in situ measurements. The sensor chip is environmentally friendly and consists of a bismuth working electrode, an integrated reference electrode, a gold counter electrode, and microfluidic channels. The sensor was successfully tested for detecting and quantifying lead and cadmium ions. The applications of the sensor chip were also demonstrated with in situ measurements of Cd (II) concentration in soil and groundwater samples. This paper proposes an affordable heavy metal sensor featuring an on-chip planar Bi working electrode, an integrated Ag/AgCl reference electrode, and microfluidic channels (refer to Fig. 1.6a). This development utilizes standard biomedical microelectromechanical systems (BioMEMS) fabrication technology. The intended characteristics of the sensor include low cost, suitability for mass production, minimal analyte consumption and waste generation, rapid sensing times, and user-friendly operation. Conventional microfabrication processes were employed to produce the compact heavy metal sensor, as illustrated in Fig. 1.6b. Regarding the fabrication process utilizing an e-beam metal evaporator, a 100nm gold layer and a 100nm bismuth (Bi) layer were deposited onto a 3-inch blank cyclic olefin copolymer (COC) substrate. Subsequently, a 2 μ m thick S1818 photoresist was spin-coated and patterned on the COC substrate using photolithography. The designed structures for the electrodes underwent etching via Bi and gold etchant. An Ag/AgCl ($\sim$ 1 μ m) layer was electroplated onto the gold seed layer to establish the reference electrode. Integrating the polymer microfluidic chip reduces the proposed sensor's analyte consumption, waste generation, and sensing time. They developed plastic injection molding and UV adhesive bonding techniques for high-throughput polymer biochip fabrication, providing a summarized overview in Fig. 1.6b. The detection and quantification of Pb (II) and Cd (II) were statically performed using anodic stripping voltammetry inside the microchannels, in the

Pb (II), the limit of detection was 8 ppb for 60 s deposition, and in the Cd (II), the limit of detection was 9.3 ppb for 90 s deposition.

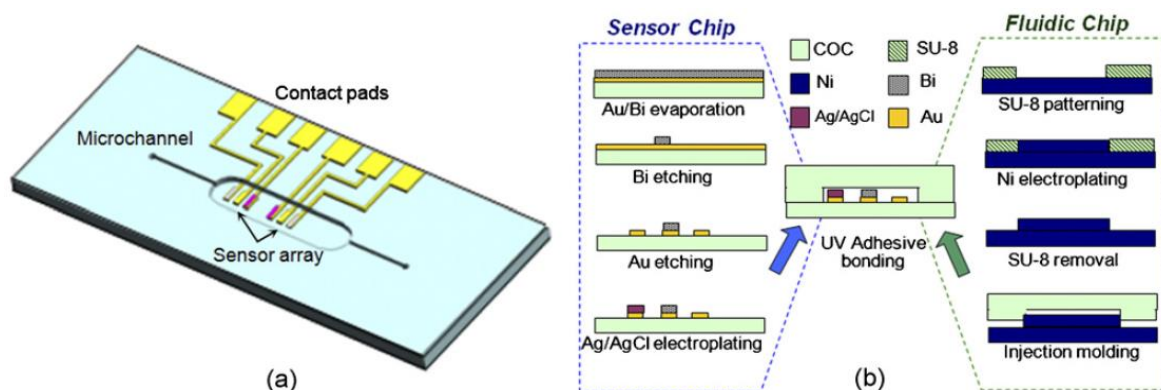


Fig. 1.6 (a) The schematic representation of the on-chip heavy metal sensor array employing microfabricated bismuth (Bi) electrodes integrated with microfluidic channels, and (b) an overview of the fabrication procedures³⁶. Used with permission from Elsevier.

In their paper, Wooseok Jung et al. introduced a reusable polymer lab chip sensor designed for continuous and on-site monitoring of heavy metals, focusing on lead³⁷. The miniaturized sensor features a microfabricated silver working electrode, replacing conventional mercury and bismuth electrodes, an integrated silver counter and quasi-reference electrode, and microfluidic channels (see Fig. 1.7). It aims to facilitate on-site environmental monitoring without disrupting or contaminating the sensing environment during reuse. The sensor's reusability was evaluated through forty-three consecutive SWASV measurements in non-deoxygenating standard solutions, demonstrating high repeatability and linearity over a wide concentration range of 1–1000 ppb for Pb (II). The developed sensor chip can be reused for on-site measurements with minimal sample volumes.

Their study also characterizes new Ag electrodes for continuous heavy metal detection on polymer lab-on-a-chips. This solid-state sensor offers reusability and effective detection of stable peak potentials for on-site SWASV measurements of heavy metal ions without producing toxic waste. Microfabrication technology is utilized to create a fully integrated sensor with a planar Ag working electrode, Ag counter/quasi-reference electrode, and microfluidic channel, as shown in Figure 1.8.

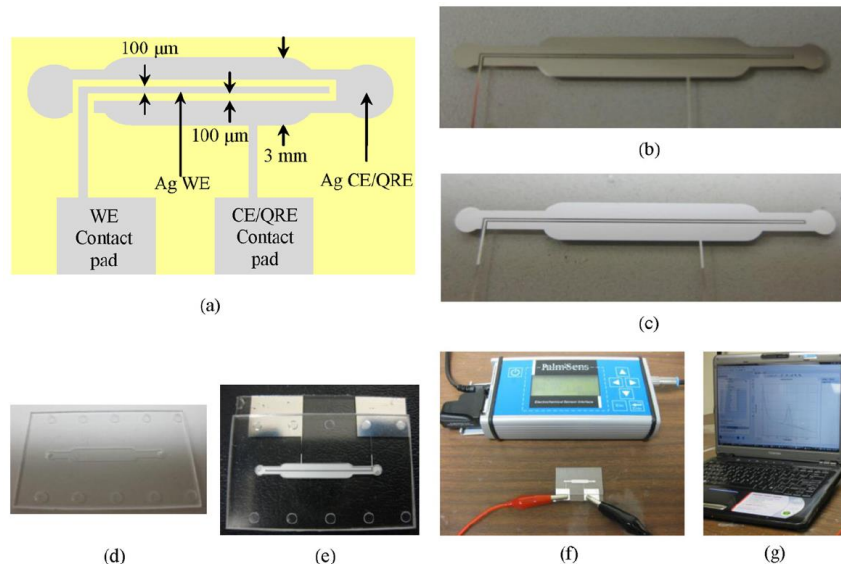


Fig. 1.7 (a) A schematic illustration of the reusable on-chip heavy metal sensor utilizing microfabricated Ag electrodes, (b) The composition of the sensor electrode consisting of 10nm Ti and 100nm Ag following PR lift-off, (c) The sensor electrode post-Ag electroplating, resulting in a thickness of 1.7μm Ag, (d) The fluidic chip post-injection molding, (e) The complete chip encompassing both the sensor and the microfluidic channel, (f) The potentiostat paired with the chip for measurement, and (g) The display of the measurement plot shown on a personal computer via PSTrace software³⁷. Used with permission from Elsevier.

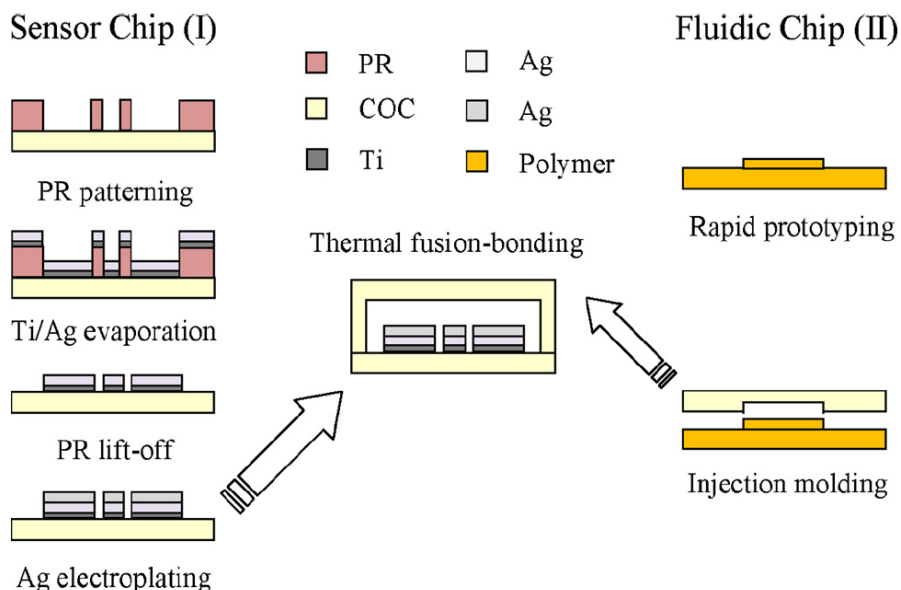


Fig. 1.8 The fabrication processes for the sensor chip (I) and the fluidic chip (II)³⁷. Used with permission from Elsevier.

Zou et al. introduced an on-site analyzer system for continuous monitoring of heavy metals employing disposable polymer lab-on-a-chips (lab chips) and a constant flow sensing approach³⁸. The system, comprised of a plastic fluidic motherboard with microchannels, valves, pump, control circuits, wireless communication module, potentiostat, and LabVIEW control, facilitates automatic long-term water sampling and heavy metal measurement. Utilizing square wave anodic stripping voltammetry with a microfabricated planar bismuth electrode on the lab chip enhances sensing performance, further bolstered by continuous flow sensing propelled by the analyzer. Testing on soil pores and groundwater samples from a lab-scale reactor demonstrated successful on-site measurement of Cd (II) concentration changes. The analyzer, characterized by compact size, low weight, low power consumption, rapid detection, ease of use, flexible sampling, and automatic controls, offers promising prospects for long-term deployment in heavy metal detection. Additionally, its adaptability to various electrochemical detection methods and sampling schemes enhances its utility for on-site monitoring of diverse environmental pollutants (see Fig. 1.9).

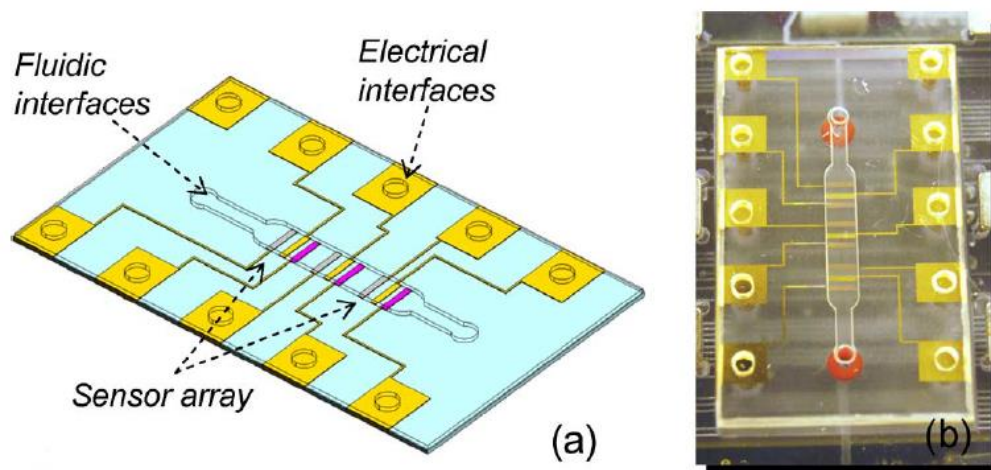


Fig. 1.9 (a) schematic view of the polymer lab chip with array of three sensors and (b) the photograph of the fabricated chip³⁸. Used with permission from IEEE.

A microanalytical system utilizing a combination of microfluidics and electrochemical detection was developed by Lin et al.³⁹, employing a multi-layer lamination method to fabricate the microfluidic platform (Fig. 1.10). Laser-machined adhesive-backed polyimide gaskets were used to create fluidic microchannels within the device. Key components, including microfabricated

piezoelectrically actuated pumps and a microelectrochemical cell, were designed as plug-in modules for easy integration into the microfluidic platform, allowing for rapid component interchangeability and repair akin to "plug and play" functionality found in personal computers. The system's capabilities were demonstrated by detecting lead and chlorophenols, showcasing its potential for on-site environmental characterization and real-time non-invasive biomonitoring of toxic chemical mixtures. This device examined the 0 to 200 ppb concentration of Pb in the sample solution, and the calibration curve was reported using the SWV technique.

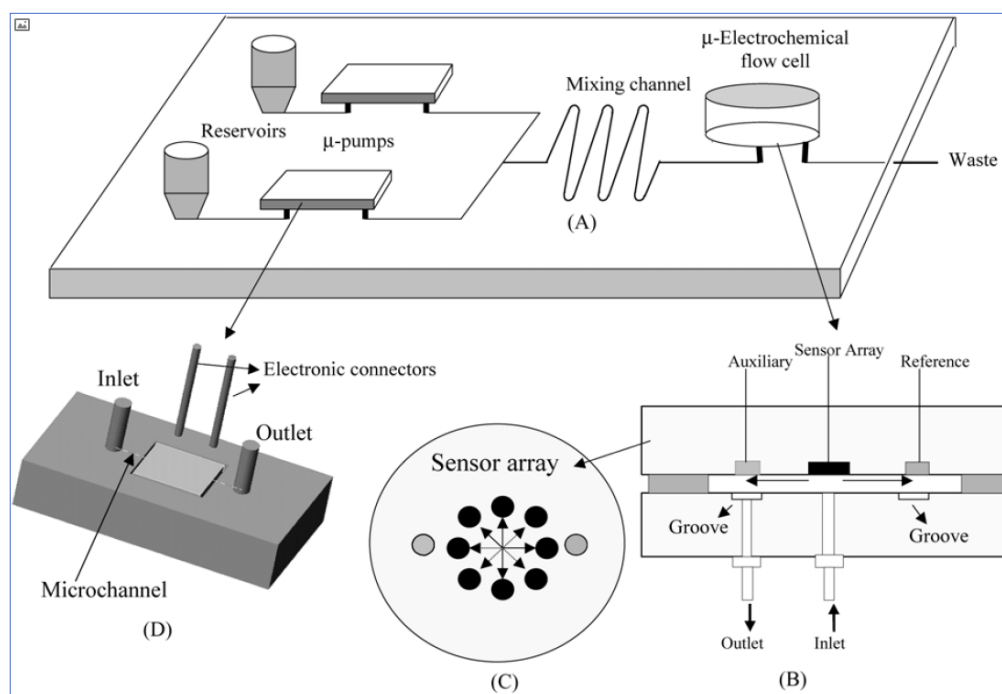


Fig. 1.10 (A) Schematic of electrochemical-microfluidic system, (B) Plug-in microelectrochemical cell, (C) Bottom view of cell with eight working electrode arrays, (D) Plug-in micropump³⁹. Adapted with permission from Kluwer Academic Publishers.

Paper-Based Microfluidic Electrochemical Sensors for Lead Detection

Shen et al. have developed a microfluidic electrochemical carbon-based sensor (μ CS) for detecting trace heavy metals⁴⁰. This sensor utilizes a microfluidic paper channel combined with a novel three-dimensional electrode layout, where pristine graphite foil serves both the conductive pad and the working electrode. The μ CS performs exceptionally in detecting Cd^{2+} and Pb^{2+} ions, achieving

detection limits of 1.2 $\mu\text{g/L}$ and 1.8 $\mu\text{g/L}$, respectively, through square-wave anodic stripping voltammetry.

The device is portable, reusable, and highly reproducible, making it suitable for on-site monitoring. The graphite foil's unique properties, including abundant edge sites facilitating metal deposition, contribute to the sensor's effectiveness. The arrangement of the electrodes, brings the working electrode closer to the counter electrode, promoting a more uniform and effective electric field. This configuration is expected to enhance the reductive deposition of metal ions onto the graphite-foil working electrode.

A novel disposable electrochemical lateral flow paper-based sensing device is proposed by Medina-Sánchez et al.⁴¹ for detecting heavy metals, particularly lead and cadmium, in aqueous samples. Demonstrating quantification within the range of 10 to 100 ppb with a limit of detection of 7 ppb for lead and 11 ppb for cadmium, this device leverages the filtering properties of its paper platform for sample pretreatment, making it effective even with turbid matrices without requiring prior filtration. The device offers simplicity, affordability, fabrication, and portability, making it promising for various point-of-care applications in environmental monitoring, public health, and food safety. Moreover, it successfully detects pollutants in complex matrices such as mud and seawater, showcasing its potential for diverse applications, including detecting heavy metals in biological samples like blood. The sensitivity of electrochemical stripping signals for lead and cadmium and their detection in complex samples underscores the device's versatility and reliability in various real-world scenarios. Figure 1.11 illustrates the process of layer-by-layer chip construction and presents the appearance of the completed device.

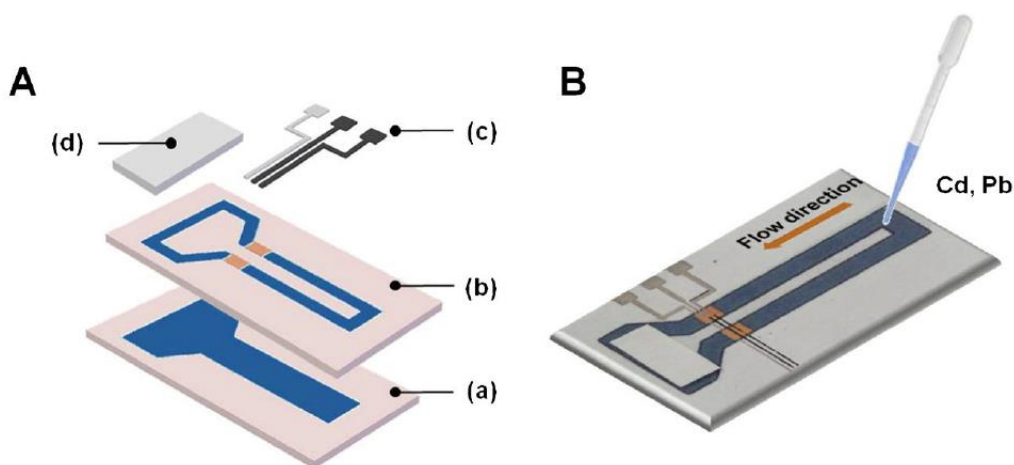


Fig. 1.11 The schematic of a lab-on-paper device. (A) depicts various components of the chip: (a) the bottom layer, (b) the channel layer, (c) the RE (in grey color), WE (located in the center), and CE, and (d) the absorbent pad. (B) shows the completed device⁴¹. Used with permission from Springer.

Jianjun Shi et al. developed a microfluidic device utilizing filter paper strips and screen-printed carbon electrodes (SPCE) for the direct detection of Pb(II) and Cd(II) in aqueous samples⁴². This portable device, featuring direct electrochemical detection, demonstrated the quantification of multiple analytes without the need for sample pretreatment. With SWASV, the device exhibited excellent analytical performance, detecting Pb (II) and Cd (II) from 0 to 100 ppb with low limits of detection (2.0 ppb for Pb(II) and 2.3 ppb for Cd(II)). Moreover, it displayed good selectivity and stability when tested with gas-dissolved salty soda water samples and physically contaminated groundwater. The device's simplicity, low cost, ease of fabrication, and portability make it promising for point-of-care applications in environmental monitoring, public health, and food safety. By enabling direct electrochemical measurements on original samples, such as gas-dissolved and physically contaminated water, the paper-based microfluidic device offers a practical solution. Its mechanism, driven by capillary action, ensures a steady flow of fresh samples across the SPCE surface, enhancing analysis efficiency. This practical application underscores its potential for further miniaturization and enhancement in medical, food safety, and environmental monitoring domains.

LIG-based Microfluidic Electrochemical Sensors for Lead Detection.

The study was done by Ding et al. to fabricate electrodes via direct laser sintering on commercial polymer films, eliminating the need for complex surface modifications⁴³. These porous carbon electrodes, integrated into a 3D-printed flow reactor, require minimal analyte for detection. Numerical simulations demonstrate that flow-based detection enhances sensitivity compared to stirring conditions, with a limit of detection of approximately 0.0330 mg/L for Pb²⁺. Optimizing laser irradiation parameters significantly influences the electrochemical performance of the electrodes.

The study indicates that the electrode process is diffusion-controlled, with electrochemical deposition typically performed under agitation. Simulation and experimental results highlight the superiority of laminar flow in continuous flow reactors over stirring in batch reactors for improving electrode surface dynamics and sensitivity. The enrichment process significantly impacts the sensitivity of lead ion detection, typically requiring solution stirring to decrease the diffusion layer thickness. Flowing the solution facilitates the practical approach of lead ions to the electrode surface, enhancing their adsorption onto the porous carbon electrode.

1.5. Research Gaps in the Use of Microfluidic- Electrochemical Sensors for lead detection

New devices with greatly enhanced detection limits, sensitivity, and selectivity are being developed as part of state-of-the-art research on Pb detection in microfluidic-based electrochemical sensors based on nanomaterials^{44–46}, modification of electrodes' surface using IIP (ion imprinted polymers)^{47–49}, developing electrode surface modification conductive polymer material^{50–52}, laser-induced surface modification^{53,54}, electrode surface modification employing nanoparticles^{55–59}, modifier-free microfluidic electrochemical sensors for heavy-metal detection^{40,60} has been in the focus of the research community. Regardless of their potential, current MES has limitations and constraints. Although much research has been conducted to improve electrode materials and surface modifications in microfluidic electrochemical sensors using various methods, this thesis will not primarily focus in improving electrode materials or

surface modifications. Instead, we will focus on improving the MES's performance by changing the curvature of the microchannel that guides the sample towards the electrodes.

1.5.1. Constraints of Fluid Control and Fluid-Electrode Interactions (*Sensitivity and Detection Limit*):

One of the areas for improvement associated with microfluidic electrochemical sensors is the limited attention given so far to fluid dynamics and fluid-electrode interactions within the microchannels. Typically, straight microchannels have been integrated with electrodes to make the sensors, and the effects of channel dimensions and flow rates on sensor performance have yet to be studied extensively. Flows within microchannels are dominantly laminar, which can limit the interaction of target analytes with the electrode surfaces^{61–63}.

Dean flow is a phenomenon that occurs in curved channels or pipes through which a fluid is flowing. It results from the centrifugal force acting on the fluid as it moves through the curved geometry. This force causes the fluid to experience secondary flow patterns, characterized by forming vortices perpendicular to the main flow direction. Dean flow has applications in microfluidics, particularly in microscale devices where fluid flow is confined to small channels. In microfluidic systems, Dean flow can significantly influence the transport and mixing of fluids. Understanding and controlling Dean flow is crucial for optimizing the performance of microfluidic devices in various applications such as lab-on-a-chip systems, chemical synthesis, biological analysis, and microscale heat exchangers. Researchers and engineers utilize Dean flow to enhance mixing efficiency in microfluidic channels. By exploiting the secondary flow induced by Dean flow, they can achieve rapid and efficient mixing of different fluids, which is essential for various biochemical assays, reactions, and analyses conducted within microfluidic platforms^{64–68}. Despite its significant applications in microfluidic systems to enhance fluid mixing and transport, Dean flow has received no attention in microfluidic electrochemical sensors.

1.5.2. Complicated Fabrication Techniques (*Miniaturization and Integration*)

Microfluidic electrochemical sensors often require intricate fabrication processes involving multiple steps and specialized equipment. These techniques typically involve photolithography, soft lithography, metal deposition, or other microfabrication methods, which demand high expertise and precision. The complexity of fabrication can lead to increased production time and complexity, as well as potential issues with reproducibility and scalability.

1.5.3. Expensive Equipment Requirement (*Cost-effectiveness*)

Fabricating microfluidic electrochemical sensors often necessitates using expensive equipment such as photolithography systems, microfabrication tools, and specialized deposition equipment. These high-cost instruments are essential for achieving the desired features and dimensions of microfluidic channels and electrodes. The requirement for such equipment adds to the overall cost of sensor development. For instance, specific fabrication methods, such as Physical Vapor Deposition (PVD), require cleanroom facilities to maintain precise environmental conditions. Cleanroom facilities are expensive to operate and maintain.

1.5.4. Lack of Continuous Detection (*Real-time Monitoring*)

Some microfluidic electrochemical sensors face limitations in achieving continuous detection capabilities. For example, paper-based MES cannot be used for continuous contamination monitoring. These sensors have benefits like portability, simplicity, and low cost but frequently sacrifice performance metrics essential for continuous monitoring.

1.6. Thesis Objectives

Our goal was to address the abovementioned gaps and develop a low-cost, easy-to-fabricate MES with high sensing performance. The curved microchannel in the microfluidic part of the sensor should be capable of disrupting the laminar flow over the channels but in a controlled manner to enable investigation of the effect of induced flow on electrode fluid interaction and subsequent detection performance enhancement.

In this research, we hypothesized that by employing and strengthening the Dean flow over MES electrodes, one may be able to improve mass transport towards the electrodes and enhance the readout current of the SWV measurement, hence boosting the performance of the sensor in terms of LOD, LOQ, sensitivity, and detection range.

To achieve this goal, we pursued the objectives below:

1.6.1. Objective 1: Simplifying the Fabrication and Integration Process of Microfluidic Electrochemical Sensor

As mentioned, MES's fabrication and integration process often poses significant challenges due to their intricate nature and the requirement for precise manufacturing techniques. Our first objective includes exploring alternative materials, simplifying multi-step microfabrication processes, and reducing the cost of mass production of the MES. By addressing this objective, we aim to simplify the fabrication and integration processes of MESs.

1.6.2. Objective 2: Investigating the Effect of Secondary Flow on Enhancing Analyte-Electrode Interaction

This objective evaluates how curvature in microfluidic channels affects electrochemical measurements, focusing on employing the well-known redox couple of potassium ferricyanide and potassium chloride (KCl) as the supporting electrolyte. The primary focus involves fabricating a microfluid part of the sensor featuring curved and straight microchannel components to analyze the effects of curvature on the output signal in electrochemical measurements.

1.6.3. Objective 3: Translating Findings to Lead Detection in Drinking Water

Building on the results obtained in Objectives 1 and 2, the third objective aims to extrapolate the findings to detect Pb in drinking water. This entails validating the applicability of the results obtained from SWV measurements with the potassium ferricyanide/KCl redox couple to the broader context of heavy metal detection, specifically targeting lead.

SO1: The first step includes SWV detection of specific concentrations of Pb using the optimum device from previous investigations and comparing the results with those of a straight microchannel.

SO2: In the second step, we would like to run SWV with various concentrations of the Pb injected into the sensor to obtain the calibration curve and sensor parameters such as sensitivity and LOD.

Through rigorous experimentation and analysis, we seek to demonstrate that the insights gained from Objectives 1 and 2 regarding the influence of curvature on electrochemical measurements can be generalized to the detection of lead in aqueous samples. Extending our investigation to a real-world scenario, such as lead contamination in drinking water, we aim to underscore our research findings' practical relevance and utility.

This objective represents a crucial step towards bridging the gap between fundamental research and real-world applications. By establishing the feasibility of utilizing curvature-enhanced microfluidic sensors for lead detection in drinking water, we aim to develop cost-effective, sensitive, and reliable sensing solutions with broad societal impact.

1.7. Thesis Structure

This thesis has been organized into five chapters,

The first chapter presents lead as a hazardous heavy metal in the environment, gives a general review of traditional lead detection tools, discusses electrochemical analysis methods, reviews modern electrochemical lead sensors, highlights research gaps and issues regarding Pb detection, and outlines thesis objectives. The research's theoretical and experimental components, as well as materials and procedures, are covered in the second chapter. The third chapter reports on the investigation of the influence of using curvature geometry in the microfluidic part of the sensor, the fabrication of sensors with varying curvature radii, the application of SWV techniques, the analysis of electrochemical response under different curvature Conditions, and the discussion of findings. Chapter Four elaborates on transferring findings to lead detection in deionized and real water, validation of results from the previous chapter's results, application of findings to lead detection in drinking water, discussion of results, and conclusion. Finally, in Chapter Five, the research is summarized, and the directions for future work are proposed.

CHAPTER 2.

Materials and Methods

2.1. Introduction

This chapter explains the materials, experimental setup, and methods used to fabricate and characterize the 3-electrode MESs proposed in this thesis (Fig. 2-1). Sensors with straight (Fig. 2.1A) and curved (Fig. 2.1B) microchannels were used to investigate the effect of a secondary vortex (Dean) flow on the output electrochemical current signal of the sensors. Fig. 2.1C illustrates the layer-by-layer schematic of the straight-channel and curved-channel sensors. The fabrication process will be described in detail in the next section. SWV was used as the electrochemical measurement technique. Parametric studies on the effects of channel radius of curvature and flow rate were conducted using $K_3[Fe(CN)_6]$ as a standard solution prepared in 0.1 M KCl as a supporting electrolyte. The best-performing sensor characteristics were then used to detect various Pb concentrations in spiked and real waters. Analysis using ANOVA (analysis of variance) was conducted in Excel to assess the significance of experimental variables on the sensor's performance.

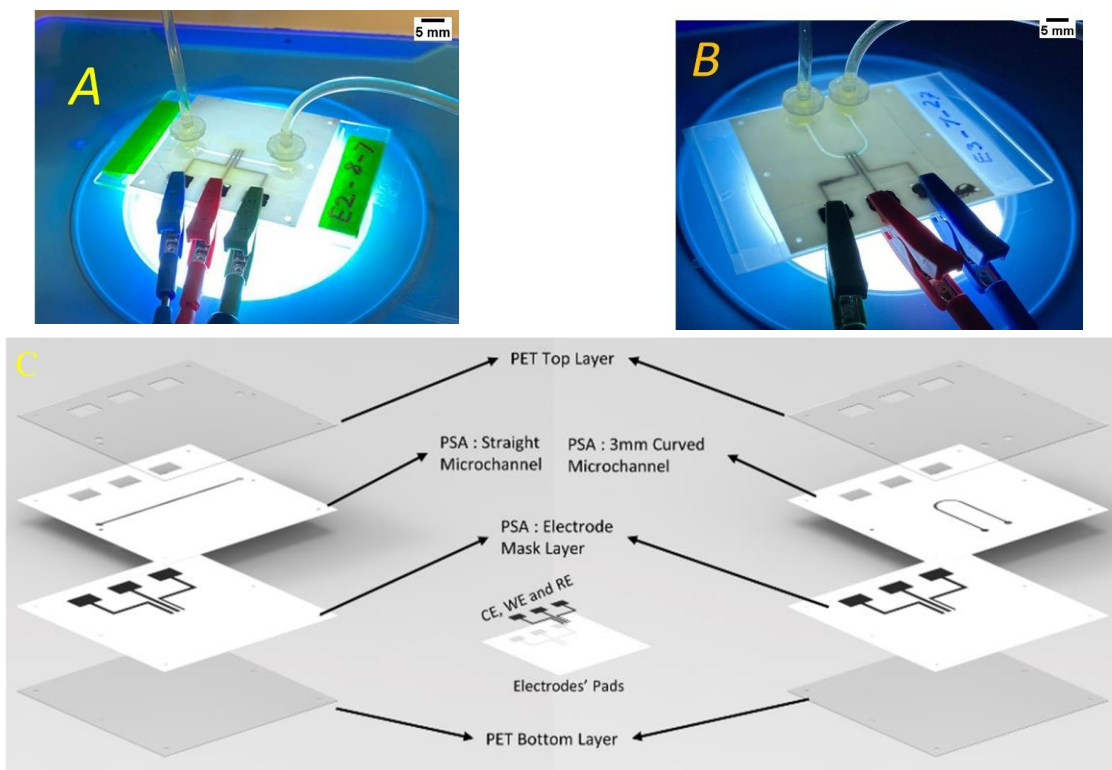


Fig. 2.1 The proposed microfluidic electrochemical sensors. Photographs of sensors with (A) a straight and (B) a curved microfluidic channel are shown. (C) Schematic of layer-by-layer composition of the two sensors.

2.2. Materials and Equipment

We used polyethylene terephthalate (PET) sheets for the top and bottom layers of the microfluidic electrochemical sensor due to its transparency, flexibility, and ease of fabrication. Both microchannel and electrode patterns were laser cut in pressure-sensitive adhesive (PSA) sheets. PET is (1) an excellent material for bonding, (2) easily can be cut into desired shapes, (3) an inert acrylic adhesive, and (4) a low-cost material for microfluidic applications. Graphene-carbon paste was used to fabricate the electrodes within the PSA layer. This electrode paste has several advantages over other materials, including (1) good printability, (2) low-temperature curing, (3) good screen residence time, (4) compatibility with low-temperature substrates such as PET, and (5) excellent electrical conductivity^{69,70}.

Table 2.1 shows the materials and consumables used in the experiments and their purpose of use. The materials were sourced from various companies, including Sigma-Aldrich (St. Louis, MO, USA), which provided KCl as a supporting electrolyte, H₂SO₄ for device cleaning, and Pb(NO₃)₂, a target analyte. The deionized water required for sample preparation was obtained from Millipore Sigma™ Milli-DI™ Water Purification System. Compounds for sensor characterization, such as K₃(Fe(CN)₆) were also acquired from Sigma-Aldrich. PDMS for microchannel and electrode conduit fabrication was sourced from Dow Corning in MI, USA. PSA were obtained from Adhesives Research in the USA, while PET layers for device assembly were procured from 3M in the USA. Tubing and sockets used for the sensor inlet and outlet were obtained from Cole-Parmer Canada in QC, Canada. Finally, graphene paste for electrode fabrication was sourced from Dycotec Materials Ltd in the UK.

Table 2.1. Materials and consumables used in this thesis and their vendors and purposes.

Material	Vendor	Purpose
KCl	Sigma-Aldrich (St. Louis, MO, USA).	Supporting electrolyte
H ₂ SO ₄	Sigma-Aldrich (St. Louis, MO, USA).	Washing the sensors
Deionized water	MilliporeSigma™ Milli-DI™ Water Purification System, USA	Base fluid sample
Pb(NO ₃) ₂	Sigma-Aldrich (St. Louis, MO, USA).	Target analyte (lead)
K ₃ (Fe(CN) ₆)	Sigma-Aldrich (St. Louis, MO, USA).	Compound used to make standard sample for sensor characterization
PDMS base and curing agent	Sylgard 184 kit from Dow Corning, MI, USA	Fabrication of microchannels and substrate for electrode fabrication
PSA (ARcare 8939)	Adhesives Research, USA	Device middle layers
PET	3M, USA	Device top and bottom layers
Tubing (ID:1/16 in, OD:1/8 in)	Cole-Parmer Canada. QC, Canada	Sensor inlets and outlets
Sockets	Cole-Parmer Canada. QC, Canada	Sensor inlets and outlets
Graphene paste	Dycotec Materials Ltd, UK	Electrode fabrication

Various equipments were used in the experiments (See Table 2.2), all chosen for accuracy and dependability. The Stereomicroscope MZ10F, obtained from Leica microsystems, allowed for the observation and manipulation of samples and precise and thorough visual inspection. The LEGATO syringe pumps, models 111 and 110, made in the USA by KD Scientific Inc., allowed

for controlled fluid delivery. A Thermoscientific Heratherm oven was used to dry the graphene-carbon hybrid electrode paste. The Utrecht, Netherlands-based PalmSens BV potentiostat was utilized for electrochemical measurements, providing accurate electrochemical reaction monitoring and control. Furthermore, the Epilog Helix Laser from the USA was used to precisely complete complex cutting and engraving tasks in the PSA sheets to fabricate the microchannels and electrode conduits.

Table 2.2. Equipment used in the experiments.

Equipment	Manufacturer
Upright microscope	Stereomicroscope Leica MZ10F, Singapore
Syringe pump	LEGATO 111 and 110, KD Scientific Inc., USA
Oven	Thermoscientific, Heratherm, USA
Potentiostat	PalmSens BV. Utrecht, Netherlands.
Laser cutting machine	Epilog Helix Laser USA
SPE	Metrohm, Canada

2.3. PDMS-Based Microfluidic Electrochemical Sensor

One of the main research goals in this project was to explore the effect of Dean flow on electrochemical readout in microfluidic sensors. Therefore, our early investigations started by fabricating the MES using PDMS (for the microchannels) and gold deposition and patterning (for electrodes), using techniques already established in the literature^{71–73}. The first chapter highlights instances of microelectrode systems employing the PDMS channel and gold electrode. In this section, we briefly describe the fabrication process of these MESs using microfabrication techniques conducted in a cleanroom environment. The sensor comprises two components: a fluidic part guiding the sample through microchannels and an electric part housing the electrochemical sensing electrodes. The fluidic part of the sensor was fabricated using photolithography and soft lithography techniques (see Fig 2.2).

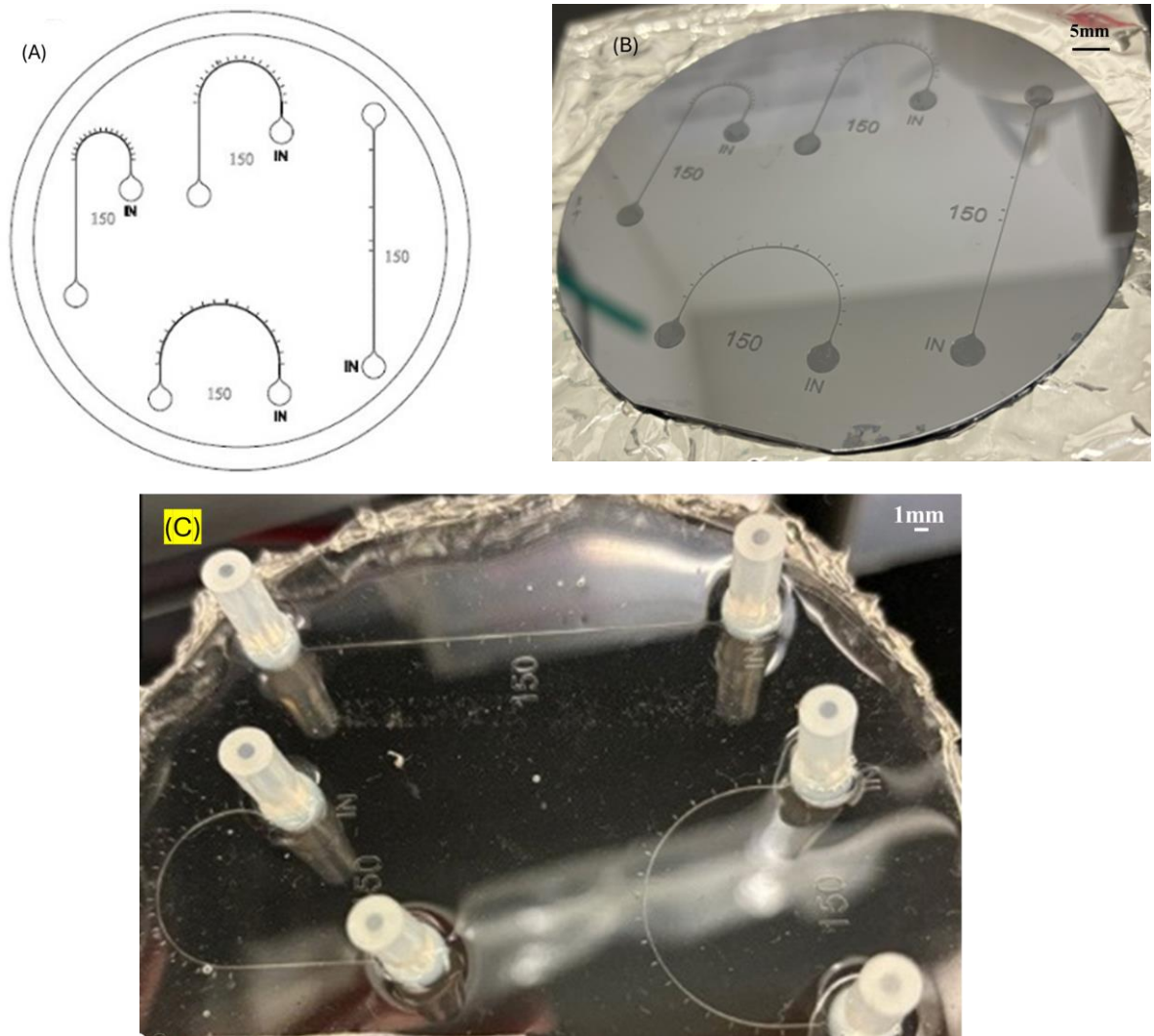


Fig. 2.2. Fabrication of microchannel using PDMS (a) Mask CAD design of channels, (b) Photolithography of microchannel patterns on the silicon wafer, (c) Soft lithography for fabrication of fluid part of the MES using PDMS

The fabrication of a PDMS microchannel was started with the design in SolidWorks software (Fig 2.2A) and creation of a photomask, translating the desired microchannel with different radii of curvature ($R=\infty$, 3mm, 6mm, and 9mm) onto a light-sensitive photoresist-coated substrate. This substrate was a clean silicon wafer, onto which the photoresist layer was uniformly applied using spin-coating techniques. Through alignment with the photomask, the microchannel pattern is transferred onto the photoresist-coated wafer via exposure to UV light. Then, the unexposed photoresist was removed, leaving behind the patterned microchannel layout on the silicon substrate (Fig 2.2B). The PDMS elastomer mixture was prepared, combining base and curing agent in 1 to

10 ratios and then degassed to eliminate air bubbles. After that, the mixture was poured over the patterned silicon wafer, ensuring complete coverage of the microchannel pattern, before curing at 80°C through oven heating. Upon curing, the PDMS layer is carefully peeled from the silicon wafer, replicating the microchannel pattern on its surface (Fig 2.2C). These PDMS microchannels were ready to be integrated with the gold-patterned electrodes to make the final sensor.

The process of depositing gold onto a glass substrate using photolithography and electron-beam (E-beam) evaporation was performed to fabricate the designed electrodes (Fig 2.3A). The electrodes included a working electrode, a counter electrode, and a reference electrode in parallel. First, a glass substrate was coated with a positive S1813 photoresist. The desired electrode patterns were formed within this coating by employing a photolithography technique similar to the one described in the previous paragraph. The photoresist-patterned glass substrate was loaded into an E-beam machine vacuum chamber and a crucible containing the gold material. Within a very low-pressure environment, an electron beam gun bombards the gold source with high-energy electrons, causing the gold atoms to evaporate. These atoms travel in a line-of-sight trajectory, depositing onto the glass substrate facing the source (on the glass within the patterned areas and on top of the photoresist on the rest of the surface). Throughout this deposition process, parameters such as deposition rate, substrate temperature, and chamber pressure are controlled and monitored to ensure the desired film characteristics. After the gold deposition was completed, the photoresist layer was removed in Acetone, and the gold layer was shaped into desired electrode designs (Fig 2.3B). Then, the microfluidic channels in Fig 2.3C were plasma bonded with the electrode layer of Fig 2.3B to produce a final sensor (Fig 2.3C).

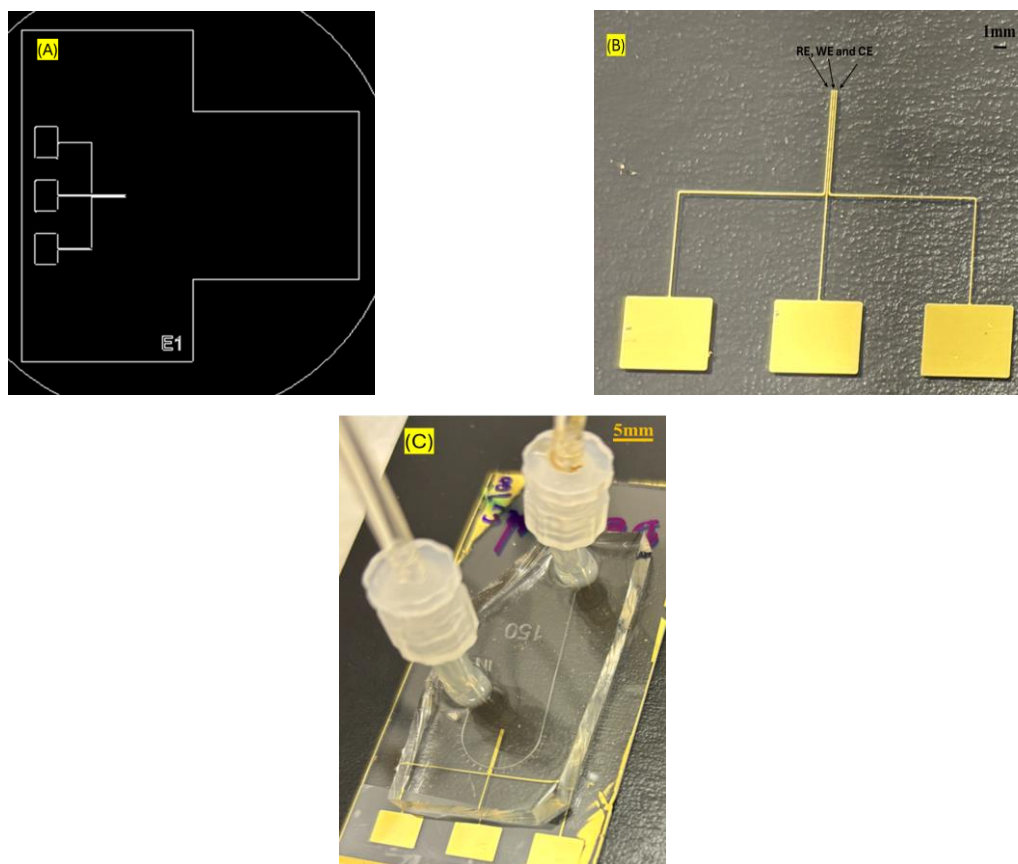


Fig. 2.3. Gold electrode fabrication and integration with PDMS microfluidic device , (a) Photomask printed at the York microfabrication facility center, (b) Three Gold electrodes deposited on the glass substrate using E-beam deposition technique and lift-off process, (c) Final MES sensor after integrating the microfluidic part and chip part.

2.4. PSA/PET-Based Microfluidic Electrochemical Sensor

As discussed in the Results chapter, after spending considerable time on the PDMS-based MES shown in Fig 2.3C, we concluded that this device fails to produce desirable results due to its fabrication complexity and cost and electrodes' delamination during SWV. This pushed us towards developing the alternative low-cost, simple-to-make, and more stable sensor shown in Fig. 2.1C. This figure shows the layer-by-layer schematics of the straight and curved-channel MESs made in PSA sheets and sealed with two PET sheet layers on top and the bottom. The sensors were designed using SolidWorks software (SolidWorks Corp., USA), focusing on electrode patterns and microchannel geometry. These sensors also consisted of two main parts, i.e., a microfluidic part and an electrochemical sensing part. The microfluidic part was designed so that the microchannel was straight or curved with different radii of curvature varying from 3 mm to 9 mm. The height of

the microchannel layer was 126 μm , bounded by the thickness of the PSA sheet. The total length of all microchannels was designed to be 38 mm.

The electrochemical sensing part consisted of three parallel electrodes including a WE, CE, and a RE. The electrodes were aligned orthogonally with respect to the microchannel axis. A typical working and counter electrode had dimensions of 900 μm in width, and a typical reference electrode had dimensions of 500 μm in width. The distance between the electrodes is set to be approximately 750 μm . We considered the impact of the geometry of the microchannel employing the non-dimensional numbers, which will be introduced in Section 2.7. Moreover, we will consider normalizing the output signal with electrode surface area to evaluate the effect of the electrode size in our experiments.

2.4.1. Laser Cutting of Microchannel and Electrodes Layers in PET Sheets

For fabricating the simple, low-cost MES, we explored the optimization process of PSA and PET sheets laser cutting parameters using a 60 W EPILOG laser cutting machine. The balance between cutting efficiency and material integrity was considered during the process. The laser cutting machine has a large area for engraving of 36" $\times$ 24" (914 mm $\times$ 610 mm) and can manage samples up to 10" (254 mm) thick. It uses an air-cooled CO₂ laser source emitting a 10.6 micrometer laser through metal/ceramic waveguide tube technology. Its maximum engraving speed is 200 in/s (5.08 m/s).

PSA sheets are sensitive materials that can quickly degrade when exposed to high heat or energy. Excessive heat can cause them to lose their strength and shape. To prevent this, we carefully adjust the power and speed of the laser during cutting. Through testing, we found the best settings for each material to minimize any damage. We did the laser cut with 100% speed and varied the power to find the range of power needed for cutting the PSA sheets. We noticed that the PSA sheet cannot be cut properly at a power lower than 13%, while the laser burns the material at a power higher than 15%. Therefore, we decided to set the power to 14% of full power, which was 8.4 W.

After this stage, we gradually reduced speed to assess its impact on the cutting process. We discovered that operating at 70% of the maximum speed yielded superior results regarding the edges and overall shape of the microchannels (See Fig. 2.4). Speeds lower than this threshold led to material burnout. We followed a similar approach for PET, determining an optimal setting of 6% power and 80% speed. However, we adjusted the number of iterations to three to ensure uniform edges while preserving material integrity. The laser cutting frequency was set at 2500 Hz based on the recommendation of our technician.

Fig. 2.4 shows examples of PSA sheets post-laser cutting, utilizing power levels between 11-14% and speeds ranging from 70-100%. The input SOLIDWORKS designs varied from 100-400 μm . Our microscopic analysis revealed an offset of approximately 145 μm between the designed values and the averaged measured values from the laser-cut parts. Consequently, we refined the designs in SOLIDWORKS to account for this offset in preparation for the subsequent step—laser cutting the electrodes and microchannel patterns.

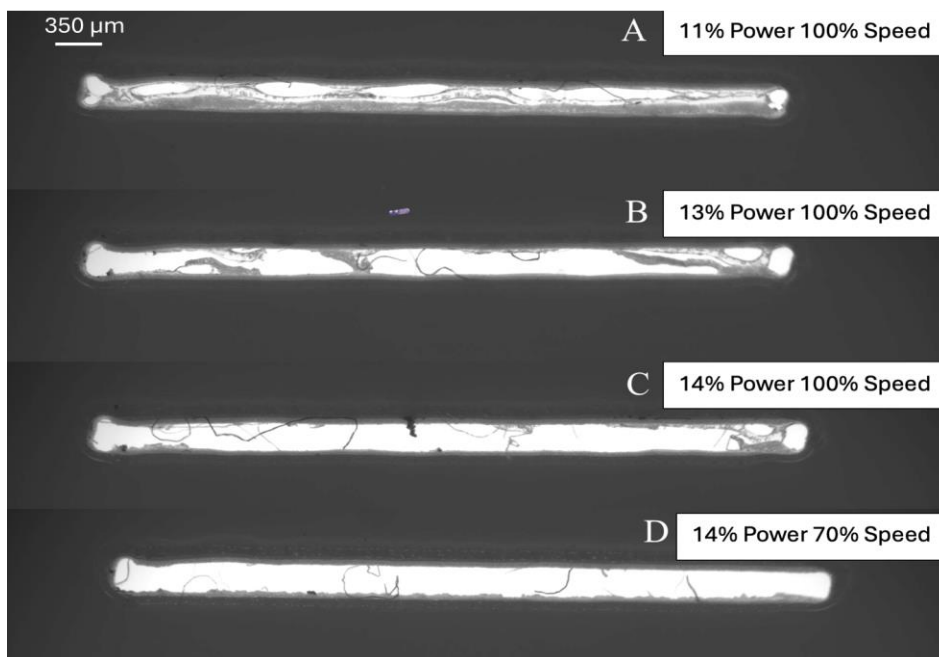


Fig. 2.4. Characterization of PSA-based microchannel fabrication using laser cutting with (A) 11% power and 100% speed, (B) 13% power and 100% speed, (C) 14% power and 100% speed, and (D) 14% power and 70% speed.

Fig 2.5 shows the example of electrodes and microchannel patterns fabrication using the laser cutting techniques and employing the setting from the characterization steps above.

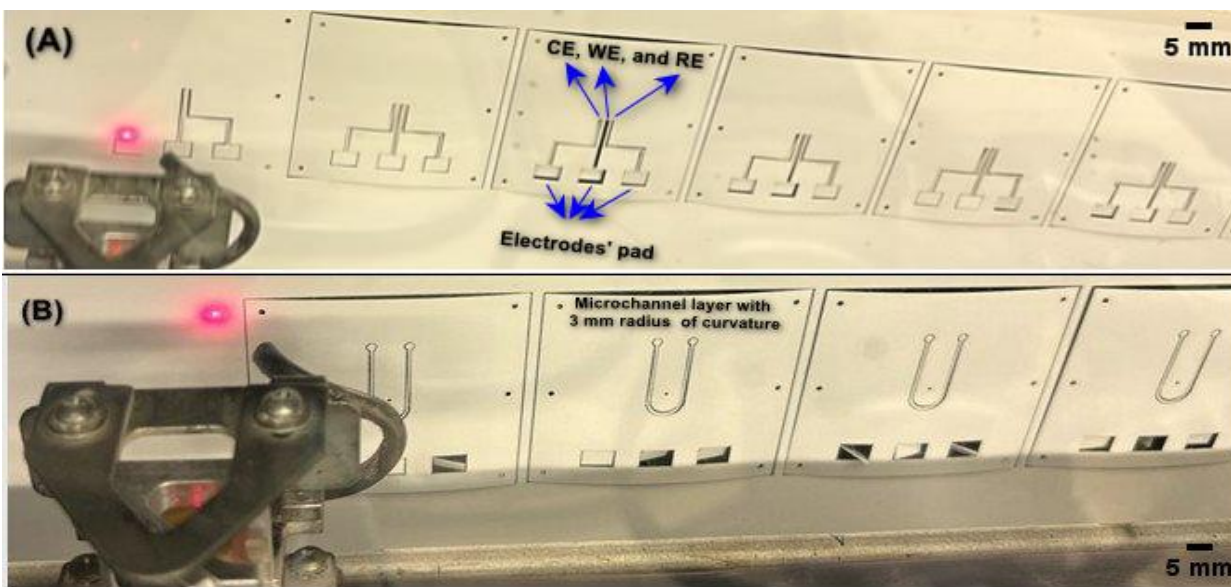


Fig. 2.5. Fabrication of various layers of the MES. Laser cutting of (A) WE, CE, and RE electrodes and (B) microfluidic curved-channel ($R=3$ mm) layer of the sensor.

2.4.2. Fabrication of Microfluidic Channel and Electrode Layers

After fabrication of straight-channel and curved-channel microfluidic part of the sensor as well as laser cutting of the electrodes pattern (Fig 2.6A), the liner on one side of the PSA sheet was peeled off (Fig 2.6B), and the sheet was attached onto a PDMS substrate (Fig 2.6C). The necessity to address shrinkage effects that arose during the electrode paste's curing process within the PSA led to the selection of PDMS as the substrate. The electrodes frequently settle at a lower position in relation to the PSA surface as a result of this shrinkage, which can cause cavities that can interfere with the fluid flow patterns inside the microchannel. We used PDMS as a substrate to lessen this problem and guarantee consistency in the electrode surface level with the PSA.

Following the curing process, the electrodes were detached from the PDMS substrate without adhering to its surface. The hydrophobic nature of PDMS prevents excessive bonding between the electrodes and the PSA electrode mask. Moreover, by leveling the electrode surface with the PSA

surface on one side in contact with PDMS, we ensured seamless integration with the microfluidic component of the sensor while the other side was sealed with a PET sheet.

After filling the electrode paste on the electrode layer (Fig 2.6D), it was placed in the oven at 80°C to solidify it (Fig 2.6E). The second liner was removed, and the PSA sheet was securely adhered to a PET sheet. (Fig 2.6F). Ultimately, the electrode layer was peeled away from the PDMS substrate and rotated to expose its front side. (Fig 2.6G and Fig 2.6H), By ensuring that all three electrodes are aligned flush with the surface of the tape, the fabrication process is finalized with meticulous precision and attention to detail.

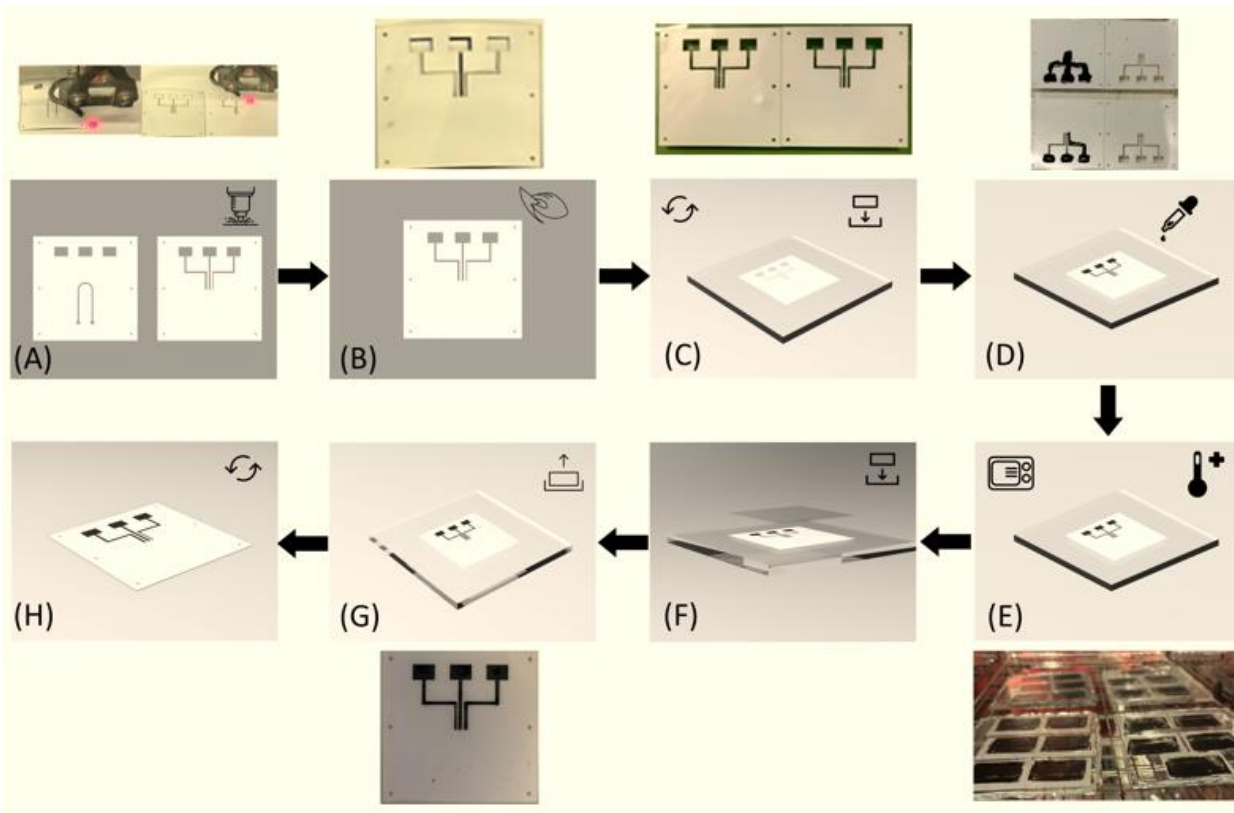


Fig. 2.6. Fabrication process of the PSA/PET-based MES. (A) Laser cutting of the electrodes and microchannels layer, (B) Peeling of the liner from one side of the tape in the electrode mask layer, (C) Placing the electrode layer on the PDMS for filling with electrode paste, (D) Applying the electrode paste, (E) Drying the electrode paste in the oven at 80 C, (F) Removing the liner from the upper side of the electrode layer and attaching PET sheet onto the back side of the electrode layer, (G and H) Removing electrode layer from PDMS substrate and turning around to the front side of the electrode layer (in front side all three electrodes are in level with the surface of the tape).

The next step of the fabrication, the integration of the electrode and microchannel layers for making the final MES, is described in the next section.

2.4.3. Integration of the Electrode and Microfluidic Channel Layers

Fig. 2.7 illustrates the integration process of the microfluidic layer with the electrode layer. Initially, the microfluidic layer was attached on top of the electrodes by removing the liner of the PSA in the two surfaces that should be connected together and using glue to establish a strong bond between them (Fig 2.7A). Following this, the microfluidic layer was enclosed by a PET layer (Fig 2.7B), ensuring the protection and containment of the channels, including their respective inlet and outlet ports (Fig 2.7C). Subsequently, 1/16 sockets were affixed onto the inlet and outlet using glue. The entire device was then placed in an oven set at 80°C for 20 minutes, allowing the glue to dry and securely fasten the sockets. Finally, tubing was connected to the sensor's inlet and outlet, completing the assembly process (Fig 2.7D).

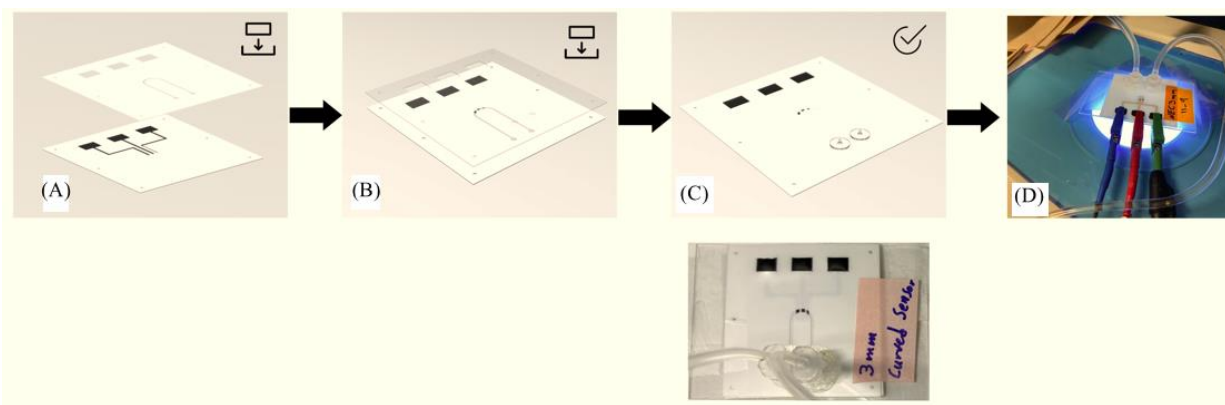


Fig. 2.7. The integration of MES electrode and microfluidic layers for making the final sensor; (A) Attaching the microchannel layer on the front side of the electrode layer, (B) Sealing the microchannel layer with PET sheet with two holes on it for placing the sockets, (C) Gluing the inlet and outlet sockets on the PET sheets, (D) Photo of completed sensor with tubing connected to the inlet and outlet.

2.5. Experimental Setup and Procedure

The experimental setup for our developed MES is shown in Fig 2.8. As depicted in this figure, MES electrodes were connected to a potentiostat for electrochemical measurements, and the microchannel inlet was attached to a syringe pump for sample injection. Sample injection was performed using three syringe pumps. One pump is dedicated to treating the electrode surface with 0.5 M H_2SO_4 and performing CV. In contrast, the second pump is utilized to rinse the microchannel with DI water before conducting SWV measurements. The third pump is employed to inject samples during SWV measurements. Additionally, the setup includes an upright microscope for process visualization and microfluidic device control to prevent bubble formation. A potentiostat for regulating the electrochemical potential was applied to the working electrode, and the resulting current was recorded during SWV measurements. A laptop equipped with PSTrace Software for data collection and subsequent analysis was used.

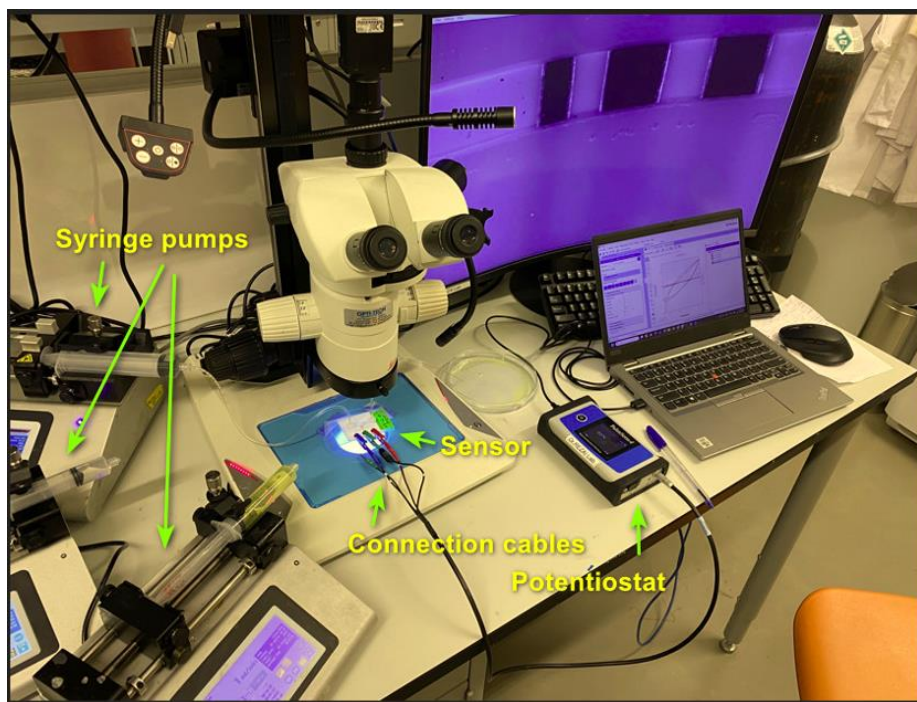


Fig. 2.8. The experimental setup consists of three syringe pumps for injecting fluid, a microscope with a mounted MES, and a large monitor displaying electrode conditions. The MES is connected to a potentiostat, and data is collected using PSTrace software during the tests.

An investigation into how curvature affects the output signal of the MES during SWV measurements of potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$) was conducted first. Subsequently, we utilized the optimized MES, which exhibited a higher peak current, to detect Pb in both DI water and real water samples. A solution comprising 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ in 0.1 M KCl served as the supporting electrolyte, renowned for its reliability and cost-effectiveness, to explore the impact of curvature on the sensor's output signal. Upon optimization, the sensor was deployed to detect Pb in water samples. The procedure involved injecting the solution into a microfluidic device, followed by SWV measurements using a potentiostat to quantify the Pb concentration.

To prepare the Pb solutions, $\text{Pb}(\text{NO}_3)_2$ salt was dissolved in DI water, and KCl salt was added until the total volume reached 1000 milliliters (mL). This initial solution contained 0.01 M Pb and 0.1 M KCl. Subsequently, serial dilution was performed. For instance, 5 milliliters (mL) of the stock solution were withdrawn, and 0.1 M KCl solution was added to achieve a final volume of 50 cubic centimeters (cc) for a 0.001 M Pb solution suitable for injection into the sensor. This process was iterated 12 times to generate a concentration range from 10^{-2} to 10^{-8} Molar. Thorough mixing was ensured at each step to guarantee homogeneity in the prepared lead solution concentrations for experimental purposes. The MES was connected to the syringe pumps, and the solution was injected into the microchannels at a flow rate varied from 1 mL/min to 5 mL/min.

The potential was applied using a potentiostat, and the resulting current was measured using SWV with the voltage range set from -1.2 V to 0.2 V, potential step 10 mV, amplitude 20 mV, and frequency 15 Hz. We also used the commercial screen-printed electrode to compare our handmade low-cost sensor with available sensors on the market. SPE with carbon WE and CE and Ag/AgCl RE was used for this purpose.

SWV analysis was also conducted using a commercially available carbon SPE to compare the outcomes with our custom-made MES. This facilitated a comparative examination of peak current variances and corresponding potentials between continuous MES and stationery SPE measurements. The working electrode's diameter was standardized at 4mm.

2.6. Square Wave Voltammetry Parameters

In SWV, the potential between the working electrode and the reference electrode undergoes a square wave pattern. Despite this variation, the potentiostat ensures a constant potential between the WE and the RE throughout each waveform segment. It achieves this by actively adjusting the voltage applied to the WE relative to the reference potential. This maintained potential allows for precise measurement of the resulting current through the counter electrode, facilitating the analysis of electrochemical reactions.

As the potential is applied to the WE, electrochemical oxidation and reduction reactions occur at the electrode surface, leading to changes in the current flow. These changes in the current response are directly related to the analyte concentration in the solution. By analyzing the current response as a function of the applied potential, SWV provides valuable information about the solution's redox behavior and concentration of analytes.

The “t equilibrium” in the SWV setting indicates the equilibration time (s), establishing how long the apparatus will wait before the measurement begins. Depending on the potentiostat model, this could be a waiting period during which the cell is turned off or at the scan's starting potential. The purpose of this parameter is to ensure that the initial currents of the scan are constant and devoid of abrupt jumps. Since our experiments were carried out in continuous injection mode, there is no need for an equilibrium state. This parameter has zero value.

The “E begin” is the initial potential applied to the electrochemical cell during the measurement and is known as the starting potential, and the symbol E represents it. The final potential applied to the electrochemical cell during the measurement is indicated by the ending potential, also known as E end. During the experiments with $\text{K}_3\text{Fe}(\text{CN})_6$ and $\text{Pb}(\text{NO}_3)_2$, we decided to set the potentials at the beginning and end to -1.2 V and 0.2 V, respectively, since the literature indicates that the peak potential for $\text{K}_3\text{Fe}(\text{CN})_6$ is approximately -0.2 V, and for $\text{Pb}(\text{NO}_3)_2$, it is approximately -0.65 V.

An E step indicates a potential step. It shows the staircase step of the excitation wave in the experiment's linear sweep component. It immediately affects the resolution of the voltammograms; in our experiments, the peaks for the E step higher than 10 mV could have been sharper and more

precise. The potential step also affects the acquisition time; higher values necessitate longer acquisition times.

Amplitude displays the square component of the experiment's excitation wave's amplitude. It modifies the background signal and the signal-to-noise ratio of the resulting peaks, which are the two primary features of the resulting voltammograms. The amplitude was fixed at this value because increasing it to more than 0.02 V produced graphs with wider peak currents. The speed at which the square wave will be applied during the experiment is determined by frequency.

We can determine the SWV scan rate by multiplying the frequency by the step potential. The speed at which the potential is swept between two limits (from a starting to an ending potential) is determined by the scan rate. A higher scan rate results in a quicker sweep of the potential. We found that increasing the frequency beyond 15 Hz resulted in fluctuating current vs. potential graphs, hence it was set to 15 Hz.

2.7. Microfluidic Electrochemical Sensor Data Analysis and Statistics

The collected data from SWV experiments were analyzed, and various statistical techniques were employed to find the significance of the effect of flow rate and channel geometry on the output signal. In the first phase, according to the first objective, using $K_3[Fe(CN)_6]$, the flow rate and channel curvature effects were examined to determine how microfluidic channel curvature influenced the sensor's output signal. Then, in the next phase, for achieving the second objective, the straight-channel sensor and curved-channel sensor data from $Pb(NO_3)_2$ detection experiments at different concentrations were analyzed to find the sensor's parameters, including sensitivity, LOD, and LOQ.

The LOD, LOQ, and sensor sensitivity were calculated based on the generated Pb dose-SWV peak current response curves to report the sensor performance. Experimental data was gathered by observing the sensor's reaction to predetermined concentrations of the analyte. Following this, the blank sample's Standard Deviation (SD) should be calculated. Subsequently, the sensor's performance can be evaluated using the following equations,

$$LOD = \frac{3 \times \text{SD of the blank}}{\text{Slope of the dose-response curve}} \quad (2-1)$$

The LOD for the sensor was established by analyzing the standard deviation of the SWV measurements taken at the lowest concentration of lead in our serial solutions, which was 10^{-8} M. Typically, LOD is determined by evaluating the variability in measurements at the blank solution level. However, in our case, the current amount was zero in SWV measurements of blank samples. To address this, the standard deviation of the lowest detectable concentration, which was 10^{-8} M in our experiments, was calculated. This concentration was used as a reference point to evaluate the sensor's capacity to detect even trace amounts of the analyte. To be more precise, the LOD was calculated as three times the standard deviation of the SWV measurements made at this lowest concentration.

The calibration curve can be drawn by measuring the sensor response to known concentrations of the analyte. For the LOQ, which is the lowest concentration of analyte that can be reliably quantified with acceptable accuracy and precision, the following formula was used:

$$LOQ = \frac{10 \times \text{SD of the blank}}{\text{Slope of the dose-response curve}} \quad (2-2)$$

Having the slope in the linear range of the calibration curve, the sensitivity of the sensor can be reported.

To determine the impact of experimental variables (such as electrode geometry and microfluidic flow rates) on the sensor's performance, an ANOVA was carried out at the 95% confidence level using Excel software to examine how the sensor type (straight-channel or curved-channel) and flow rate influence the sensor's peak current. This analysis allows us to gauge the impact of these factors on the output signal and determine whether our initial hypothesis holds true or not.

In addition to the ANOVA analysis, a series of t-tests were performed to further investigate specific pairwise differences between the means of various radii of curvature within each flow rate group. These t-tests allowed for a more focused examination of the influence of microfluidic channel curvature on the sensor's output signal at different flow rates. The results of these t-tests provided additional confirmation of the significance of curvature as a factor affecting the sensor's performance, complementing the findings from the ANOVA analysis. Notably, the obtained p-

values from the t-tests corroborated the evidence against the null hypothesis, reinforcing the conclusion that microfluidic channel curvature plays a significant role in modulating the sensor's response, thereby contributing to a comprehensive understanding of the sensor's behavior under varying experimental conditions. One asterisk (*) represents a p-value less than 0.05, indicating statistical significance at the 95% confidence level. Two asterisks (**) denote a p-value less than 0.01, indicating even higher statistical significance at the 99% confidence level. Three asterisks (***) show a p-value less than 0.001, indicating extremely high statistical significance at the 99.9% confidence level.

2.8. Conclusion

The fabrication and characterization of microfluidic electrochemical sensors proposed in this thesis have been comprehensively described, highlighting both the materials and methods employed, as well as the experimental setup and analysis procedures. These sensors, featuring both straight and curved microchannels, were meticulously designed and fabricated using advanced microfabrication techniques in a cleanroom environment.

PET sheets and PSA layers were utilized for their transparency, flexibility, and ease of fabrication. Graphene-carbon paste was chosen for electrode fabrication due to its favorable properties such as good printability, low-temperature curing, and excellent electrical conductivity. The experimental setup involved the integration of various advanced tools and equipment for accurate and reliable measurements, including syringe pumps, a potentiostat, an oven, a laser cutting machine, and a microscope.

Parametric studies were conducted to investigate the effects of channel radius of curvature and flow rate using $K_3[Fe(CN)_6]$ as a standard solution. The optimized sensor characteristics were then utilized for the detection of various Pb concentrations in spiked and real waters. Statistical analysis, including ANOVA and t-tests, was performed to assess the significance of experimental variables on the sensor's performance. Results demonstrated the significance of microfluidic channel curvature on the sensor's response, with statistically significant differences observed across different flow rates and sensor types.

Furthermore, the determination of the sensor's performance metrics such as LOD, LOQ, and sensitivity provided valuable insights into its analytical capabilities. These metrics were calculated based on experimental data obtained from known concentrations of the analyte, enabling a comprehensive evaluation of the sensor's performance.

CHAPTER 3.

Novel Microfluidic Electrochemical Sensor to Investigate the Effect of Dean Flow on Square Wave Voltammetry

3.1. Introduction

To promote the widespread application of MES, it is imperative to simplify the fabrication and integration process, better understand and leverage the interactions between flow patterns induced by the geometry of the microchannels, and improve analyte detection through electrode design, and so on. This chapter summarizes the results from experiments conducted to address the objectives of this project.

Objective 1 aims to address the inherent complexities of sensor development by investigating alternative materials and simplifying multi-step fabrication processes in order to improve future mass production techniques. New methods, transitioning from a PDMS/gold electrode MES to a PSA/silver paste electrode MES, were used to accomplish this as explained in Chapter 2. We will summarize our findings in sections 3-1 of this chapter.

Objective 2 explores the complex relationship between analyte-electrode interaction and convective Dean vortex flow in microfluidic channels concurrently. This objective is to investigate how channel geometry affects sensor performance by examining the effect of channel curvature on electrochemical measurements using well-known sample solution containing 5 mM potassium ferricyanide in 0.1 M KCl.

The output peak current in SWV measurement depends on parameters such as concentration, diffusion coefficient of the sample solution, concentration boundary layer (region near an electrode where the concentration of electroactive species differs significantly from the bulk solution) thickness, electrode surface area, microchannel height, flow rate, etc ^{74,75}. The thickness of the diffusion layer is the region in the vicinity of an electrode where the concentrations differ from their value in the bulk solution. The thickness of the diffusion layer is the imaginary vertical distance from the electrode surface where the concentration approaches asymptotically the value

in the bulk solution near the electrode surface ⁷⁶. We hypothesized that secondary Dean flow may affect the output signal in the electrochemical measurements ^{1,2,77–79}. Electrochemical test results with curved-channel MES proved this hypothesis, explained thoroughly in sections 3-2.

3.2. Cost and Fabrication Simplicity of Various Microfluidic Electrochemical Sensor Devices

As discussed in Chapter 2, the first set of MESs were made from PDMS microchannels bonded to gold-on-glass electrodes (Fig. 2.2 and Fig. 2.3) due to the wide availability of this technique. Using photolithography and physical vapor deposition (PVD), gold was first patterned on the glass to make the electrodes. Then, by employing the soft lithography technique, the PDMS-based microchannel was created as the microfluidic part of the sensor. Finally, these two parts were bonded together to form the final sensor. SWASV was performed in high-concentration sample solutions such as 10^{-4} M $\text{Pb}(\text{NO}_3)_2$ prepared in 0.1 M KCl supporting electrolyte. The pre-deposition voltage and time were set to be -1 V and 60 s, respectively, and frequency and amplitude were 15 Hz and 0.02 V, respectively.

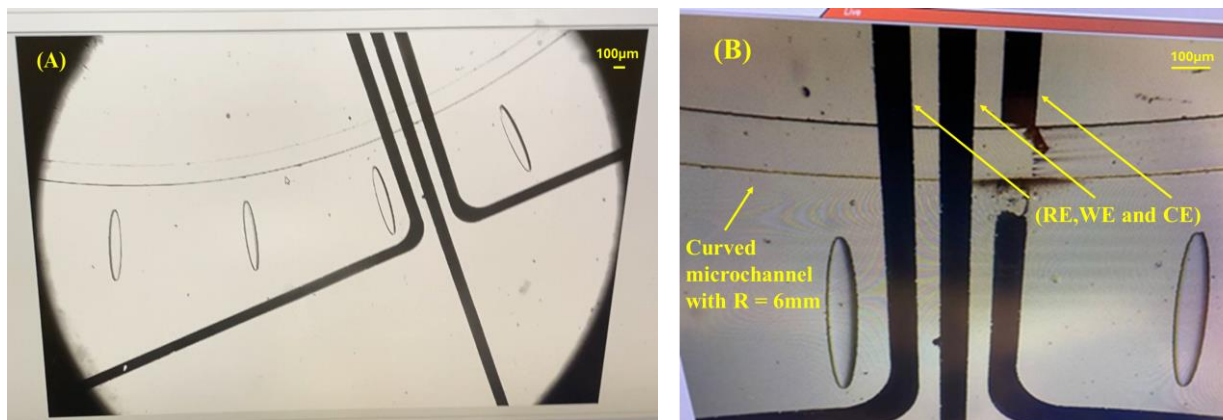


Fig. 3.1 SWASV measurements of PDMS channel/gold electrode-based MES. (A) Microscope image of three RE, WE, and CE on a large screen before running the SWASV. (B) CE corroded away during running the SWASV because of the chemical reaction between the CE and sample solution. SWASV parameters setting was as follows: The pre-deposition voltage: -1V, pre-deposition time: 60s, frequency: 15 Hz, and amplitude: 0.02 V. 10^{-4} M $\text{Pb}(\text{NO}_3)_2$ prepared in 0.1 M KCl was used as a test solution.

Fig. 3.1A shows the three electrodes before running the SWASV. The inverted microscope and the large screen in this photo were used to monitor the electrodes during the SWASV. As shown in Fig. 3.1B, the SWASV test resulted in CE detached within the microchannel after applying a -1 V potential for 60 seconds. The method's vulnerability was apparent after SWASV, which revealed that this fabrication method is unsuitable for continuing electrochemical measurements, especially in high-concentration solutions. Chemical reactions between the CE material and the sample solution content may cause the counter electrode's destruction and detachment. There is a chromium (Cr) layer underneath the gold layer to enhance the bonding between the gold electrode and the glass substrate in the sensor. During square wave anodic stripping voltammetry, both gold and chromium undergo oxidation processes at the electrode interface. Gold typically undergoes controlled oxidation and reduction cycles, maintaining its structural integrity. However, chromium oxidation can lead to the formation of chromium oxides, which may weaken the adhesion between the chromium layer and the glass substrate, ultimately causing detachment of the gold electrode. Therefore, the specific oxidation of chromium is more likely to contribute to the observed detachment of the gold counter electrode from the substrate surface.

Another drawback was the fabrication complication and price of each MES using this method. Significant equipment investments and continuous maintenance are required for cleanroom facilities, and expensive materials like photoresists, substrates, and deposition materials like gold or platinum are needed for processes like photolithography and lift-off. Moreover, careful monitoring and optimization of all fabrication processes—from mask design to photolithography, deposition, and lift-off—are essential in cleanroom settings. However, these processes frequently take several days, hindering rapid prototyping and development advancement. Using the cleanrooms at York University each sensor costs about eighty dollars and fabrication process took at least three hours to complete. We decided to develop another method for several reasons mentioned above and switched to PSA and PET material and laser cutting technique as described in section 2.4 of Chapter 2. This low-cost method offered scalability, enabling the mass production of sensors in large quantities to meet market demands.

We also decided to examine the possibility of using the commercial SPE for our sensor fabrication process. The cost-effectiveness of commercial SPEs (which is 8\$ per each) compared to the traditional cleanroom methods aligns well with our goal of scalable production to meet market

demands. However, upon further examination, it became evident that the electrode configuration of available SPE options would limit our ability to study the effect of curvature on the output signal. Integrating curved microfluidic structures with our desired sensor geometry in the SPE proved to be unfeasible. This realization prompted us to explore alternative approaches. We switched to laser cutting of PSA and PET to make a MES as described in Section 2.4 of Chapter 2. This method enabled us to maintain cost-efficiency (using this method and the material, the estimated cost for MES was less than 2\$ per sensor) while accommodating the necessary experimental parameters crucial for our research objectives.

In this research, a successful fabrication and integration technique aimed at simplifying the production process of MES was achieved. Carving electrodes and microchannels on PSA with laser cutting technology employs a more efficient and economical fabrication method instead of the more conventional ones. Layer-by-layer assembly is made more accessible, and manufacturing costs are reduced when PSA is used as the substrate material, which adds to the overall simplicity of fabrication. The microfluidic channel layer's integration with the electrode layer is straightforward in the developed fabrication process. Therefore, we decided to continue with PSA-PET-based MES.

Paper-based microfluidic electrochemical sensors (their price typically is about \$10 per square meter⁸⁰) may be within our sensor's price range, but their primary drawback is their inability to be used for monitoring of samples in a continuous flow manner. It is important to note that continuous-flow monitoring and fluid dynamics control on top of electrodes is not feasible for SPEs or paper-based microfluidic electrochemical sensors.

3.3. Effect of Channel Curvature on Square Wave Voltammetry of Potassium Ferricyanide

As per the second objective of the thesis, we hypothesize that the output signal of a curved-channel MES may be higher than that of straight-channel MESs. The question is what might happen to the sensor's peak current when the microfluidic layer's geometry changes from straight to curved microchannel design. To examine this, SWV measurements were conducted with PET-PSA based

ME. All measurements were carried out at a constant flow rate of 3 mL/min to evaluate the sensing performance of different types of MESs, from straight-channel sensors (Sensor 1) to sensors with curved microchannels (Sensor 2: R=9 mm, Sensor 3: R=6 mm, Sensor 4: R=3 mm). SWV responses of these sensors are displayed in Fig 3.2. The t-test statistical analysis result of the peak current densities can also be seen in the inset of this picture.

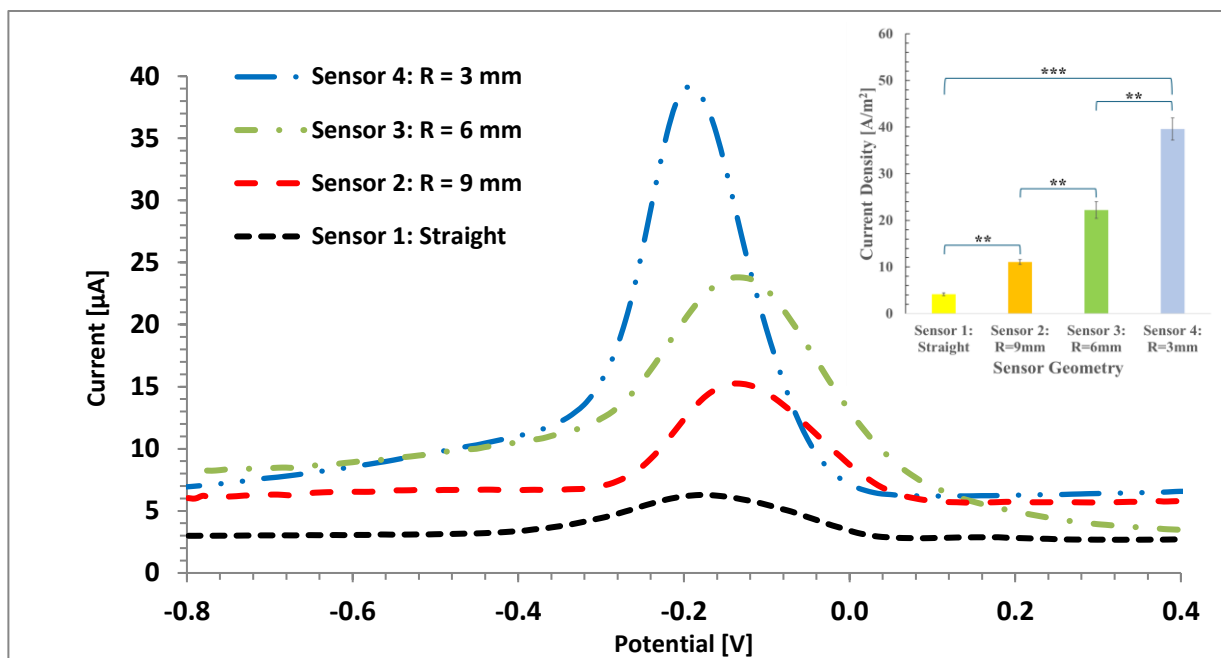


Fig. 3.2 Current as a function of the potential in SWV measurements for different types of MESs (straight- and curved-channel sensor with R=9mm, 6mm, and 3mm) at a 3 mL/min flow rate. The sample solution is 5 mM $K_3[Fe(CN)_6]$ prepared in 0.1 M KCl. The inset compares the significance of the peak current increase for various types of sensors using t-test analysis. (N=4 SWV runs in one device per condition). One asterisk (*) corresponds to a p-value less than 0.05, indicating statistical significance at the 95% confidence level. Two asterisks (**) represent a p-value less than 0.01, indicating statistical significance at the 99% confidence level. Three asterisks (***) show a p-value less than 0.001.

As can be seen, the straight-channel peak magnitude was the lowest among all the sensors, and the curved-channel sensor with R = 9mm was the lowest peak current among the curved-channel sensor, and as radii of curvature decreased to R= 3mm, the signal increased. As can be seen in Fig 3.2 inset, the current density increases by 4 folds as R reduces from R= 9 mm to R= 3 mm, signifying the effect of channel curvature and Dean flow on SWV of Potassium Ferricyanide. The

SWV was done with KCl alone, and there was not any peak in the range of potential shown in Fig 3.2.

From the literature⁸¹ the corresponding potential for peak current for potassium ferricyanide is +0.2 V to +0.3V vs. Ag/AgCl RE, while for SWV measurement it was around -0.2 V vs graphene-carbon hybride RE. We can attribute this observation to RE material we used in our research. Ag/AgCl electrodes consist of a silver and silver chloride while our RE, on the other hand, comprises carbon materials that can influence the electrode performance, including potential stability and resistance to fouling. The carbon-based REs may exhibit different electron transfer kinetics compared to traditional reference electrodes like Ag/AgCl, contributing to potential drift⁸².

For more elaboration on the observed phenomenon in curved-channel sensors, we can attribute it to Dean vortices forming within the curved microchannel cross-section and influencing the kinetics of electrochemical reactions. These vortices continuously mix analyte ions with supporting electrolyte ions and transport them toward the electrode surface as they circulate within the channel. This enhanced mixing of sample solution, which contains the target analyte ion and supporting electrolyte ions, is hypothesized to reduce concentration gradients, ensuring a more uniform analyte distribution near the electrode. In other words, in microfluidic electrochemical sensors with curved microchannels, convective transport facilitates analyte movement from the bulk solution towards the electrode surface more rapidly than diffusion alone, and the counter-rotating vortices effectively reduce the thickness of the concentration boundary layer by supplying more ions from bulk to vicinity of the electrodes. Consequently, analyte ions experience higher momentum in reaching the electrode surface within the vortices, leading to faster reaction rates and increased peak currents in electrochemical measurements.

3.4. Effect of Flow Rate on Square Wave Voltammetry of Potassium Ferricyanide

After noting the significant improvement in SWV performance in MESs due to channel curvature, which is attributed to secondary fluid flow, a series of experiments were conducted at various flow rates to investigate the effect of flow rate on the peak current in SWV measurements. Our interest

in exploring different flow rates stemmed from previous observations highlighting the superior performance of curved microchannels in SWV. By systematically varying flow rates, we aimed to determine the relationship between flow velocity and SWV enhancement, thereby deepening our understanding of the complex fluid dynamics within the sensors. These experiments were conducted using the curved-channel sensor with the highest peak current, which was the sensor with $R=3$ mm radii of curvature (hereafter, we name it curved sensor), and the sensor with the lowest peak current, which was the straight-channel sensor (hereafter, we name it straight sensor). The findings are depicted in Fig. 3.3 and Fig 3.4 respectively.

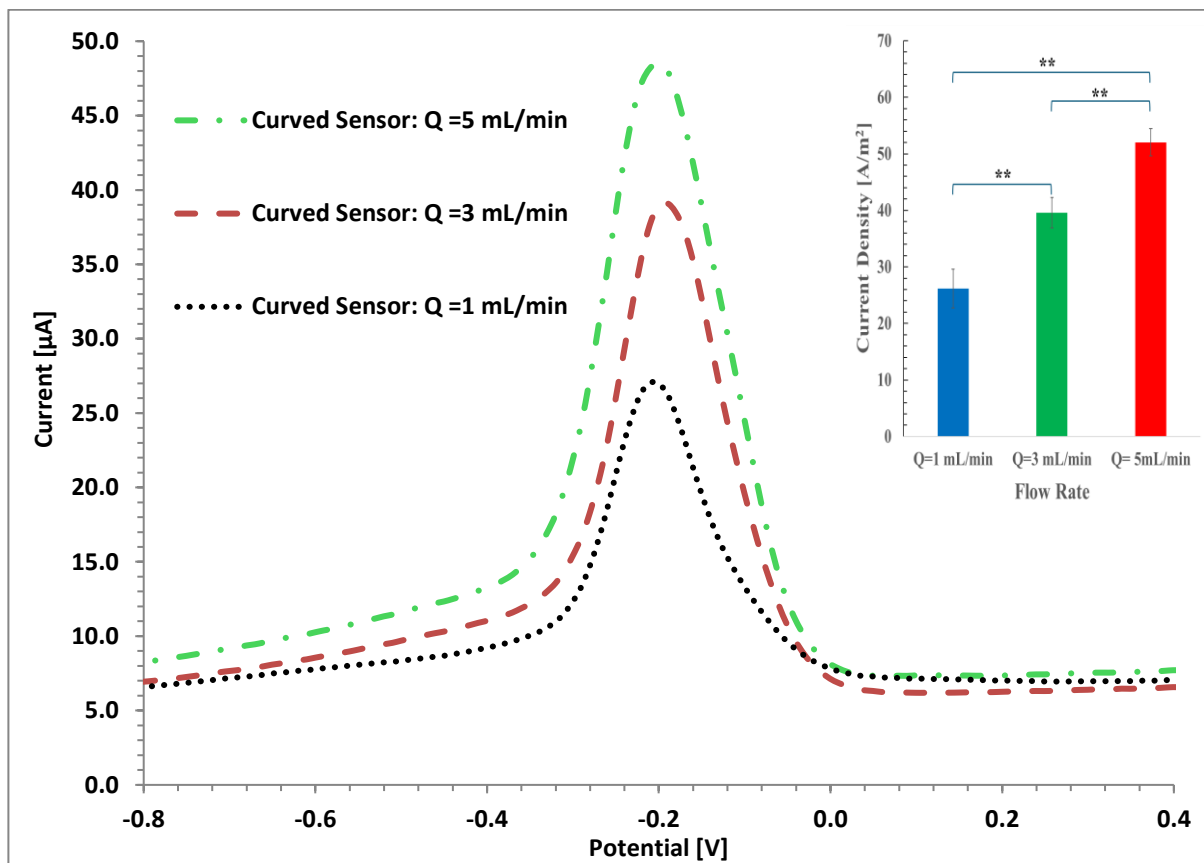


Fig. 3.3 Current as a function of the potential in SWV measurement for $R=3$ mm curved MES at different flow rates (1-5 mL/min). The sample solution is 5 mM $K_3[Fe(CN)_6]$ prepared in 0.1 M KCl. ($N=4$ SWV runs in one device per condition). Inset: compares how significant the peak current increase is for curved sensors by varying the flow rate using t -test analysis.

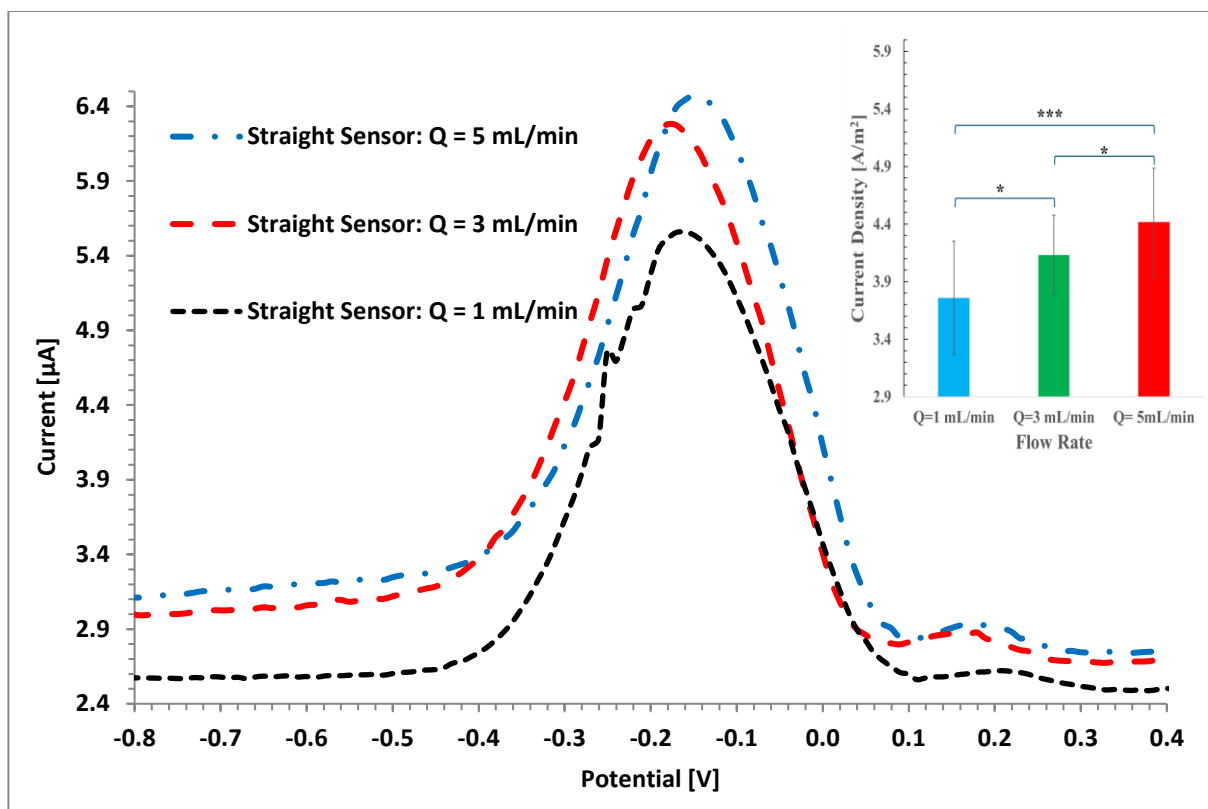


Fig. 3.4 Current as a function of the potential in SWV measurement for the straight-channel MES at different flow rates (1-5 mL/min). The sample solution is 5 mM $K_3[Fe(CN)_6]$ prepared in 0.1 M KCl. (N=4 SWV runs in one device per condition). The inset compares the significance of the peak current increase for a straight sensor by varying the flow rate using t-test analysis.

Fig 3.3 shows that the peak current increases by increasing the flow rate for the 3 mm sensor, which has the highest output signal. Also, the inset of this figure shows that varying flow rates significantly affect the peak current with a p-value lower than 0.01 (presented by two asterisks in the figure). Fig. 3.4 show the result of the same experimental test with the straight sensor. As seen in this figure, while the peak current is substantially lower than the curved sensor, it still increases when the flow rates increase. The t-test analysis shows a p-value lower than 0.05 (one asterisk) when the test is done between 1mL/min and 3mL/min and between 3 mL/min and 5 mL/min. The significance of the flow rate effect on peak current density (the peak current value divided by working electrode surface area) is more apparent when we compare the impact of flow rates between 1 mL/min and 3 mL/min. The t-test result gives us a p-value lower than 0.001, depicted with three asterisks in the figure. As shown in this figure, the peak current demonstrates a positive

correlation with the increasing flow rate, which is in agreement with the established theory in electrochemistry^{75,83,84}. From Equation 1-2, the peak current has direct proportionality with $(1/3)$ root of flow rate. Our results show the direct proportionality of these two parameters for both straight and curved sensors. However, the percentage of increase in the peak current for the 3 mm sensor is about 131%, while for the straight sensor, it is about 21% under the same conditions. This is most probably due to the Dean flow that strengthens with the flow rate in the curved channel, while the laminar flow in the straight channel is not affected by the flow rate significantly (Re raises from 32.4 to 162.4 when the flow rate increases from 1 to 5 mL/min, maintaining the flow regime under laminar conditions).

3.5. Peak Current Density Comparisons for Curved- and Straight-Channel Microfluidic Electrochemical Sensors

This section illustrates the outcomes of five sets of SWV measurements in two separate figures for comparative analysis. The relationship between sensor curvature and MES performance will be shown using the flow rate and Dean number effect on the current density of the sensor. By employing SWV, the investigation aims to unravel how varying sensor geometries influence electrochemical behaviors.

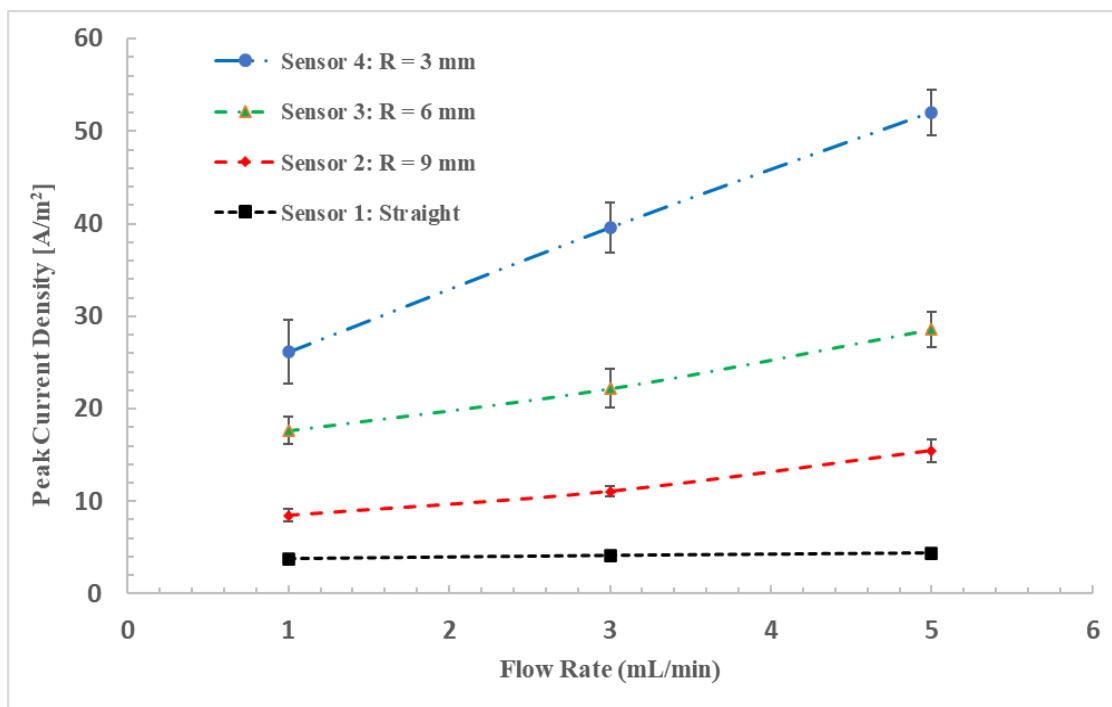


Fig. 3.5 Peak current density as a function of the flow rate for the four types of MESs (straight- and curved-channel sensors with $R=9\text{mm}$, 6mm , and 3mm). The working electrode surface area is 0.9 mm^2 , 0.88 mm^2 , 0.89 mm^2 , and 0.86 mm^2 for the straight sensor, 9 mm , 6 mm , and 3 mm sensor, respectively. ($N=4$ SWV runs in one device per condition). The sample solution is $5\text{ mM K}_3[\text{Fe}(\text{CN})_6]$ prepared in 0.1 M KCl .

Fig. 3.5 reveals that increasing the flow rate results in a rise in current density for both straight and curved sensors, with the effect being more pronounced in curved sensors. It can be easily seen that the current density increases at each specific flow rate (from 1 mL/min to 5 mL/min) by changing the type of sensor from straight to curved. Notably, the 3 mm sensor consistently exhibits the highest current density at a given flow rate, while the straight sensor consistently shows the lowest. Specifically, the current density in the 3 mm sensor increases significantly from 21.9 A/m^2 to 50.8 A/m^2 as the flow rate escalates from 1 mL/min to 5 mL/min , indicating its heightened sensitivity to flow rate changes. The steeper slope observed in the current density vs. flow rate curve for the 3mm sensor suggests that secondary flow effects become more significant in tighter curvatures, where stronger vorticities are present. These findings highlight the considerable influence of sensor curvature on the relationship between flow rate and current density, with curved sensors demonstrating greater responsiveness to changes in flow rate compared to straight sensors.

Fig. 3.6 presents the current density data as a function of the Dean number (Eq 2.2), reflecting the vortices' strength based on the Dean number values for different MESs. As defined in Chapter Two, the Dean number for the straight sensor remains zero across all flow rates. However, as the Dean number increases, the current density also rises for the curved sensors. Notably, the 3 mm sensor graphs exhibit steeper slopes, indicating a higher rate of increase with Dean number. Furthermore, a notable observation from this figure is that the highest peak corresponds to the 3 mm sensor at specific Dean numbers for curved sensors. This suggests that at the same Dean number, geometries with sharper radii of curvature display higher current density. Consequently, to enhance sensor performance from the perspective of increasing current density, minimizing the radius of curvature could be a beneficial suggestion.

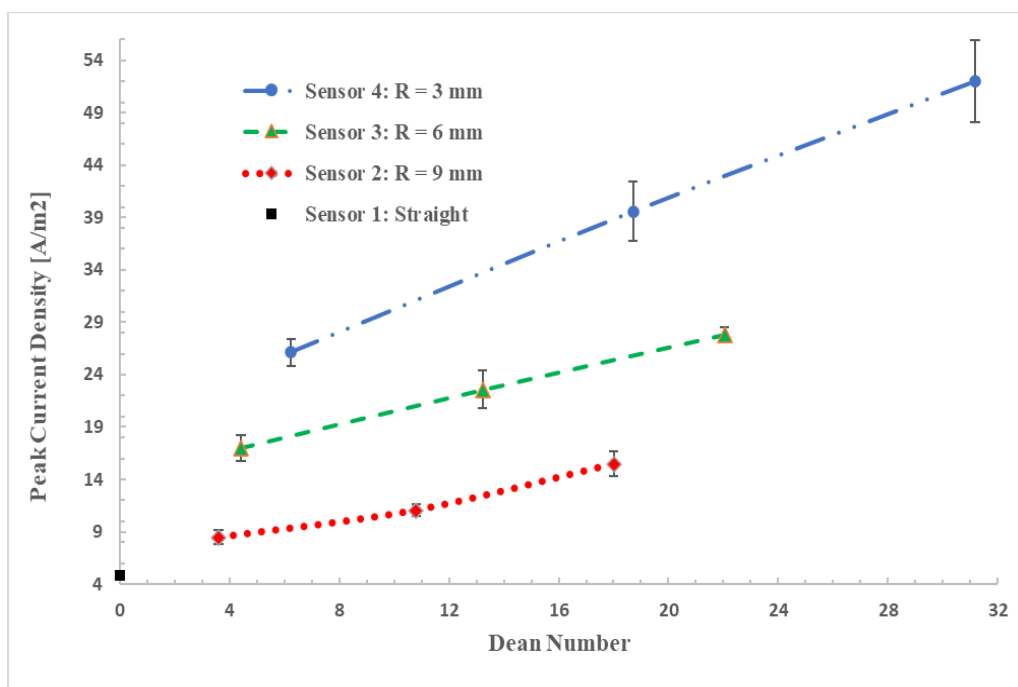


Fig. 3.6 Peak current density as a function of the Dean number for the four types of MESs (straight- and curved-channel sensors with $R=9\text{mm}$, 6mm , and 3mm). The maximum injection flow rate is 5 mL/min for all devices. The working electrode surface area is 0.9 mm^2 , 0.88 mm^2 , 0.89 mm^2 , and 0.86 mm^2 for the straight sensor, 9 mm , 6 mm , and 3 mm sensor, respectively. ($N=4$ SWV runs in one device per condition). The sample solution is 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ prepared in 0.1 M KCl .

3.6. Comparing Microfluidic Electrochemical Sensor with Commercial Screen-Printed Electrode

We did electrochemical measurements to compare our device with the commercial carbon screen printed electrode (CSPE) introduced in Chapter 2⁸⁵. According to the literature review in Chapter 1, SPEs have gained significant attention due to their portability, disposability, and ease of mass production. CSPEs are widely used for electrochemical sensing applications due to their excellent conductivity, chemical stability, and compatibility with various analytes. As indicated in the previous section, our sensor is less expensive than SPEs, and the main disadvantage of SPEs is that they cannot be used for fluid handling or continuous monitoring while performing electrochemical measurements.

In this research, electrochemical measurements were conducted to compare the performance of our novel carbon graphene hybrid paste sensor with a commercial carbon SPE. The electrochemical measurements were performed using the same potentiostat system. The working electrode and the counter electrode were carbon and graphene-carbon for SPE and our sensor, respectively, while the reference electrode was silver for the commercial SPE and carbon graphene hybrid paste for our sensor. The sample solution consisted of a 5 mM potassium ferricyanide ($K_3[Fe(CN)_6]$) prepared in a 0.1 M KCl electrolyte.

Fig 3.7 compares the electrochemical performance of the commercial carbon SPE and our novel carbon graphene hybrid paste sensor.

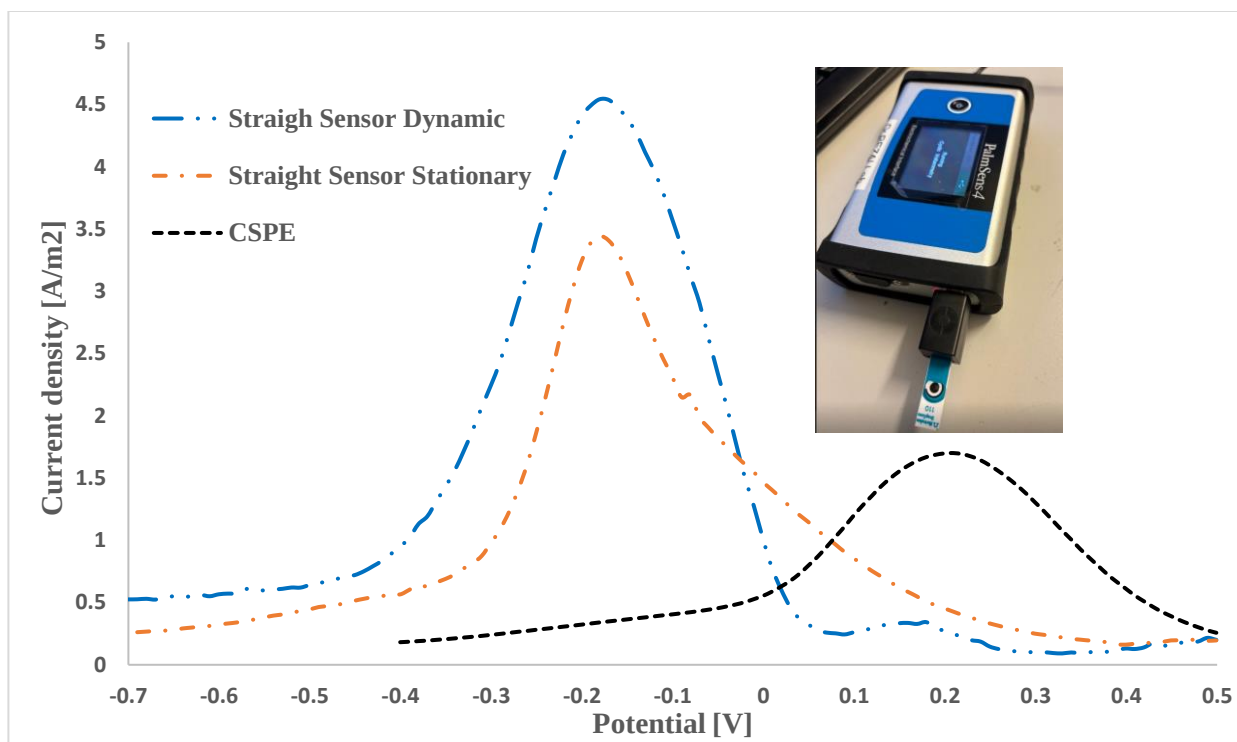


Fig. 3.7 Current density as a function of the potential in SWV measurement for comparing the commercial CSPE with our straight-channel MES. The inset shows the SPE connecting to the potentiostat for SWV measurements. The sample solution is 5 mM $K_3[Fe(CN)_6]$ prepared in 0.1 M KCl. WE and CE are carbon for SPE, and the reference electrode is silver.

One notable observation is that even a straight sensor geometry without sample injection generates a higher current density than commercial SPE. The curved-channel MES data is not shown as it produces even more significant results than the straight-channel MES. This difference in current density can be attributed to several factors. One reason can be the electrode's surface area, which is 0.86 mm^2 for the straight sensor and 12.56 mm^2 for SPE. Another reason can be the distance between the electrode surface and the analyte. In MES, this distance is small due to the confined microscale dimensions of the microchannels. This reduced diffusion distance can result in faster mass transfer rates and higher current densities. As a 3rd justification for the higher current density observed with our sensor suggests enhanced electrochemical activity, which can be attributed to the unique properties of the carbon graphene hybrid paste. Carbon graphene hybrids offer advantages such as increased surface area, improved conductivity, and enhanced electron transfer kinetics, resulting in superior electrochemical performance.

Additionally, the graph shows the corresponding potential for our sensor and the commercial SPE at which the peak current occurs. There is a difference in the peak current potential between the two electrodes. The variation in the reference electrode material is the cause of this discrepancy. Our sensor incorporates a carbon graphene hybrid paste reference electrode, whereas the commercial SPE uses a silver reference electrode. Because of differences in the electrode potential and electron transfer kinetics, the choice of reference electrode material can significantly impact the measured potential—the same sample solution used for all experiments.

3.7. Statistical Analysis of the Results

In previous sections, we showed that the effectiveness and performance of the MESs depend on various factors, including flow rate and geometric curvature, both of which can influence the sensor's output signal. In this section, we explore the impact of these factors on the peak current of the developed MES in SWV measurements, employing a statistical analysis.

Every pair of different sensor types—straight-channel and curved-channel sensors with different curvature radii—was analyzed using the t-test. Furthermore, the effect of flow rate on the sensor's peak current was analyzed, and the findings are appended to the corresponding figures. Using the ANOVA analysis, we wanted to determine whether the means of the peak current is the same for all factor-level combinations. In other words, to confirm the t-test results, which examined two pairs of experiments, two-factor ANOVA with a replication test was performed to support the claim that flow rate and curvature significantly affect the peak current. Table 3.1 shows the ANOVA test results, another confirmation of the hypothesis. This table shows two main factors: flow rate and sensor type. Each factor has multiple levels.

The null hypothesis for a two-factor ANOVA test asserts that there are no appreciable differences in the means of the peak current between the various flow rates and sensor types. In other words, it implies that during SWV tests, the sensor's peak current significantly changes when the flow rate changes or when we change the curvature of the channel. The null hypothesis is rejected if the test's p-value is less than the predefined significance level, which, in our case, is 0.05 (95% confidence level).

Table 3.1 Two-factor ANOVA test for proving the effect of curvature on the MES output signal

ANOVA							
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>	<i>Result</i>
Flow rate	989.0666	2	494.5333	156.6322	1.72452E-18	3.259446	TRUE
Type of the sensor	8398.554	3	2799.518	886.6836	8.84199E-34	2.866266	TRUE
Interaction	692.8602	6	115.4767	36.57462	6.85988E-14	2.363751	TRUE
Within	113.6625	36	3.157291				
Total	10194.14	47					

The results in Table 3.1 indicate that the type of sensor and the use of curved microchannels significantly increase the sensor's peak current. The results also indicated that there are significant interactions between the factors. When interactions are significant, analyzing the two factors separately using one-way ANOVA is advisable.

3.8. Conclusion

In this Chapter, Initially, we started with a conventional fabrication approach, utilizing PDMS microchannels bonded to gold-on-glass electrodes for making the MES, which, despite its wide availability, posed challenges such as CE detachment and high fabrication costs associated with cleanroom processes.

Transitioning to PSA/PET and laser cutting technology offers a compelling solution. These technologies enable efficient fabrication and integration of MES at a fraction of the cost compared to available methods. The layer-by-layer assembly process is streamlined, and using PSA as a substrate material enhances simplicity and reduces manufacturing expenses. Additionally, PSA-PET-based MES demonstrates the potential for continuous monitoring applications, addressing the limitation of paper-based microfluidic sensors.

By leveraging low-cost fabrication methods and substitute materials, the developed PSA-PET-based MES offers a viable alternative for rapid prototyping and mass production, meeting market demands for affordable and scalable sensor solutions.

The analysis clarifies how crucial microfluidic channel curvature is in affecting the functionality of MES. After extensive testing and analysis of electrochemical measurements with the potassium ferricyanide and KCl redox couple, it was clearly shown that sensors with curved microchannels—especially those with more tight curvatures—show noticeably higher peak currents than straight sensors.

The observed improvement in sensor performance is attributed to the formation of Dean vortices within the curved channels, which facilitate convective transport and mitigate concentration gradients near the electrode surface. This phenomenon accelerates analyte movement towards the electrode, promoting faster reaction rates and increased peak currents in electrochemical measurements. Additionally, secondary flows within curved channels contribute to convective-diffusive mass transfer near the electrode, further augmenting sensor performance.

Furthermore, comparative analysis with commercial carbon screen-printed electrodes highlights the superiority of MES in terms of both performance and cost-effectiveness. Even a straight sensor geometry without sample injection demonstrates higher current density than commercial SPEs, underscoring the potential of MES for various electrochemical sensing applications.

The statistical analyses, including t-tests and two-factor ANOVA, corroborate the significant impact of curvature and flow rate on sensor performance, further validating the research findings. These insights provide valuable guidance for optimizing MES design and fabrication processes, ultimately enhancing their analytical potential for applications including environmental monitoring, biomedical diagnostics, and chemical analysis. In particular, the 3 mm sensor emerges as a promising candidate for detecting Pb in drinking water, our next objective of the thesis.

CHAPTER 4.

Lead Detection in Drinking Water Using the Developed Straight- and Curved-channel Microfluidic Electrochemical Sensors

4.1. Introduction

Earlier sections outlined our goals and strategy for conducting electrochemical tests on the $K_3[Fe(CN)_6]$ solution. In Chapter 4, we apply our findings to determine how to detect Pb in water samples. Leveraging the insights from Objective 2, this chapter presents the results obtained from the experimental validation of our approach to address Objective 3: Detecting lead in simulated and real water samples.

The first step involved employing SWV to perform comparative detection of specific lead concentrations using the best-performing device identified in our previous investigations in Chapter 3, compared to a straight-channel sensor. The chosen device featured a curved microchannel measuring 900 μm in width, 126 μm in height, and 38 mm in length, with a radius of curvature of 3 mm. This device was compared to one with a straight microchannel, with the same width, height, and length, to evaluate the effects of Dean flow in curved channels on sample mixing and the efficacy of lead detection.

Subsequently, we investigated the impact of pre-deposition voltage and time on the performance of both straight-channel and curved-channel sensors. Conducting SWASV measurements of Pb at 10^{-4} M in 0.1M KCl electrolyte, we compared the peak current responses across different pre-deposition times. Then we investigated the detection capabilities of our sensors across a range of Pb concentrations, from 10^{-8} M (2.072 $\mu\text{g/L}$) to 10^{-4} M (20.72 mg/L), using SWASV and applying the selected pre-deposition voltage and time from the previous step. Tests were conducted with straight-channel and curved-channel sensors in deionized (DI) water spiked with lead ions and 0.1 M KCl as the supporting electrolyte. The sensor parameters, such as sensitivity, LOD, and LOQ, were determined in this step.

Finally, similar measurements were performed on real water. The sensors demonstrated the ability to detect lead in complex environmental matrices. DI water provides a controlled environment that facilitates validating our sensing approach under ideal conditions, enabling us to assess its output signal without interference. On the other hand, real water samples introduce the complexity of environmental matrices, offering insights into the practical applicability of our methodology in real-world scenarios.

4.2. Comparison of Microfluidic Electrochemical Sensor with Straight and Curved Microchannels for Pb Detection

Based on the knowledge gained in Chapter 3, we aimed to investigate if adding curved-channel microfluidic components to a sensor can improve its performance and increase the MES's capacity to detect heavy metals like Pb ions. As stated previously, we aim to explore the possible effects of switching from a straight-channel sensor design to a curved-channel sensor design on Pb detection sensitivity and LOD.

A 1 mM concentration of $\text{Pb}(\text{NO}_3)_2$ was prepared in a solution containing 0.1 M KCl. Subsequently, SWV measurements were conducted using straight- and curved-channel sensors. Fig. 4.1 compares the current density as a function of the potential for a MES with a curved microchannel (hereafter called curved-channel sensor) with a radius of curvature of 3 mm and a MES with a straight microchannel (hereafter called straight-channel sensor) at different flow rates. For a better comparison of the effect of sensor type and flow rate on lead detection in the microfluidic electrochemical sensor, all experiments were performed without any pre-deposition of the ions on the electrode surface and only with a bare electrode at a concentration of 10^{-3} M of $\text{Pb}(\text{NO}_3)_2$ prepared in 0.1 M KCl solution.

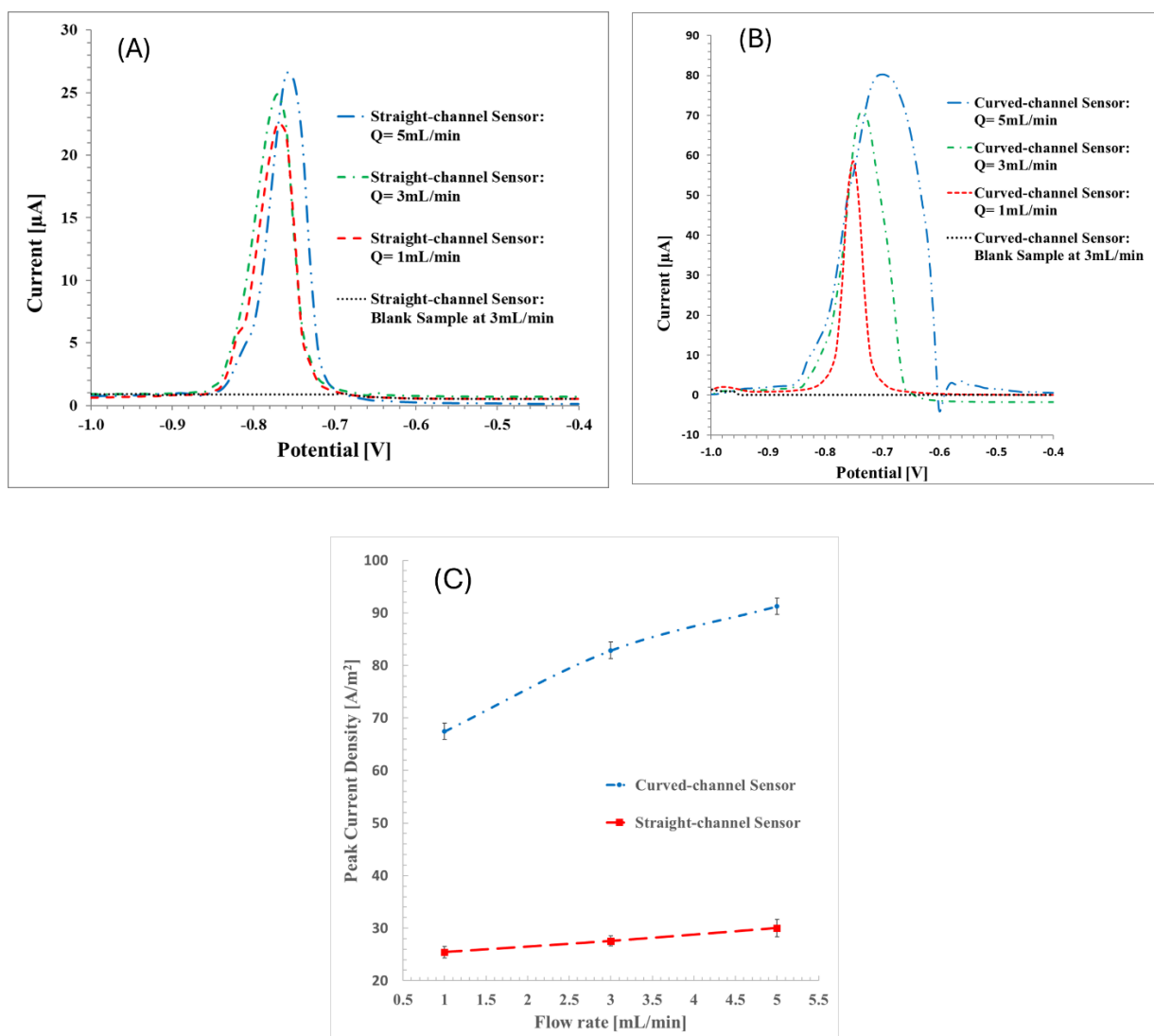


Fig. 4.1 SWV detection of Pb in straight and curved channel MESs. Comparison of SWV current response as a function of applied potential for MESs with (A) straight and (B) $R=3$ mm curved microfluidic channels at different flow rates (1-5 mL/min). (C) SWV peak current density as a function of flow rate for the curved- and straight-channel sensors ($N=4$ SWV runs in one device per condition). All samples consist of 1 mM $\text{Pb}(\text{NO}_3)_2$ and 0.1 M KCl in DI water. A blank sample of 0.1 M KCl in DI water was also tested as a negative control.

Fig. 4.1A illustrates the SWV current versus voltage measurements conducted on straight-channel sensors at different flow rates. A blank sample was first injected through the sensor, and the measured current was zero, which was the potential range we used for SWV. This figure only displays our results at a 3 mL/min flow rate in the straight channel sensor since the sensor response

remained similar across all other flow rates for the blank sample. However, when the straight channel was tested with lead-spiked KCl electrolyte, there was a SWV peak current at the voltage of -0.78V. This figure also shows a slight enhancement in the peak current as the flow rate increases in the straight-channel sensor.

Fig. 4.1B illustrates the SWV current as a function of potential conducted on curved-channel sensors at different flow rates. The blank sample showed a similar no-current response in this sensor, while the addition of Pb resulted in a significant current peak, which was stronger than the straight-channel sensor. Similar to the findings for $K_3[Fe(CN_6)]$ solutions from the previous chapter, it can be seen that microfluidic electrochemical sensors with curved microchannels exhibit superior sensing capabilities compared to straight-channel sensors in Pb detection. This improvement can be attributed to the formation of Dean vortices within the curved channels, which enhance convective transport, reduce concentration gradients, and accelerate analyte movement toward the electrode surface, leading to increased reaction rates and peak currents, as discussed in Chapter 3. Curved channels with secondary flows and recirculation zones can prolong analyte residence times, allowing for longer contact with the electrode surface. We took advantage of smaller, sharper curvatures' radii, known to improve SWV measurement performance, to increase fluid circulation and hasten analyte transport. Curved sensors' strong secondary flow facilitates the interaction between the electrode and analyte by obstructing the passage of fluid particles through the sensing region and extending the duration of interaction with the electrode surface. Combining these factors and parameters is responsible for the observed improvement in sensing performance.

From Fig. 4.1B, it can also be seen that there is a slight shift in the peak potential when the flow rate increases. A reference electrode made of graphene-carbon paste was used for all sensors. This type of electrode works differently from the standard ones like Ag/AgCl or saturated calomel electrode (SCE) in terms of its ability to maintain a stable potential between the working and reference electrodes. As a result, we anticipate seeing some differences in the potential corresponding to the peak current in the SWV measurements because of using the carbon-based reference electrode^{86,87}.

In other words, the observed differences in Fig. 4.1B in corresponding peak potentials when repeating experiments with a Graphene-carbon reference electrode compared to Ag RE can be

attributed to various factors influencing the electrochemical behavior of the system. Carbon-based electrodes possess distinct surface properties, including surface functional groups and conductivity, which can significantly impact analyte adsorption and desorption kinetics during electrochemical reactions. Additionally, the potential stability of carbon electrodes may differ from that of Ag electrodes, leading to variations in the applied potentials and observed peak potentials in SWV measurements. Moreover, differences in electrolyte composition and electrode polarization behavior between the two types of reference electrodes further contribute to the observed discrepancies^{88,89}.

Fig. 4.1C shows the peak current density (peak current divided by working electrode surface area) of the SWV measurement as a function of flow rate for straight-channel and curved-channel sensors. The curved-channel sensor had higher current density than the straight-channel sensor at all flow rates. Another important observation that can be used to confirm the effect of secondary flow on output signal is the rate of increase of peak current in two types of sensors. The rate of increase of peak current density for the curved-channel sensor was higher than that for the straight-channel sensor. For instance, varying the flow rate from 1 mL/min to 5 mL/min caused an increase of 18% for straight sensors, while this was 35% for curved sensors. As can be seen, similar to our previous finding for $K_3[Fe(CN)_6]+KCl$ solution, in SWV measurement, the peak current density for the curved-channel sensor was at least twice that of the straight one in all ranges of flow rates. This figure also shows that the peak current increases for curved and straight sensors as the flow rate increases.

4.3. Square Wave Anodic Stripping Voltammetry for Better Comparison of Pb Detection in Curved and Straight Sensors

In this section, using the advantages of the SWASV, we want to allow for the preconcentration of trace amounts of metal ions onto the electrode surface, which enables better quantification of lead ions concentrations, making it suitable for comparison of the straight-channel and curved-channel results at lower concentrations. After the characterization of SWASV parameters, the effect of pre-deposition voltage and time is investigated on the performance of both straight-channel and

curved-channel sensors. For this purpose, SWASV of Pb at 10^{-4} M prepared in 0.1M KCl electrolyte was conducted in straight- and curved-channel ($R=3$ mm) sensors. In all measurements, the concentration was one order of magnitude lower compared to the SWV measurement in the previous section.

Reducing the concentration of the solution from 10^{-3} M to 10^{-4} M in SWASV measurements mitigates potential issues associated with electrode fouling and limiting current effects. At higher concentrations, such as 10^{-3} M, the deposition of Pb ions onto the electrode surface can be substantial, leading to the formation of fouling layers that hinder electron transfer and cause a change in electrode surface property^{90,91}. Additionally, when the electrochemical reaction rate has reached its maximum value due to the availability of reactants at the electrode (due to the high concentration of the target analyte), then maximum current would be achieved in SWV, which is called “limiting current”. This can manifest as a plateau in the current response in the current versus potential graphs. Lowering the concentration to 10^{-4} M reduces the flux of Pb ions toward the electrode surface, minimizing the likelihood of limiting current effects and mitigating electrode fouling. This adjustment enables more reliable and reproducible SWASV measurements and selects the pre-deposition potential and time for the next step with lower concentrations.

Selecting the optimal pre-deposition time in sensor experiments involves a delicate balance between several key factors, including the need for a prompt experimental response and the desire for a higher output signal. Initially keeping the pre-deposition time fixed at 60 seconds, the applied potential varied from -0.8 to -1.3 volts. The observed trend in peak current versus potential was ascending. A potential of -1.2 volts was selected instead of -1.3 volts to prevent undesired electrochemical reactions that could cause electrode damage, hydrogen evolution, and high background noise under highly negative voltage conditions^{40,92,93}. Another consideration was selecting a pre-deposition time which is the important factor for a timely experimental response. A shorter pre-deposition time, such as 60 seconds, offers the advantage of quicker results, reducing the overall experiment duration and enabling faster data collection. However, this expedited timeline must be balanced against the need for sufficient interaction between the sensor surface and the target analyte. Short pre-deposition time may not allow adequate time for the accumulation of Pb ions on the surface of the electrode, potentially compromising the sensitivity or accuracy of the measurement. Therefore, it must be increased to ensure it does not sacrifice signal quality or

accuracy. On the other hand, a longer pre-deposition time—300 seconds, for example—allows for a higher signal response during test runs. Longer durations provide more opportunities for the Pb ions and working electrode to interact, which improves signal strength. This benefit, though, comes at the cost of an extended experiment's duration, which is not always viable or practical, particularly for applications with a need for quick analysis or real-time monitoring. Moreover, high pre-deposition times may raise the possibility of saturation effects or unintended side reactions, which could result in incorrect readings. In some previous research, it has been shown that with increasing the pre-deposition time after a specific point, there is a reduction in peak current⁹⁴. On the other hand, even though a longer pre-deposition period might improve signal strength, it must be weighed against the requirement for prompt data collection.

Samples were injected through the sensors and SWASV was run at different time frames. Current as a function of potential at each time frame was collected for both straight- and curved-channel sensors (Fig. 4.2A and Fig. 4.2B) and the peak current density as a function of time was plotted in Fig. 4.2C. Four replicate experiments were conducted at each set of conditions.

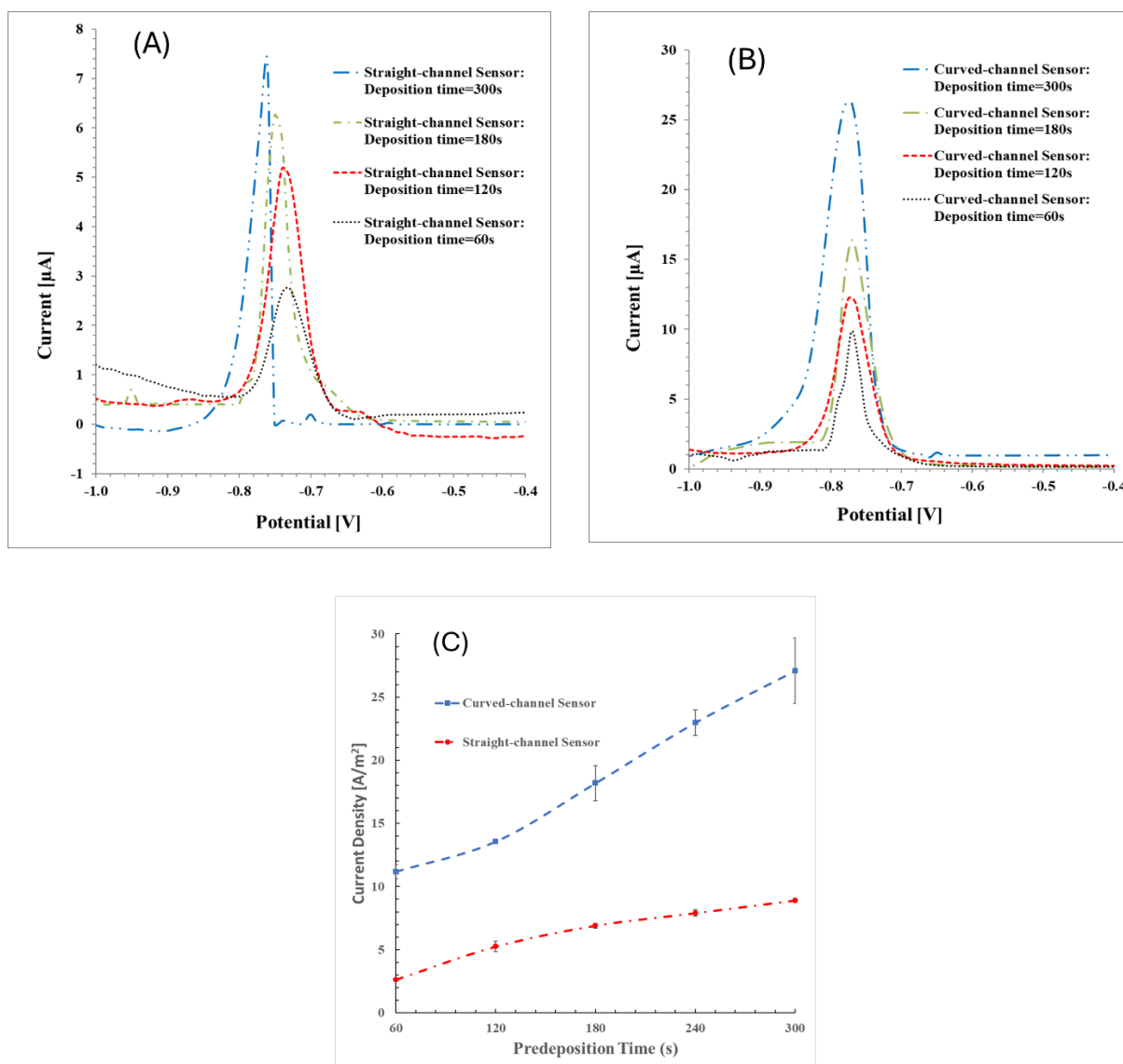


Fig. 4.2 SWASV detection of Pb in straight and curved channel MESs. Comparison of SWASV current response as a function of applied potential for MESs with (A) straight and (B) $R=3$ mm curved-channel sensor by varying pre-deposition time (60-360 s); (C) SWASV Peak current density as function of pre-deposition time for straight- and curved-channel sensor; ($N=4$ SWASV runs in one device per condition). All samples consist of $0.1 \text{ mM Pb}(\text{NO}_3)_2$ (20.72 mg/L) and 0.1 M KCl in DI water.

Fig. 4.2A and Fig. 4.2B show the impact of pre-deposition time on the peak current in both straight- and curved-channel sensors. These figures reveal that in all ranges of the pre-deposition time, which varies from 60s to 300s, the peak current for the curved sensor is higher than the straight one. This observation is expected because longer pre-deposition times allow more analytes to

accumulate on the electrode surface. Fig. 4.2C represents the peak current density as a function of the pre-deposition time for the straight- and curved-channel sensors. As can be seen, it is evident that when the deposition time is increased, the response (peak current density) of the straight-channel and curved-channel sensors increases. The slope of the current density versus time graph is sharper for the curved-channel sensor because, during the pre-deposition, the circulating vortex flow in the cross-section of the curved-channel of the sensor may cause more ions to accumulate on the electrode surface compared with the straight-channel sensor⁹⁵⁻⁹⁷. Another reason supporting this finding could be attributed to the secondary flow dynamics, which promote enhanced electrode surface coverage by analyte ions during the pre-deposition process. Considering the information mentioned above, pre-deposition time of 180 seconds was selected for our future experiments. This intermediate value allows a moderate amount of time for the sensor surface to interact with the analyte, facilitating a strong signal response. At the same time, it ensures a reasonable experimental turnaround time, enabling timely data collection without sacrificing signal quality.

4.4. Lead Dose-Response Curves of the Straight- and Curved-Channel Microfluidic Electrochemical Sensors

In this section, we investigated the ability and characteristics of our sensors to detect Pb at different concentrations from 10^{-8} M (2.072 $\mu\text{g/L}$) to 10^{-4} M (20.72 mg/L). The 10^{-8} M concentration is below the MAC threshold. For all SWASV measurements, the predisposition voltage was set to -1.2V, and the pre-deposition time was selected to be 180s, as discussed before. Different concentrations of lead ions were added to deionized water, with the addition of 0.1 M KCl serving as a supporting electrolyte during serial dilution. All SWASV tests were performed by injecting the sample solution through the sensor at a 3 mL/min flow rate. Fig. 4.3 shows the dose-response of the straight-channel and curved-channel sensors at various concentrations of lead.

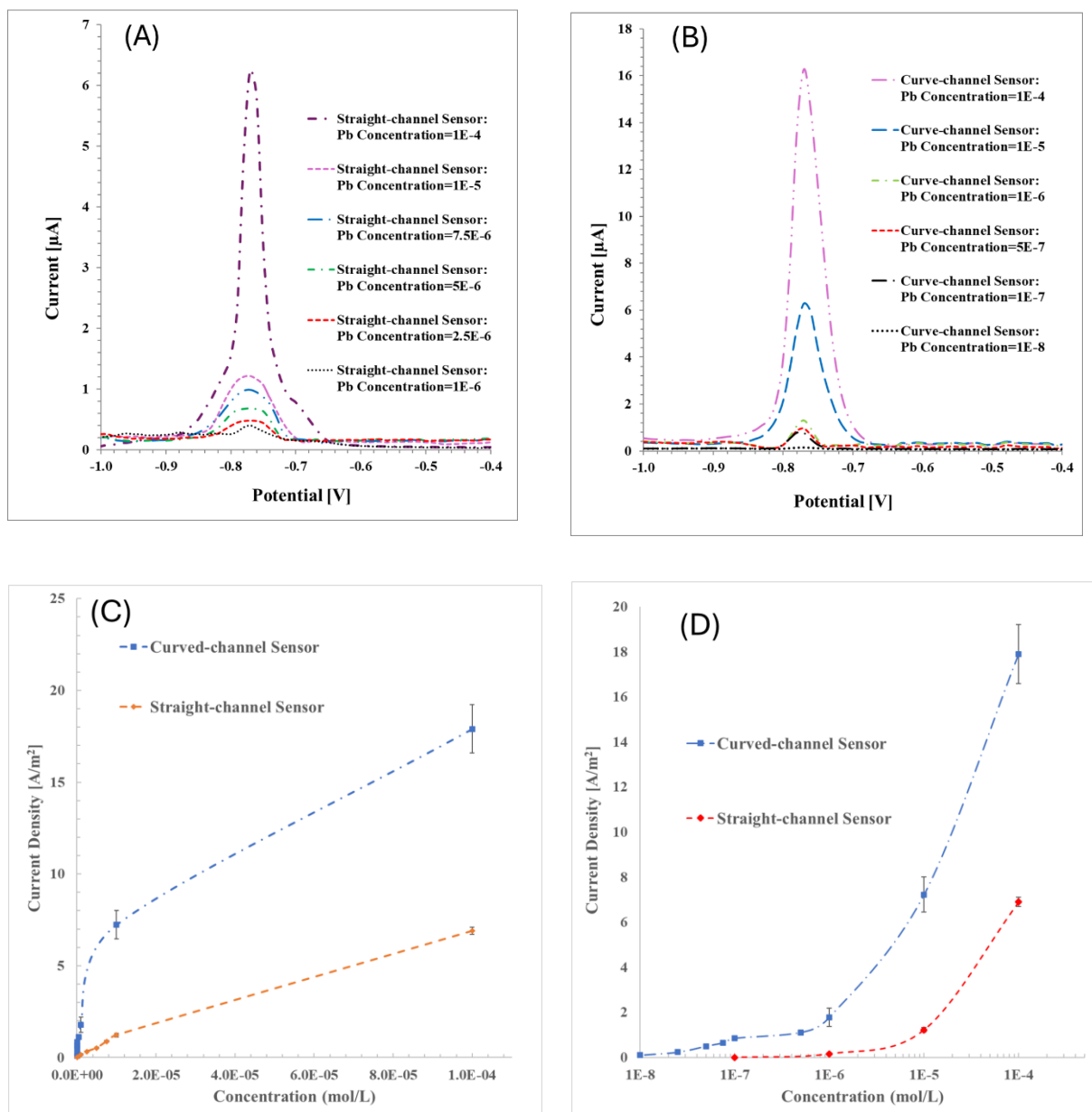


Fig. 4.3 SWASV-based Pb dose-response curves of straight and curved channel MESs. Comparison of SWV current response as a function of applied potential for MESs with (A) straight and (B) $R=3$ mm curved-channel sensor at different Pb concentrations (10^{-8} - 10^{-4} M); (C) SWASV peak current density as a function of concentration for the curved- and straight-channel sensors; (D) SWASV peak current density as a function of concentration (logarithmic scale) for the curved- and straight-channel sensors ($N=4$ runs in one device per condition). All samples consist of serial diluted concentration of $\text{Pb}(\text{NO}_3)_2$ and 0.1 M KCl in DI water.

The straight-channel sensor response for variation of Pb concentration from 10^{-6} M to 10^{-4} M is depicted in Fig. 4.3A. The figure shows that swiping the potential from -1 V to -0.4 V peak current occurs at around -0.78 V, and increases as the concentration of Pb increases. As can be seen the minimum concentration that was measured with this sensor is 10^{-6} M. For the curved-channel sensor, the same SWASV measurement was performed as can be seen in Fig. 4.3B. Again, similar to Fig. 4.3 sweeping the potential from -1 V to -0.4 V peak current occurs around -0.78 V, and the peak current increases as the concentration of Pb increases. The minimum concentration measured was 10^{-8} M, which is significantly (Based on the two-way ANOVA test, the p-value was 1.2×10^{-10}) higher than the straight-channel sensor. By comparing peak current against the potential for both curved-channel and straight-channel sensors, it can be found that the electrochemical response of the curved-channel sensor is higher than the straight one at the same concentration. In fact, Fig. 4.3A and Fig. 4.3B show that the curved-channel sensor exhibits a higher peak current for all concentrations compared to the straight-channel sensor. The reason behind this observation was explained previously in Sections 4-2 and 4-3.

Fig. 4.3C shows the peak current density of Pb-spiked DI water at various concentrations (dose-response curve) for both curved and straight sensors. The y-axis represents the peak current density, measuring the peak current per unit area of the working electrode. This figure reveals a higher current density by varying the lead concentration for the curved sensor type, offering higher sensitivity and efficiency in detecting lead ions. As discussed in more detail in the next sections, the straight channel could not detect the concentration below 10^{-6} M (207.2 $\mu\text{g/L}$), while the curved sensor could detect concentrations as low as 10^{-8} M (2.072 $\mu\text{g/L}$).

Implementing a logarithmic scale along the x-axis, the peak current density of Pb-spiked DI water at various concentrations was redrawn in Fig. 4.3D. This adjustment enables a more detailed depiction of the information and better representation of the data, offering enhanced clarity and insight into the trends observed within the dataset.

4.4.1. Assessment of Straight-Channel Microfluidic Electrochemical Sensor Performance in Pb Detection in DI Water

To assess the performance of the straight sensor, a detailed examination of the dose-response curves is necessary, along with the calculation of the LOD, LOQ, and sensitivity of this sensor. Fig. 4.4A and Fig. 4.4B illustrate the peak current as function of potential in linear range and corresponding calibration curve of the straight-channel sensor respectively.

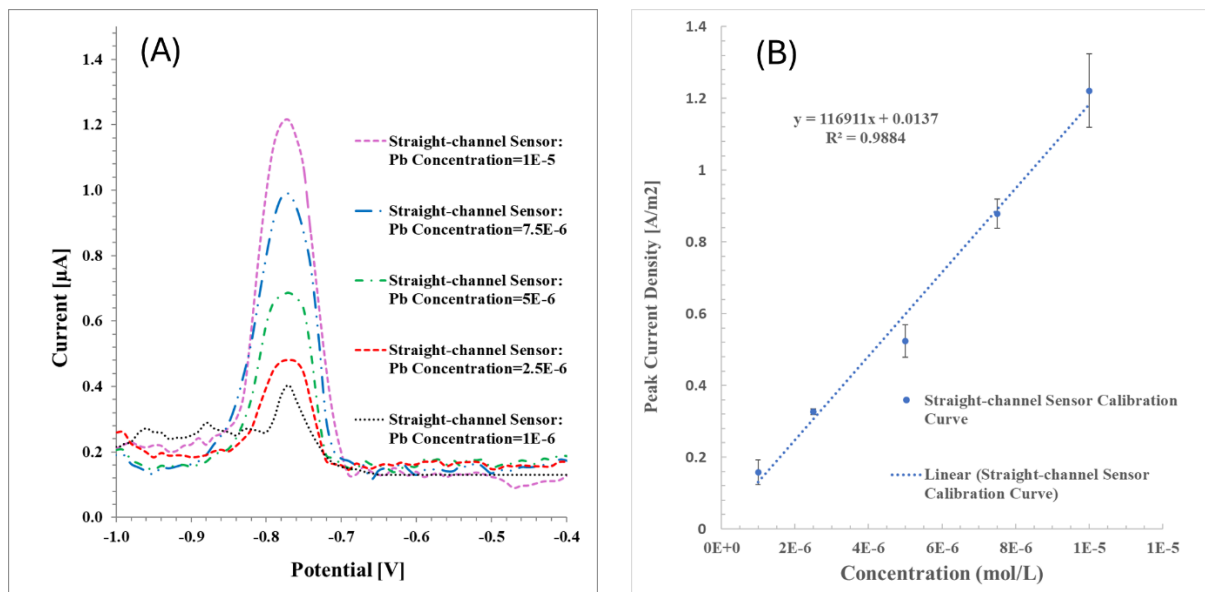


Fig. 4.4 Pb dose-response curve in linear range for the straight-channel MES. (A) Current as a function of potential for various concentrations of Pb (10^{-6} M to 10^{-5} M) in DI water for Microfluidic electrochemical sensors with the straight microchannel. (B) Peak current density as a function of concentration, i.e., the dose-response curve for the straight-channel sensor. The sample solution consists of different concentrations of Pb prepared in 0.1 M KCl.

Getting the peak current values from Fig. 4.4 A and calculating the peak current density and plotting these values against the concentration, the calibration curve was drawn (Fig. 4.4B).

According to the R-square values obtained for the best curve fitting through the various concentration points in the SWASV tests for this sensor, the linear range is determined to be from 10^{-6} M to 10^{-5} M. Based on the calibration curve in Fig. 4.4B, the sensor's LOD and LOQ were 8.7×10^{-7} (0.18 mg/L) and 2.9×10^{-6} (0.6 mg/L), respectively. The sensor's sensitivity was $0.105 \text{ A}/\text{M}$ ($0.5 \mu\text{A}/(\text{mg}/\text{L})^{-1}$).

4.4.2. Assessment of Curved-Channel Microfluidic Electrochemical Sensor Performance in Pb Detection in Deionized Water

To assess the curve-channel MES performance and compare it with the straight-channel sensor, the same procedure for the straight-channel was repeated. From Fig. 4.3C, we can visually observe that there are two ranges in the graph that curved-channel sensor seems to show a linear behavior within. For finding and verifying these two ranges, the R-square test on various ranges was performed. Based on the R-square values displayed in Table 4.1, which is obtained from best curve fitting through the various concentration points in the SWASV tests for this sensor (Fig. 4.3C), these two linear ranges were quantitatively determined for the curved-channel MES.

Table 4.1 R-square values for determining the linear range in curved-channel sensor

	Concentration [M]	Concentration [$\mu\text{g/L}$]	R-square value
1 st linear range investigation	10^{-8} to 5×10^{-8}	2.072 to 10.36	0.9978
	10^{-8} to 7.5×10^{-8}	2.072 to 15.54	0.9857
	10^{-8} to 10^{-7}	2.072 to 20.72	0.991
	10^{-8} to 5×10^{-7}	2.072 to 103.6	0.6539
2 nd linear range investigation	10^{-7} to 10^{-6}	20.72 to 207.2	0.9653
	10^{-7} to 10^{-5}	20.72 to 2072	0.9968
	10^{-7} to 10^{-4}	20.72 to 20720	0.9272

From Section 4-3 and based on our observation in Figure 4-3, we found that the curved-channel sensor shows bimodal behavior in the range of the concentrations we run the SWASV. From Table 4-1 can be seen as we increase the range of concentration from 10^{-7} M to 5×10^{-7} M, the R square value reduces from 0.991 to 0.6539, which leads us to decide the linear range should be 10^{-8} M to 10^{-7} M. The same procedure was continued to detect the second linear range which was 10^{-7} M to 10^{-5} M. Figure 4-4 shows the SWASV graphs and calibration curves for the MES with curved

microchannels in the two linear ranges determined above. The figure is divided into four panels, each depicting specific aspects of the sensor's behavior and calibration.

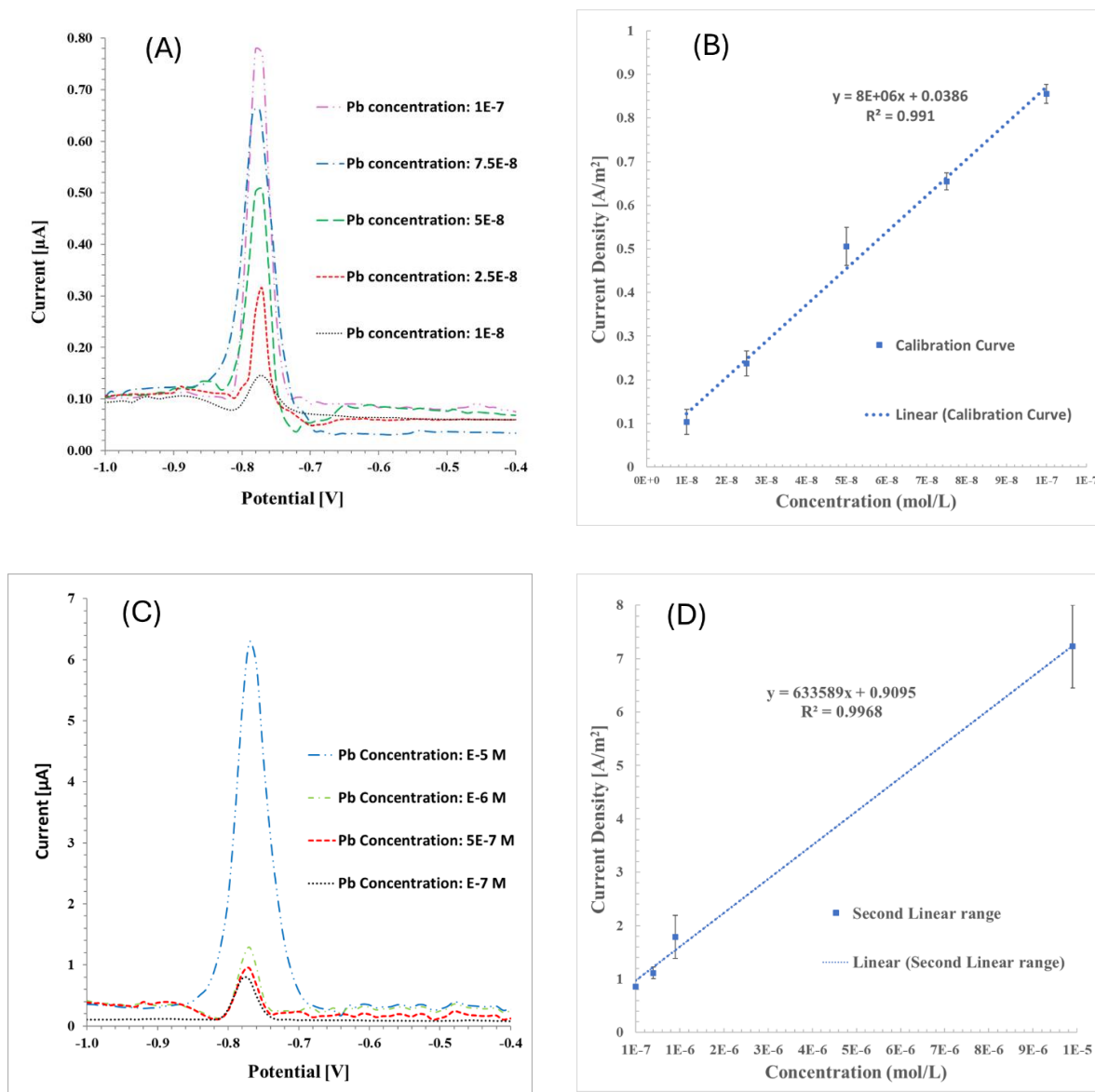


Fig. 4.5 Dose-response curve in linear range and calibration curve for curved-channel sensor. (A) Current as a function of potential for a trace amount of $\text{Pb}(\text{NO}_3)_2$ (10^{-8} M to 10^{-7} M) in DI water with 0.1 M KCl as supporting electrolyte; (B) Current density as a function of concentration: calibration curve for the curved-channel sensor in the concentration of $\text{Pb}(\text{NO}_3)_2$ from 10^{-8} M to 10^{-7} M; (C) Current as a functional of potential for a higher concentration of Pb (10^{-7} M to 10^{-5} M) in DI water with 0.1 M KCl as supporting electrolyte; (D) Second linear range: linear best-fit line for curved-channel sensor in the concentration of $\text{Pb}(\text{NO}_3)_2$ from 10^{-7} M to 10^{-5} M. The sample solution consists of different $\text{Pb}(\text{NO}_3)_2$ (aq) concentrations prepared in 0.1 M KCl.

Fig. 4.5A compares peak current versus potential for trace amounts of lead (ranging from 10^{-8} M to 10^{-7} M) spiked in deionized (DI) water. This panel initially assesses the sensor's sensitivity and response to varying concentrations of lead ions.

Fig. 4.5B displays the curved sensor's first linear range and calibration curve, focusing on the concentration range of lead ions from 10^{-8} M to 10^{-7} M. The calibration curve facilitates quantitative analysis by determining lead ion concentrations based on the observed peak currents. The linearity of the curve within this concentration range indicates the sensor's reliability and accuracy in measuring low concentrations of lead ions in DI water samples. According to this calibration curve, the LOD, LOQ, and the sensitivity of the sensor were 1.09×10^{-8} M (2.26 $\mu\text{g/L}$), 3.63×10^{-8} M (7.53 $\mu\text{g/L}$), and 6.88 A/M ($0.0332 \mu\text{A}/(\mu\text{g/L})^{-1}$), respectively.

These essential values reveal a significant improvement for the curved channel sensor in enhancing the sensitivity and lowering the LOD. The sensitivity of the curved-channel sensor is approximately 66-fold more significant than that of the straight-channel sensor. This means that this sensor produces a response approximately 66 times stronger for the same change in concentration compared to the straight-channel sensor. On the other hand, The LOD of the curved-channel sensor is approximately 75-fold smaller than that of the straight-channel sensor. Similarly, the LOQ of the curved-channel sensor is approximately 79 fold smaller than that of the straight-channel sensor. This means the curved-channel sensor can reliably quantify lead concentrations approximately 79 times lower than the straight-channel sensor.

Fig. 4.5C compares peak current versus potential to higher concentrations of lead ions (ranging from 10^{-7} M to 10^{-5} M) spiked in DI water. This figure shows how the device behaves at higher concentrations and evaluates the sensor's performance across a higher concentration range. The trends in peak current variation versus potential that have been observed provide information about how the sensor responds dynamically to rising lead ion concentrations.

Fig. 4.5D presents the second linear range for the curved sensor, covering the concentration range of lead ions from 10^{-7} M to 10^{-5} M. Similar to Fig. 4.5B, this curve provides a quantitative relationship between the sensor's response and the lead ion concentration but focuses on a higher concentration range. The sensor's suitability for detecting moderate to high concentrations of lead ions is confirmed by establishing another linear relationship within this concentration range. The

sensitivity of the sensor in this range is 0.544 A/M ($0.0026 \mu\text{A}/(\mu\text{g/L})^{-1}$), which is smaller than the first linear range (lower concentration range) sensitivity—comparing these parameters for two kinds of MESs. It was found that the curve-channel sensor has better performance than the straight-channel sensor in terms of detection limit and sensitivity.

Table 4.2 presents the LOD, deposition time, electrochemical techniques, and characteristics of the different kinds of MES published to date for comparison to our curved-channel MES⁹⁸. This table compares the electrodes' material, detection limit, deposition time, and electrochemical methods used in the literature with the present work.

Table 4.2 Comparison of the MESs performance and characteristics with our research.

Author	Ion	Electrode	LOD	Deposition time	Technique	Characteristics
Jothimuthu <i>et al.</i> ⁹⁹	Pb, Cd, Mn	Bi			SWASV	Wide negative potential window
Jung <i>et al.</i> ³⁷	Pb	Planar Ag	$0.55 \mu\text{g L}^{-1}$	300 s	SWASV	Reusable
Wang <i>et al.</i> ¹⁰⁰	Pb	Au film array	$0.8 \mu\text{g L}^{-1}$	300 s	CV	An artificial counterpart of a shark's olfactory sensor with an increased surface area
Hong <i>et al.</i> ¹⁰¹	Pb, Cd	Microporous Mn_2O_3 -modified screen-printed electrode	$0.2 \mu\text{g L}^{-1}$	300 s	DPASV	Good selectivity and excellent reliability
Nantaphol <i>et al.</i> ¹⁰²	Pb, Cd	Boron-doped diamond paste electrodes	$1 \mu\text{g L}^{-1}$	300 s	SWASV	A wide potential window, a very low background current, and a high sensitivity
Zou <i>et al.</i> ³⁶	Pb, Cd	Planar Bi	$9.3 \mu\text{g L}^{-1}$	90 s	ASV	Does not require introducing additional Bi ions and alteration of the sample pH, low-cost
Zou <i>et al.</i> ³⁶	Pb,	Planar Bi	$9.4 \mu\text{g}$	90 s	ASV	Digitized and automated

Zhou <i>et al.</i> ³⁵	Cd Pb, Cd	Pt	L^{-1} 150 μg L^{-1}	10 s	CV	A convenient and low-cost method for the fabrication
Hong <i>et al.</i> ¹⁰³	Pb	S-shaped Screen-printed electrode	0.3 μg L^{-1}	120 s	SWASV	
Shi <i>et al.</i> ⁴²	Pb, Cd	Screen-printed carbon electrodes	2.0 and 2.3 μg L^{-1}	120 s	SWASV	Paper-based microfluidic devices
Williams <i>et al.</i> ¹⁰⁴	Cd	Six gold working electrodes			DPASV	
Medina-Sanchez <i>et al.</i> ⁴¹	Pb, Cd	Graphite ink	7 and 11 μg L^{-1}	120 s	SWASV	Paper-based microfluidic devices
Chaiyo <i>et al.</i> ¹⁰⁵	Pb, Cd	Boron-doped diamond electrode	0.1 μg L^{-1}	120 s	ASV	A wide potential window, a low background current, chemical inertness, and a readily renewable surface
Shen <i>et al.</i> ⁴⁰	Pb, Cd	Pristine graphite foil	1.8 μg L^{-1} and 1.2 μg L^{-1}	60 s and 180 s	SWASV	Modifier-free
Avuthu <i>et al.</i> ⁴⁰	Pb, Hg	Carbon electrode			CV	Phenanthroline-based selective detection
Nipapan Ruecha <i>et al.</i> ¹⁰⁶	Zn, Pb, Cd	Graphene-polyaniline (G/PANI) nanocomposite electrode	1, 0.1, and 0.1 μg L^{-1}		SWASV	Low-cost alternative solution for determination of heavy metals
Present work	Pb	Graphene-carbon hybrid paste	2.26 $\mu g/L$	180s	SWASV	Low-cost, easy to fabricate, modifier-free electrodes, capable of fluid handling

Our sensor, utilizing a graphene-carbon hybrid paste and employing SWASV for detection, presents a detection limit of 2.4 $\mu g/L$ for Pb, slightly higher compared to specific sensors such as

Boron-doped diamond electrodes by Chaiyo et al. ⁹⁸, Au film array by Wang *et al.* ³⁶ or pristine graphite foil by Shen et al. ⁴⁰. However, it falls within the range reported by other studies, which shows the excellent performance of our MES without any treatment or modification of the electrodes. For the sensors with lower detection limits in the table, it is notable that many of them are fabricated using metal deposition techniques, which tend to be very expensive. For instance, the Au film array by Wang et al. ³⁶ is produced through costly metal deposition methods, which are very expensive and cannot be employed in mass production of the MES. Additionally, it is worth mentioning that the only modifier-free microelectrode sensor in the literature is paper-based, as seen in the work by Shen et al. ⁴⁰ While this sensor achieves a lower detection limit, it is not suitable for fluid handling and continuous monitoring due to its paper-based nature. With a deposition time of 180 seconds, our sensor's characteristics include being low-cost, easy to fabricate, modifier-free, and capable of fluid handling, aligning well with the need for contaminant detection in environment, such as lead detection in drinking water. Therefore, our sensor's advantages make it a good alternative for practical heavy metal detection applications.

4.5. Curved-Channel Microfluidic Electrochemical Sensor Performance in Pb Detection in Real Water

To accurately determine the presence and concentration of lead in environments where human exposure may occur and to ensure that the results are applicable to real-world scenarios and regulatory standards, real water sample testing is required for lead detection. After completing the SWASV tests with simulated water, the capability of the devices was examined with real water samples obtained from the Southern Ontario surface water river system. We decided to test a real water sample spiking the Pb at three concentrations. The 10^{-8} M concentration of $\text{Pb}(\text{NO}_3)_2$ was spiked in the real water sample and diluted to three concentrations C1, C2, and C3, which are 1.09×10^{-8} M (2.26 $\mu\text{g/L}$), 2.4×10^{-8} M (or 5 $\mu\text{g/L}$), and 3.63×10^{-8} M (7.52 $\mu\text{g/L}$) respectively. Results are presented in Fig. 4.6. The maximum allowable lead concentration regulated by Health Canada, is 5 $\mu\text{g/L}$ ¹¹ in drinking water.

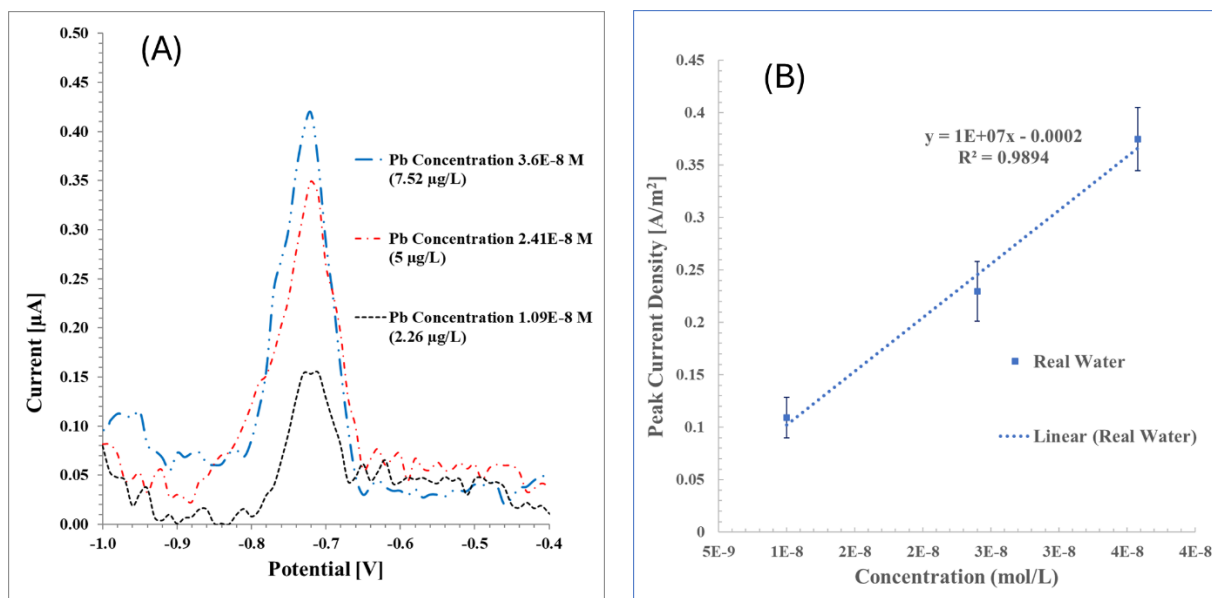


Fig. 4.6 SWAVV detection of Pb in real water sample in $R=3$ mm curved channel MESs; (A) current as a function of potential for a trace amount of Pb spiked in a real water sample (Southern Ontario surface water river system) (B) Peak current density values as a function of concentration for three critical concentrations: $C1= 1.09 \times 10^{-8}$ M (2.26 $\mu\text{g/L}$), $C2= 2.4 \times 10^{-8}$ M (or 5 $\mu\text{g/L}$), and $C3= 3.63 \times 10^{-8}$ M (7.52 $\mu\text{g/L}$); The injection flow rate was 3 mL/min. ($N=4$ SWASV runs in one device per condition).

Fig. 4.6A compares the current versus potential for the trace amount of lead spiked in real water samples using curved-channel MES. The data reveal distinct electrochemical responses corresponding to different lead concentrations, demonstrating the sensors' ability to detect lead in complex environmental matrices. As expected, higher lead concentrations elicited greater peak currents, indicating a proportional relationship between lead concentration and sensor response.

Fig. 4.6B illustrates peak current density values as a function of concentration. We previously obtained LOD and LOQ in DI and tested the same concentrations in real water here. Then, we observed that the signal was strong in these concentrations. The LOD was determined for the previous experiment with DI water to be 1.09×10^{-8} M (2.26 $\mu\text{g/L}$). A peak current was also observed for the lead sample prepared in real water at this concentration. Approaching the LOQ at 3.6×10^{-8} M, we observed that the sensor response closely aligns with the MAC established by Health Canada at 5 $\mu\text{g/L}$ for lead detection. Fig. 4.6B underscores the significance of three key

concentrations: LOD, LOQ, and MAC, illustrating the detection range of the microfluidic electrochemical sensors developed in our study.

Recovery is a criterion for evaluating the precision of analytical techniques used to identify target analytes in real samples^{107,108}. Recovery is the extent to which the target analyte's measured concentration agrees with the known or expected concentration. This measure is essential for assessing how well the electrochemical technique quantifies analytes among complex matrices in real-world samples, like real water samples. Moreover, the computation of recovery helps identify possible error sources, such as matrix effects and instrumental constraints, which makes the analytical approach easier to optimize and validate. The recovery (%) was calculated using the formula:

$$Recovery(\%) = \frac{\text{Measured concentration} - \text{Original concentration}}{\text{Spiked concentration}} \times 100\% \quad (4-1)$$

Where, the measured concentration is the estimated concentration of lead in the spiked real water sample obtained by extrapolating from the calibration curve. The original concentration is the concentration of lead in the real water sample before spiking. Since there was no detectable lead in the original real water sample we tested, this value is zero in our case. The spiked concentration represents the known concentration of lead added to the real water sample, which are C1, C2, and C3, mentioned at the beginning of this section.

The recovery percentage for the SWASV test with real water was between 95.8% and 109.6%. A recovery close to 100% suggests that the analytical method accurately quantifies the target analyte in the real water samples. The recovery percentage obtained from real water SWASV indicates that our MES was capable of providing reliable detection results.

4.6. Conclusion

In this chapter, we presented the experimental validation of our approach to detecting Pb in aqueous samples using MES. Leveraging SWV, we assessed the efficacy of sensors with straight and curved microfluidic channels. Our investigation included DI water and real water samples

spiked with specific concentrations of Pb, offering insights into our methodology's practical applicability.

The outcomes showed that curvature-enhanced microfluidic sensors are superior to straight counterparts in detecting lead. We found that curved sensors consistently had higher current densities through SWV measurements, regardless of the flow rates and lead concentrations. The creation of Dean vortices inside the curved channels, which promote convective transport, lessen concentration gradients, and quicken analyte movement towards the electrode surface, is responsible for this improvement in sensing capabilities. Secondary flows and recirculation zones further influenced Analyte residence times, which extended the analyte's interaction with the electrode surface and improved sensing performance.

Furthermore, we found that the peak current increment rate increased noticeably for curved sensors instead of straight sensors, suggesting that secondary flow plays a significant part in improving the output signal. Differences in fluid dynamics and electrode characteristics are responsible for the observed shift in peak current potential; the secondary flow supplies extra energy for electrochemical reactions close to the electrode surface.

Pre-deposition time optimization uncovered a trade-off between signal quality and fast experimental response. We ensured enough interaction between the sensor surface and the analyte by choosing an intermediate pre-deposition time of 180 seconds. This allowed us to achieve a strong signal response without compromising experimental turnaround time.

In SWASV experiments with simulated and real water samples, the curved microfluidic sensors exhibited higher sensitivity and efficiency in detecting lead ions than straight sensors. The calibration curve constructed for different lead concentrations highlighted the sensors' reliability and accuracy, confirming their suitability for quantitative analysis across a wide concentration range.

Our sensors showed the ability to detect lead levels at the MAC suggested by Health Canada, the LOQ obtained in simulated water experiments, and the limit of detection LOD when tested with actual water samples from the Southern Ontario surface water river system. These findings

highlight our research's practical relevance and societal impact, which provide dependable, sensitive, and reasonably priced sensing solutions to address lead contamination in drinking water.

Moreover, our sensor, employing a graphene-carbon hybrid paste and SWASV for detection, achieves a detection limit of 2.26 $\mu\text{g/L}$ for Pb. While slightly higher than specific sensors utilizing expensive metal deposition methods, it falls within the range reported by other studies, demonstrating its good performance without requiring treatment or modification of electrodes. Notably, it stands out as a low-cost, easy-to-fabricate, modifier-free, and fluid-handling-capable option, addressing limitations of paper-based sensors with lower detection limits but unsuitable for continuous monitoring. With these advantages, our sensor presents a promising solution for practical heavy metal detection, particularly in environments like drinking water, aligning well with the need for effective contaminant monitoring.

CHAPTER 5.

Thesis Summary and Recommendations for Future Work

This thesis has delved into the potential of incorporating curved microchannels into microfluidic electrochemical sensors to enhance their ability to detect lead in water. By addressing fabrication challenges and simplifying the production process, we aimed to make these sensors more accessible and cost-effective.

Even though the MESs are suitable for on-site and portable monitoring, their lower sensitivity and higher LOD of MES compared to laboratory-based analytical methods used to analyze water (e.g., ICP-MS) is another constraining factor for water quality monitoring when high accuracy and precision are needed. This encourages researchers to concentrate on lowering LOD and increasing sensitivity using various techniques.

We gained valuable insights into improving sensor performance through experiments exploring the effects of curvature on the sensor output signal, mainly focusing on the geometry of the microfluidic part of the sensor interaction between analytes and electrodes. Our research has demonstrated that curved-channel MES offer increased sensitivity and a lower limit of detection compared with straight-channel MES for lead detection.

5.1. Summary of Findings and Limitations of the Work

In this section, we summarize the findings based on the specific objectives identified in Chapter 1.

Objective 1: Simplifying the Fabrication and Integration process of the MES.

While significant progress has been made in advancing microfluidic electrochemical sensors for various applications, including lead detection in drinking water, there remain notable limitations in current designs, fabrication techniques, and testing methodologies.

One fundamental limitation is the complexity and cost of available fabrication methods, which often involve multi-step processes and specialized equipment. Despite efforts to streamline fabrication through innovative approaches, challenges persist in achieving straightforward, low-cost, consistent, and scalable production. This hinders the widespread adoption of MES in the commercial water quality monitoring market.

To achieve Objective 1, we focused on streamlining the MES device fabrication and integration processes, resulting in significant advancements in simplifying multi-step fabrication techniques, exploring alternative materials, and reducing production costs.

A new sensor production process was developed using materials like PSA, PET, and laser cutting technology, reducing the complexity and cost of the available fabrication methods. Our technique's estimated cost per sensor is significantly lower than that of available methods, making our novel MES a viable option for mass production and suitable for portable and on-site water quality monitoring.

In comparison to the cleanroom fabrication method, which was high-cost and vulnerable during electrochemical measurement, and existing alternatives such as SPEs and the PB-MES, our developed PSA-PET-based MES offers a significant cost advantage. By leveraging innovative fabrication techniques and cost-effective materials, our MES presents an economical solution without compromising continuous monitoring capabilities.

Objective 2: Investigating the Effect of Convective Vortex Flow on Enhancing Analyte-Electrode Interaction.

In Objective 2, the investigation delved into the impact of convective vortex flow, caused by a curvature microchannel design, on analyte-electrode interaction within the MES. By designing experiments and leveraging the sample solution of potassium ferricyanide and KCl, insights were gained into how channel curvature influences electrochemical measurements. Extensive testing and analysis have demonstrated that sensors with curved microchannels, particularly those with

tighter curvatures (small radii of curvature, e.g., $R = 3$ mm in our study), exhibit significantly higher peak currents than straight-channel sensors. This performance improvement is attributed to the formation of Dean vortices within the curved channels, which enhance convective transport and mitigate concentration gradients near the electrode surface. Additionally, secondary flows within curved channels contribute to convective-diffusive mass transfer, further augmenting sensor performance.

Statistical analyses, including t-tests and two-factor ANOVA, further validate the significant impact of curvature and flow rate on sensor performance. Comparative analysis with commercial CSPEs highlights the superiority of our MES in terms of performance and cost-effectiveness. Even straight-channel sensor geometries without sample injection demonstrate higher current density than commercial SPEs, underscoring our MES's potential for various electrochemical sensing applications.

Objective 3: Translating Findings to Lead Detection in Drinking Water

The final goal of our efforts to achieve the last objective was to apply research findings to the detection of lead in drinking water. Through systematic experimentation and analysis, the viability of curved-channel MES for lead detection was successfully demonstrated, building on the developments from Objectives 1 and 2. This showed the sensor platform's potential for practical uses by determining its suitability for detecting particular lead concentrations, producing calibration curves, and figuring out essential sensor parameters like sensitivity and LOD. The calibration curves constructed across various lead concentrations affirmed the reliability and accuracy of our sensors, underscoring their suitability for quantitative analysis over a broad concentration range. The straight-channel sensor showed the LOD and sensitivity of $180\text{ }\mu\text{g/L}$ and $0.0005\text{ }\mu\text{A}/(\mu\text{g/L})$, respectively. The LOD and sensitivity for the curved-channel sensor were $2.4\text{ }\mu\text{g/L}$ and $0.033\text{ }\mu\text{A}/(\mu\text{g/L})$, respectively, which shows considerable improvement in the sensor performance by changing the geometry compared with the straight-channel sensor.

As was previously mentioned, a curved-channel sensor with $R = 3\text{ mm}$ showed promise as an electrochemical measurement tool, marking a significant advancement in detecting analytes in samples. The results of this study can be used to drive MES technology forward, opening the door to more effective and affordable sensor options for various uses, including the detection of heavy

metals in drinking water. Continuing our research in developing and validating curvature-enhanced MES, the detection of Pb in deionized and real water samples was performed.

By leveraging the SWV electrochemical technique, the performance of sensors featuring both straight and curved channels was evaluated. Our results demonstrated that curved-channel sensors are superior to straight-channel sensors when it comes to lead detection. The microfluidic channels' curvature promoted the formation of Dean vortices, which improved convective transport, lessened concentration gradients, and sped up the migration of the analyte toward the electrode surface. Secondary flows and recirculation zones, which prolonged analyte residence times and encouraged more substantial interaction with the electrode surface, further enhanced this improvement in sensing capabilities.

Using a 180-second duration, we optimized the pre-deposition time as part of our methodology. To ensure sufficient interaction between the sensor surface and the target analyte, this decision balanced signal quality with experimental response time. The superiority of curved microfluidic sensors in detecting lead ions was demonstrated in later experiments that used SWASV with deionized (DI) water and real water samples from the Southern Ontario surface water river system. The accuracy and dependability of our sensors were validated by calibration curves built across a range of lead concentrations, underscoring their applicability for quantitative analysis at specific concentrations. The developed curved-channel sensor exhibited the capability to detect lead levels around Health Canada's MAC, which is 5 µg/L in drinking water, underscoring its potential in detecting low concentrations of Pb in water.

The limitations of this study included:

Even after proving the curvature effect on the output signal using the standard $K_3[Fe(CN)_6]$ solution, it appears essential to validate this phenomenon in real-world scenarios through practical sensing and detection, such as Pb detection on-site or with various real water samples collected from different sources. Optimizing the electrochemical methods in the presence of other interferences and contamination is necessary to maximize effectiveness across different analytes and environmental conditions.

In this study, curved microchannels were used to enhance mixing within microchannels and encourage more contact between the target analyte and the electrodes. This is the first attempt to use this fluid dynamic phenomenon to enhance MES performance, and much work can be done to improve the findings in this research.

5.2. Significance of Findings

In comparison to cleanroom fabrication methods, which typically incur costs of approximately \$80 per sensor, and existing alternatives such as MES that uses the SPEs for electrochemical tests (the price is around \$8 per each SPE), our developed PSA-PET-based MES offers a substantial cost advantage. Through innovative fabrication techniques involving laser cutting technology and the use of cost-effective materials, our MES achieves an estimated production cost of less than \$2 per sensor. This significant reduction in cost positions our device as a highly economical solution for electrochemical sensing applications. Moreover, while the paper-based MESs present a lower-cost alternative, their limitation in continuous monitoring and fluid control capability underscores the significance of our PSA-PET-based MES, which offers affordability and ensures reliable and continuous monitoring capabilities, addressing critical needs in various sensing applications.

We quantitatively compared our results with state-of-the-art commercial MESs for Pb detection. We found that the curved-channel sensor developed in this project exhibited the LOD of approximately 2.26 $\mu\text{g/L}$ which is around Health Canada's maximum allowable concentration, i.e., 5 $\mu\text{g/L}$. underscoring its potential to detect even lower concentrations by applying some electrode surface treatment or modification which is proposed in the next section. In addition, fluid handling was used to pump the fluid through a curved microfluidic part of the sensor, which was a unique characteristic of our MES.

5.3. Recommendations for Future Research

5.3.1. Investigating the Combined Effect of Curved Microfluidics and Modified Electrodes

Future research must examine the synergistic effects of modified electrode surfaces and curved microfluidic channels. Sensor selectivity and sensitivity can be increased by ionophore drop-casting or electrode surface modification techniques like ion imprinted polymer (IPP)^{47,109–112}. The interplay between electrode modification types and microfluidic channel curvature in influencing sensor performance can be explained, and these parameters can be optimized for specific analytical applications.

5.3.2. Exploring the Influence of Sample Solution Properties on Microfluidic Electrochemical Sensors Performance

Understanding how sample solution characteristics like viscosity, diffusion coefficient, and other rheological behaviors impact sensor performance is crucial for optimizing sensor design and operation, as well as its application to non-water samples like blood and milk monitoring. Future research could examine the implications of these features on mass transfer and electrochemical reactions at the electrode surface. Techniques like rheological characterization and microfluidic mixing can be used to learn more about how variations in sample solution properties affect sensor response and to develop strategies to mitigate adverse effects. It would be great information to test the adding additives like polyethylene oxide (PEO) to sample solutions and introducing non-Newtonian behavior to the sample to see its influence on the sensor performance. Further studies could examine how ion transport to the electrode surface is affected by non-Newtonian behavior, like normal stress in the non-Newtonian fluid, and how this affects the signal. Using computational modeling and experimental validation, designing sensors with improved sensitivity and detection limits may be possible by comprehending the underlying mechanisms governing non-Newtonian fluid dynamics in microfluidic electrochemical systems.

5.3.3. Simulating and Optimizing Electrochemical Reactions in Varied Geometrical Configurations

Comprehensive models of electrochemical reactions across different geometric sensing regions and electrode configurations can provide valuable insights for optimizing sensor performance. Further research should focus on developing complex computational models that predict the impact of geometric factors on signal response and validate these predictions through experimental validation. By systematically investigating and after the model is validated against the offered experimental data in this thesis, different electrode configurations and microfluidic design parameters such as microchannel width and height and electrode size and distancing can be examined with these models. The optimal designs that include the results of the mentioned parameters' effects on the sensor performance can be optimized.

5.3.4. Formulating Mathematical Predictive Models for Peak Current in Microfluidic Electrochemical Sensors

Future research on MES can focus on creating predictive models that connect peak currents with experimental parameters. Researchers can produce empirical correlations that accurately predict sensor performance under various conditions by carefully examining the relationships between geometric, fluidic, and electrochemical components. The predictive accuracy of these models can be raised by incorporating data-driven strategies like machine learning algorithms. These predictive models can direct the creation of next-generation MES with improved analytical capabilities and are helpful tools for sensor optimization.

5.3.5. Implementing Electro-Microfluidic Separation Techniques for Preconcentration

Preconcentration techniques have been studied to enhance the sensors' performance^{113–119}. It can be hypothesized that incorporating electro-microfluidic separation techniques into sensor platforms will help lower the LOD and boost sensitivity in the future. Future studies could look

into novel ways to preconcentrate target analytes before they reach the sensing region of the sensor. Investigating techniques like electrokinetic trapping and dielectrophoretic can help concentrate target analytes more precisely while minimizing interference from background species. Optimizing the design of microfluidic separation modules and their seamless integration with electrochemical sensor platforms can improve analytical performance.

5.3.6. Leveraging Droplet-Based Microfluidics for Enhancing the Sensitivity of the Microfluidic Electrochemical Sensors

Combining droplet-based microfluidics with microfluidic electrochemical sensors may increase the likelihood of achieving a lower LOD and higher sensitivity^{120–124}. Future research endeavors could investigate innovative techniques for creating and altering droplets that contain target analytes and attaching them to sensor electrodes. Sensor performance may be improved by carefully regulating droplet volumes and improving mass transport to the electrode surface through strategies like droplet merging bursting in the sensing region. To further maximize the benefits, droplet-based microfluidic concepts combined with curved microfluidic channels can lead to superior analytical capabilities.

5.3.7. Exploring Curvature-Induced Peak Current Performance Using Various Electrochemical Techniques

This study has only examined curvature-induced peak current performance using square wave voltammetry. Future research should explore other electrochemical approaches to provide more information about the effect of channel curvature on device behavior. For example, techniques such as DPV, CA, and EIS offer new perspectives on the kinetics and mechanisms of electrochemical reactions at curved electrode interfaces. For example, using the DPV method and optimizing the parameters, we can compare the values for the peak current density of the SWV method. Similarly, CA can provide detailed information about reaction kinetics by monitoring the current response at a constant applied potential over time. By analyzing the transient behavior of electrochemical processes at curved electrode interfaces, we can clarify the influence of curvature

on reaction rates and charge transfer mechanisms and see how the effect of curvature would change with time. EIS can offer valuable insights into the interfacial properties and charge transfer dynamics at curved electrodes by probing the impedance response of the system over a range of frequencies. This would enable us to investigate phenomena such as double-layer capacitance, charge transfer resistance, and mass transport limitations, providing a comprehensive understanding of the electrochemical behavior in curved geometries.

5.3.8. Investigation of other Analytes and Multiplex Microfluidic Electrochemical Sensor Platforms

Analyzing the development of versatile sensor platforms can significantly increase their utility and adaptability. These platforms should be able to detect several analytes at once or carry out complementary sensing tasks. Future research can concentrate on combining various sensing regions and electrochemical techniques to detect multiple analytes selectively. The results have wide-ranging implications for sensing and detecting biological targets, other heavy metals, antibiotics, microplastics, precious metals, etc.^{125–130}.

5.3.9. Investigation of Environmental Stability and Long-Term Reliability

It is helpful to assess the environmental stability and long-term reliability of microfluidic electrochemical sensors under actual operating conditions to guarantee their practical utility and commercial viability. In the future, sensor performance over extended periods can be evaluated through studies on accelerated aging and comprehensive durability testing. Factors such as mechanical stress, humidity exposure, temperature swings, and chemical interference can be carefully investigated to improve the robustness of packaging and sensor materials and identify potential failure modes. Developing protocols for in-situ calibration and recalibration of sensors can enable long-term deployment of those sensors in field applications such as industrial process control systems and environmental monitoring networks. This process can also minimize drift and ensure consistent performance over an extended period.

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