

Electrochemical Tablet-based Sensor for Stable and Reproducible Biosensing

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Abstract

Electrochemical Tablet-based Assay for Stable and Reproducible Biosensing

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Tablet-based assays are particularly promising because of their portability and long shelf life; however, nearly all reported formats rely on optical readouts that are slow, depend on external readers, and are prone to color interference. Electrochemical biosensors provide a faster and more sensitive alternative but often require enzyme immobilization, a multi-step process that can reduce activity, complicate fabrication, and limit scalability and reproducibility.

In response to these challenges, this thesis introduces an electrochemical tablet (eTab) platform in which all reagents are encapsulated within a pullulan matrix and released upon dissolution to produce a ready-to-use solution. As a proof of concept, glucose and lactate eTabs were developed, exhibiting linear detection ranges of 0–15 mM and 0–4 mM, with detection limits of 0.11 and 0.14 mM, respectively, covering their physiological concentrations in plasma. The eTabs demonstrated excellent reproducibility ($RSD < 6\%$) and they maintained their initial performance with less than 10% signal deviation after 30 days of storage at room temperature, whereas comparable solution-phase reagents showed progressively increasing signal drift. Both assays achieved over 90% recoveries in human plasma and showed negligible interference from common interferents.

By combining the durability of tablet-based assays with the analytical performance of electrochemical sensing, this work establishes a simple, stable, and reproducible platform for next-generation POC diagnostics, with potential applications in biomedical testing, environmental monitoring, and food-safety analysis.

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Abbreviations

Abbreviation	Full Form
AChE	Acetylcholinesterase
ADH	Alcohol Dehydrogenase
ATP	Adenosine Triphosphate
AuNP	Gold Nanoparticle
BSA	Bovine Serum Albumin
CA	Chronoamperometry
CA	Cellulose Acetate
CE	Counter Electrode
CGM	Continuous Glucose Monitoring
ChOx	Cholesterol Oxidase
CV	Cyclic Voltammetry
DET	Direct Electron Transfer
DNA	Deoxyribonucleic Acid
DPV	Differential Pulse Voltammetry
EBFC	Enzymatic Biofuel Cell
EBT	Eriochrome Black T
EC	Electrochemical

EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminetetraacetic acid
eTab	Electrochemical Tablet
EIS	Electrochemical Impedance Spectroscopy
FAD/FADH ₂	Flavin Adenine Dinucleotide (oxidized/reduced form)
GOx	Glucose Oxidase
HBV	Hepatitis B Virus
HRP	Horseradish Peroxidase
HIV	Human Immunodeficiency Viruses
IDA	Indoxyl Acetate
KCl	Potassium Chloride
LED	Light Emitting Diode
LFA	Lateral Flow Assay
LOD	Limit of Detection
LOx	Lactate Oxidase
LSV	Linear Sweep Voltammetry
MPA	3-Mercaptopropionic Acid

NAD ⁺ /NADH	Nicotinamide Adenine Dinucleotide (oxidized/reduced form)
PANI	Polyaniline
PDMS	Polydimethylsiloxane
PMMA	Polymethyl Methacrylate
PMS	Power Management System
POC	Point-of-Care
RE	Reference Electrode
RNA	Ribonucleic Acid
RPA	Recombine polymerase amplification
RSD	Relative Standard Deviation
SAM	Self-Assembled Monolayer
SPE	Screen-Printed Electrode
SWV	Square-Wave Voltammetry
TMB	Tetramethylbenzidine
WE	Working Electrode
WHO	World Health Organization

Chapter 1: Introduction and Objective

In this chapter, a brief introduction to point-of-care (POC) diagnostic devices is presented, with a particular focus on tablet-based assays and electrochemical biosensors. Their advantages and limitations are discussed. The chapter continues with the objectives of this research project and concludes with an outline of the thesis structure.

1.1 Background

Affordable and portable diagnostic platforms are central to timely disease detection and effective patient management.^{1–3} However, many conventional assays still rely on bulky instruments, trained personnel, and complex workflows, which limit accessibility in resource-constrained settings. To overcome these barriers, POC testing enables rapid and reliable analysis in simple, low-cost formats that can be performed directly at the site of need.^{4,5}

The World Health Organization's REST-ASSURED criteria emphasize the importance of real-time connectivity, ease of sample collection, scalability, transferability, affordability, sensitivity, specificity, user-friendliness, and robustness in developing practical diagnostic tools.^{1,6,7} Within this framework, tablet-based assays have gained significant attention due to their portability, long shelf life, integrated reagents, and low production cost.^{8–10} However, most reported systems rely on optical detection, which is often slow, prone to interference from sample color or turbidity, and typically limited to qualitative or semi-quantitative analysis. These drawbacks reduce their suitability for true field applications requiring high sensitivity and quantitative precision.

In parallel, electrochemical biosensors have become the most widely employed class of biosensors and an integral part of modern analytical chemistry, valued for their rapid response, operational simplicity, and relatively low cost.¹¹ Their portability and reliable signal transduction make them especially well-suited for POC applications.¹² Handheld glucometers exemplify the success of this technology in diabetes management.¹³ Nevertheless, biorecognition element immobilization on the electrode surface, an essential step in most electrochemical biosensors, can adversely affect enzyme activity and stability, complicating fabrication and reducing reproducibility.

To address these challenges, this thesis introduces an electrochemical tablet (eTab) platform that combines the operational simplicity, rapid manufacturability, and storage stability of tablet-based assays with the robust analytical performance of electrochemical sensing. In this approach, all reagents are encapsulated within a dissolvable polysaccharide matrix, eliminating immobilization steps and enabling a ready-to-use, stable, and reproducible electrochemical testing format.

Diabetes mellitus is the most prevalent endocrine disorder affecting carbohydrate metabolism. This chronic condition arises from insulin deficiency or resistance, leading to persistent hyperglycemia and blood glucose concentrations outside the normal physiological range of 80–120 mg/dL (4.4–6.6 mM). It remains a major global health burden and a leading cause of morbidity and mortality, contributing to severe complications such as cardiovascular disease, nephropathy, neuropathy, and retinopathy. Many of these outcomes can be mitigated through strict self-monitoring and regulation of blood glucose levels.¹⁴ In addition to its clinical impact, diabetes continues to increase in prevalence. In the United States, adult diabetes rates were estimated at 9.6% ($\approx$ 20.4 million individuals) between 2003 and 2006, with projections exceeding 48 million cases by 2050. Because of its high prevalence and the necessity of tight metabolic control, glucose has become the most routinely measured clinical biomarker, and glucose biosensors currently account for nearly 85% of the global biosensor market.^{15,16}

Alongside glucose, lactate serves as another essential biomarker with significant physiological and clinical relevance. Lactate is produced during anaerobic glucose metabolism, and elevated levels are indicative of metabolic imbalance or inadequate oxygen supply. Normal blood concentrations range from 0.5–1.5 mM but may increase to 12–25 mM during strenuous exercise, marking the onset of fatigue and defining the lactate threshold in sports physiology. Clinically, excessive lactate accumulation is associated with lactic acidosis and a wide spectrum of pathological conditions, including shock, respiratory or hepatic failure, and tissue hypoxia. It is also used as a diagnostic indicator during labor and delivery, cancer progression, and neurological events such as stroke and traumatic brain injury. Because lactate reflects both metabolic and physiological status, rapid and accurate lactate monitoring is essential in medical diagnostics, critical care, and performance assessment, leading to extensive efforts toward developing sensitive and dependable lactate biosensors.¹⁷

Given that both glucose and lactate provide critical insight into metabolic health and disease severity, continuous and accurate measurement of these biomarkers is of considerable clinical importance. Accordingly, a reliable POC device capable of monitoring such analytes is highly desirable. For this reason, although the biosensor platform developed in this thesis is broadly applicable, its performance is demonstrated using glucose and lactate as representative analytes.

1.2 Objectives

This thesis addresses the need for a novel POC platform that combines the operational simplicity and robust performance of tablet-based sensors with the analytical power of electrochemical detection, while overcoming the limitations of each approach.

The primary objective of this research is to develop and validate an electrochemical tablet-based bioassay that integrates the advantages of both systems. As a proof of concept, the platform is demonstrated for glucose and lactate detection in human blood plasma, two clinically relevant biomarkers associated with metabolic dysfunction and critical illness. This approach aims to provide a reliable, sensitive, and user-friendly method for biomarker monitoring at the point of care.

The study also evaluates the stability and performance of the tablet-based sensor compared to conventional solution-based assays. The encapsulation of bioreagents within pullulan, a biocompatible and biodegradable polysaccharide, enhances enzyme stability at room temperature and enables long-term storage without refrigeration. Moreover, these tablets can be readily deployed in complex biological matrices such as human blood plasma, exhibiting no significant interference from common electroactive species and providing a straightforward, efficient, and reproducible detection platform.

Together, these results highlight electrochemical tablets as a promising addition to the POC diagnostic toolbox, bridging the gap between optical tablet assays and immobilized electrochemical systems—and open new opportunities for applications in biomedical diagnostics, food safety, and environmental monitoring.

1.3 Thesis Outline

This thesis is structured into five chapters, each contributing to a comprehensive understanding of the research conducted and its outcomes.

Chapter 1 provides an overview of the study, presenting the background, objectives, and overall framework of the research.

Chapter 2 presents a comprehensive review of the relevant literature, beginning with an overview of biosensors as point-of-care (POC) diagnostic tools and tablet-based sensing platforms. It then focuses on electrochemical biosensors, their underlying detection methods, and the various enzyme immobilization techniques employed to enhance sensor performance. The chapter further discusses the materials used for immobilizing biorecognition elements and their influence on the analytical behavior and stability of biosensors.

Chapter 3 presents the recently submitted manuscript that forms the core of this research. It begins with an abstract, followed by detailed sections on materials, methods, and experimental procedures. This chapter covers the preparation and characterization of electrochemical tablets, as well as the detection of glucose and lactate in human serum. It also discusses analytical performance, interference studies, and stability tests, providing a complete overview of the experimental findings.

Chapter 4 summarizes the research outcomes, highlighting the main conclusions drawn from Chapter 3. Also, the chapter also offers recommendations and potential directions for future work in this field.

The Reference Section contains a comprehensive list of references cited throughout the thesis, documenting all relevant literature that supported and informed this research.

The Appendix (Supporting Information) provides supplementary figures, tables, and data that support the results presented in Chapter 3.

This structured approach ensures that the reader gains a clear understanding of the study—from the fundamental context presented in Chapter 1, through the literature review in Chapter 2, to the experimental work and its implications in Chapters 3 and 4, culminating in the references provided

in reference section. Together, these chapters present a cohesive and informative contribution to the development of electrochemical tablet-based biosensing platforms.

List of Publications and Conference Contributions

Publications

Rozhin Saebi, Seyed Hamid Safiabadi Tali, Rémi Rabasa-Lhoret, Sana Jahanshahi-Anbuhi*, Marc-Antoni Goulet*. *Electrochemical Tablet-Based Assay for Stable and Reproducible Biosensing*. Submitted to ***Sensors and Actuators B***.

Hamid Safiabadi Tali, **Rozhin Saebi**, Abdolali Mehrjou, Farzad Mirzaie, Zubi Sadiq, Marc-Antoni Goulet, Sana Jahanshahi-Anbuhi. *Do-it-yourself Screen Printing with Sandwiched Electrodes for Sensitive Electrochemical Paper-Based Analytical Devices (ePADs)*. Accepted 2025. ***Electrochimica Acta***.

Conference

Rozhin Saebi, Seyed Hamid Safiabadi Tali, Sana Jahanshahi-Anbuhi, Marc-Antoni Goulet. *Tackling Stability Challenges in Electrochemical Paper-Based Analytical Devices (ePADs) for Enhanced Glucose Detection*. Presented at the **Electrochemical Society Meeting**, May 2025.

Chapter 2: Literature Review

In this chapter, the fundamental concepts and methodologies related to biosensors for point-of-care applications are discussed. Emphasis is placed on the development of tablet-based biosensing platforms as innovative tools for POC diagnostics. The chapter also covers the principles of electrochemical sensing, including the most common techniques used for electrochemical detection, as well as various enzyme immobilization strategies employed to enhance biosensor performance.

2.1 Point-of-Care Diagnostics

Health care, particularly patient diagnosis and treatment, is among the most essential services that must be accessible to all communities. Yet, providing effective and equitable care remains a significant challenge, as it depends on timely diagnosis, efficient management, and improved clinical outcomes. The increasing demand for diagnostic technologies with superior analytical capabilities, especially those offering high sensitivity, selectivity, and rapid response, has intensified global research efforts toward the development of POC biosensors. These systems play a pivotal role in supporting prompt and accurate medical decision-making, particularly in resource-limited settings, emergency situations, disaster zones, and densely populated healthcare environments. Point-of-care testing is characterized by its rapid turnaround time, often delivering reliable results within minutes. Any simple, portable, and preferably non-invasive diagnostic method that can be performed in close proximity to the patient is classified as point-of-care testing.^{17–19}

2.2 Biosensors as Point-of-Care Platforms

The biosensors have proved to be the most critical component in POC diagnostics, providing rapid, reliable, and portable solutions for disease detection.²¹

Biosensors are analytical devices designed to identify the presence of a specific analyte or to measure its concentration and reaction kinetics within a given sample. A typical biosensor consists of three main components: a biorecognition element, a transducer, and a signal processor or detector, as illustrated in Figure 1.

The biorecognition element, which may include antibodies, nucleic acids, enzymes, aptamers, nanoparticles, or whole cells, confers the biosensor's specificity and selectivity toward the target

analyte. The transducer then converts the biochemical interaction between the analyte and the bioreceptor into a measurable electrical or optical signal. This signal is subsequently amplified, processed, and displayed by the detector or readout system.²²

As shown in Figure 1,²³ biosensors can be categorized based on their transduction mechanisms, including electrochemical (amperometric, potentiometric, voltametric, conductometric, impedimetric), optical (chromogenic, luminogenic, optical fiber, chemiluminescent), thermal, electronic, gravimetric, and acoustic types.

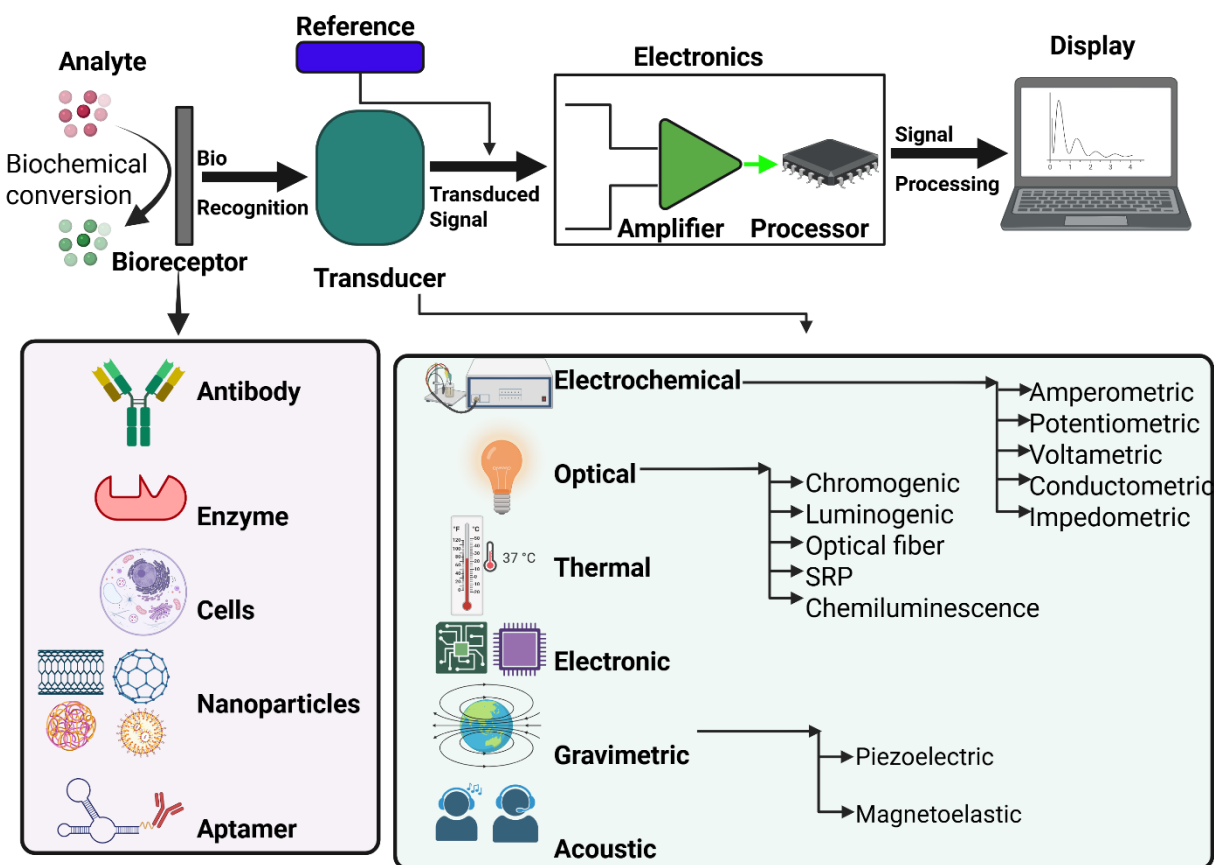


Figure 1. Schematic diagram of typical biosensor consisting of bioreceptor, transducer, electronic system (adopted from Ref [23]).

A clear example of how biosensors have enabled point-of-care diagnostics is the personal glucose meter, which remains the most widely used biosensor in healthcare.²⁴ Although enzymatic blood-glucose analysis had existed in laboratory instruments since the mid-1970s,²⁵ the introduction of the first portable, patient-operated glucose meter in the late 1980s marked a turning point in POC diagnostics,²⁶ it transformed glucose monitoring into a decentralized, quantitative, and user-

friendly diagnostic format. Its simplicity, portability, and rapid quantitative output set the standard for modern POC biosensors.

Beyond glucose monitoring, lateral flow assays (LFAs) represent another major class of POC biosensors with broad global adoption.²⁷ Although immunochromatographic principles were established earlier, LFAs became commercially significant in the 1970s with the introduction of the first home pregnancy test²⁸ and were later adapted for qualitative and semi-quantitative detection of infectious diseases,²⁹ and human immunodeficiency virus (HIV).³⁰ Other point-of-care technologies have also entered the market, further expanding the role of biosensors in decentralized diagnostics. Examples include handheld hemoglobin meters, such as those developed by Danaher,³¹ which use microcuvette technology to quantify hemoglobin directly from whole blood, and the wide range of rapid COVID-19 antigen tests that became globally adopted during the pandemic.³²

2.2.1 Enzyme-Based Biosensor

Enzyme-based biosensors are one of the most advanced and commercially successful bioanalytical devices because of a high catalytic activity and selectivity of enzymes, as well as commercial availability of purified enzymes.³³ Enzymes are large and complex biological macromolecules, typically ranging from 10 to 400 kDa, composed primarily of proteins that catalyze the rapid conversion of substrates into products.³⁴ They are nature's own catalysts, responsible for accelerating essential biochemical reactions in all living systems, including cells, tissues, and microorganisms. While other biorecognition-based biosensors are evolving quickly, enzyme biosensors still dominate biomedical use because of their exceptional specificity, efficiency, and diversity, enzymes are ideal biorecognition elements for biosensor construction, enabling the selective detection of target analytes even in complex sample matrices.³⁵

In enzymatic biosensors, one or more enzymes are immobilized onto a physical transducer, forming a hybrid system in which the biological reaction is converted into a measurable signal, most commonly an electrical or optical response, proportional to the analyte concentration. The detection principle relies on monitoring either the consumption of a co-substrate (such as oxygen or NADH) or the generation of a reaction product (such as hydrogen peroxide or electrons) resulting from the enzymatic reaction.^{36,37}

The modern concept of the enzyme-based biosensor was first introduced by Leland C. Clark and Champ Lyons in 1962, who demonstrated an amperometric glucose sensor by immobilizing glucose oxidase (GOx) on an oxygen electrode.³⁸ This pioneering work laid the foundation for the development of modern electrochemical biosensors and other sensors, many of which continue to rely on enzymatic reactions for their exceptional selectivity and sensitivity.

Generally, enzymes are classified into six major categories based on acting on organic substrates, including the following in Table 1

Table 1. Major enzyme classes and their main reaction types/functions.³⁹

Class	Main Reaction Type / Function
Oxidoreductases	Catalyze oxidation–reduction reactions involving the transfer of electrons between molecules.
Transferases	Transfer functional groups (e.g., methyl, glycosyl, or phosphate) from one molecule to another.
Hydrolases	Catalyze hydrolytic cleavage of C–C, C–O, and C–N bonds using water as a reactant.
Lyases	Catalyze the cleavage of C–C, C–O, and C–N bonds without the involvement of water molecules.
Isomerases	Catalyze intramolecular rearrangements, converting a molecule into one of its isomers.
Ligases	Catalyze the joining (ligation) of two molecules with the concurrent hydrolysis of ATP.

Conventional enzyme-based biosensors predominantly rely on optical or electrochemical transduction mechanisms, and the most widely used clinical examples target analytes such as glucose, lactate, glutamate, urea, and cholesterol.⁴⁰

One representative example of enzyme-based colorimetric biosensors is the class of paper and engineered plastic microfluidic assays, which offer a simple yet effective platform for on-site analysis. Whitesides and co-workers demonstrated this concept through a device capable of simultaneously detecting glucose and bovine serum albumin (BSA) in 5 mL of urine (Figure 2). The assay relies on the enzymatic oxidation of glucose by glucose oxidase to generate hydrogen peroxide, which subsequently reacts with peroxidase and KI to produce iodine, resulting in a visible color shift from clear to brown.⁴¹

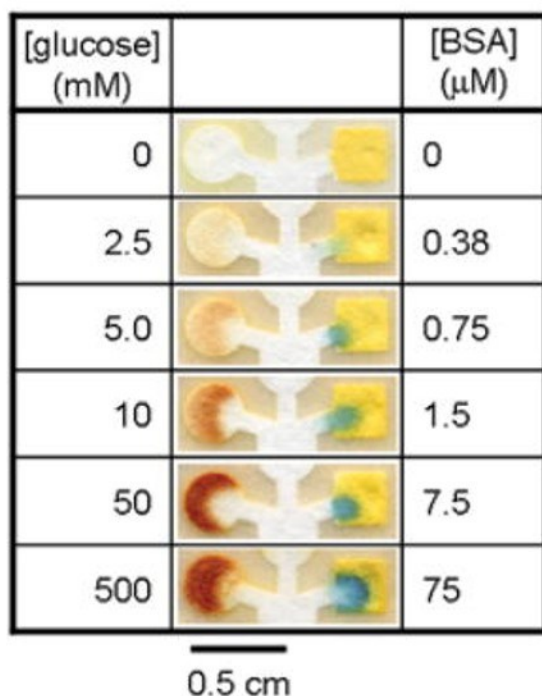


Figure 2. Detection of glucose and protein by using chromatography paper patterned with photoresist. (adopted from Ref. [40]).

2.3 Tablet-Based Assays

2.3.1 A Brief History of Tablet-Based Assays

Tablet-based assays have a long-established history and are recognized as one of the earliest formats for POC testing in modern analytical science. Their origins date back to before 1920, when methylene blue tablets were first used in Denmark for the colorimetric evaluation of milk quality and hygiene.⁴² By the 1930s, similar tablet-based approaches were applied for field testing of milk pasteurization, demonstrating their practicality for on-site analysis.⁴³

A major advancement occurred in 1941, when the Ames Company introduced *Clinitest*—a tablet-based assay designed for the semi-quantitative colorimetric detection of reducing sugars in urine, primarily for glucose monitoring and diabetes management.⁴⁴ The success of *Clinitest* led to the development of additional commercial assays such as *Acetest* for acetone detection and *Bumintest* for albumin determination in urine, thereby expanding the scope of tablet-based diagnostics.⁴⁵

The research on tablet-based sensors declined after the introduction of paper dipstick tests by Ames in 1956, which shifted attention toward simpler paper-based formats^{46,47} This trend continued with the rise of LFAs⁴⁸ and later microfluidic technologies, further reducing interest in tablet-based platforms⁴⁹ Despite this decline, tablet assays did not disappear entirely; they continued to be in use for specialized applications, such as Lovibond's water-analysis tablets, valued for their long shelf life, simplicity, and convenience.

In 2014, a pivotal study by Jahanshahi-Anbuhi et al. renewed academic interest in tablet-based systems.⁹ Instead of the traditional compression method, the researchers employed a drop-casting technique to encapsulate sensing reagents within the biopolymer pullulan, producing compact and dissolvable tablets. These tablets were successfully used for pesticide detection in water via the indoxyl acetate–acetylcholinesterase (AChE) colorimetric reaction. Importantly, pullulan encapsulation enhanced reagent stability from less than one week to over two months under ambient conditions, addressing one of the major limitations of earlier tablet formats.^{8,9}

2.3.2 Tablet Fabrication Methods

2.3.2.1 Powder Compression

Powder compression is the conventional and most widely used method for producing tablets. In this approach, powdered reagents are weighed, blended, and compacted into a solid tablet form (Figure 3). This well-established technique is highly scalable using standard industrial equipment and provides flexibility to incorporate a broad range of chemical and biological components within a single formulation.

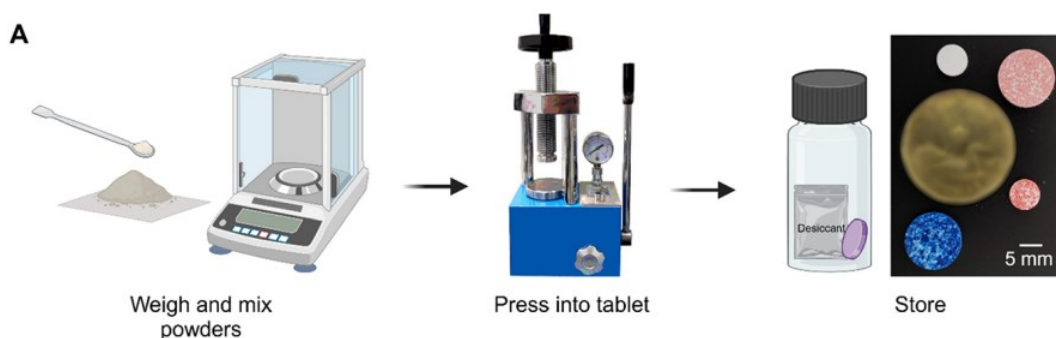


Figure 3. Schematic of tablet fabrication method via powder compression (adopted from Ref. [1]).

One of the earliest demonstrations of compressing reagents into a solid tablet format for diagnostic use was the Clinitest, commercialized by the Ames Company for estimating glucose levels in urine.⁵⁰ The test was based on the classical copper-reduction chemistry, responding to a broad range of reducing species, including glucose, through a visible color shift. Its formulation incorporated copper sulfate, sodium hydroxide, citric acid, and sodium bicarbonate, which together generated a reproducible color reaction that users could interpret semi-quantitatively by matching the resulting hue to a printed color scale. Following this innovation, tablet-based formats were explored for other clinical endpoints as well, such as total urinary protein using salicylsulphonic acid,⁵¹ and tablets designed to detect ketone bodies for monitoring diabetic ketoacidosis.⁵²

More recently, tablet diagnostics have advanced from simple chemical reactions to incorporating biological reagents. A notable example is the work reported by Udugama et al. in 2017, where the team showed that labile biomolecules could be stabilized within compressed tablets by first lyophilizing them in the presence of trehalose, an effective cryoprotectant.¹⁰ The dried reagents were blended with excipients such as mannitol (as a structural filler), croscarmellose sodium (to promote fast disintegration in under 30 seconds), and sodium stearyl fumarate (as a lubricant to facilitate clean tablet ejection). With this formulation strategy, a broad range of biomolecules, including enzymes, proteins, nucleic acids, and even quantum dots, retained functionality for at least one month at room temperature. To illustrate the potential of such stabilized tablets, the authors assembled a three-tablet, color-coded system that enabled detection of hepatitis B virus (HBV) in serum through sequential recombinase polymerase amplification (RPA), quantum-dot-based encoding, and fluorescent readout.¹⁰ This study demonstrated that compressed tablets could

support complex biochemical workflows, extending the concept well beyond simple colorimetric tests.

In subsequent work, the same group further expanded the capabilities of tablet assays by engineering multi-layered tablets designed to release reagents in a programmed order, allowing multi-step diagnostic reactions to proceed automatically without user intervention.⁵³

Another recent development introduced powder-compressed automixing tablets, designed to overcome one of the main challenges of tablet-based assays, the need for manual shaking or vortexing to dissolve reagents. These automixing tablets demonstrated strong self-mixing capabilities even in highly viscous media, enabling efficient colorimetric detection of nitrite in saliva and glucose in urine.¹

2.3.2.2 Tablets via Polysaccharide Encapsulation

Tablet fabrication via polysaccharide encapsulation involves preparing a reagent solution containing the desired analytes and dissolving film-forming polysaccharides, such as pullulan or dextran, within the same mixture.⁵⁴ Aliquots of this solution are then drop-cast and dried, forming solid tablets that contain premeasured quantities of reagents (Figure 4). This fabrication process is simple, inexpensive, and scalable, making it particularly suitable for low-cost, point-of-care diagnostic applications.

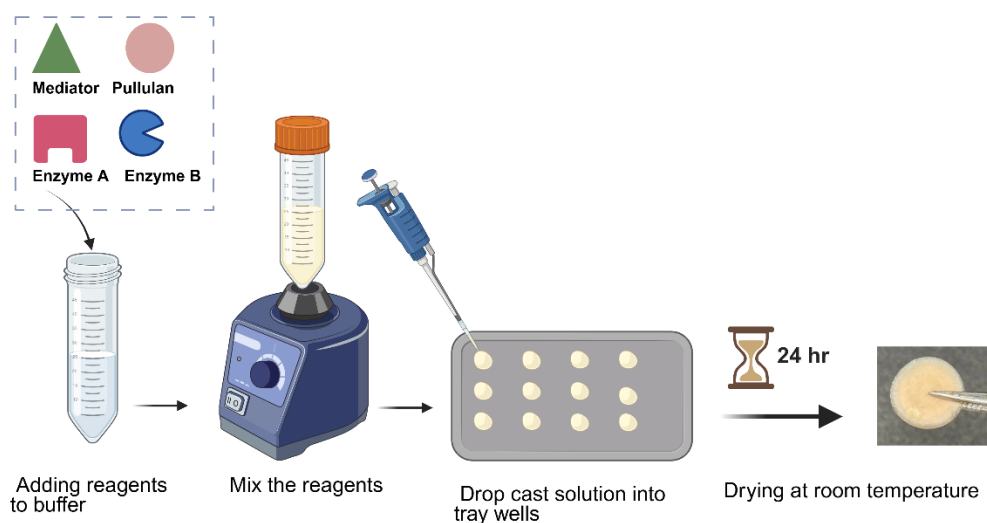


Figure 4. Schematic of tablet fabrication method via polysaccharide encapsulation.

The first demonstration of this method was reported by Jahanshahi-Anbuhi et al., who developed a colorimetric tablet-based assay for the detection of the pesticide malathion in water using the indoxyl acetate (IDA)–AChE reaction.⁵⁴ To prepare the tablets, separate IDA and AChE solutions were combined with 10–12 wt% pullulan and cast into polypropylene molds before drying. During the assay, an AChE tablet was first incubated with the water sample for five minutes, followed by the addition of an IDA tablet. In the presence of malathion, AChE activity was inhibited, leading to a reduced color intensity proportional to pesticide concentration. Encapsulation within pullulan not only simplified tablet fabrication but also significantly enhanced reagent stability under ambient conditions.

To further streamline fabrication and highlight the versatility of polysaccharide encapsulation, the same group later developed single-tablet systems for the luciferase/luciferin Adenosine Triphosphate (ATP) assay, a particularly challenging test due to the instability of its reagents.⁸ In this work, all components, including luciferase, luciferin, MgSO₄, MgCO₃, dithiothreitol, EDTA, coenzyme A, tricine, and buffer, were combined with 10 wt% pullulan and drop-cast directly onto PET sheets to form tablets without the need for molds. These tablets enabled the luminescent detection of ATP, both from standard solutions and lysed *E. coli* cells, demonstrating their robustness and ease of production.

In subsequent years, the application of polysaccharide-based tablets expanded across diverse sensing mechanisms. Sadiq et al. reported the first gold nanoparticle (AuNP)-encapsulated tablets, which utilized the plasmonic aggregation of AuNPs for colorimetric sensing.⁵⁵ Dextran was employed as the encapsulating polymer, producing tablets that exhibited excellent long-term stability (≥ 9 months) and pH-responsive behavior.⁵⁶ These dextran–AuNP tablets were later applied for detecting hypochlorite in water,⁵⁷ as well as hydrogen peroxide and uric acid in urine.^{58,59} Remarkably, the reducing properties of polysaccharides enabled *in situ* AuNP synthesis directly from gold salts in a single step, eliminating the need for additional reagents and simplifying fabrication.

Beyond their plasmonic characteristics, the nanozyme activity of AuNPs has also been exploited to develop tablet-based biosensors. In one study, pullulan was used to encapsulate AuNPs along with GOx and the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB), enabling glucose detection in diluted saliva through peroxidase-like activity that generated a glucose-dependent

gray-to-blue color shift.⁶⁰ Pullulan–AuNP tablets were also adapted for cysteamine detection in serum.⁶¹

Additional applications have further demonstrated the versatility of polysaccharide matrices. Examples include pullulan tablets with Griess reagents for nitrite detection in soil and water,⁶² dual-enzyme dextran tablets containing GOx and HRP for glucose detection in urine.⁶³ Collectively, these studies highlight the adaptability of polysaccharide tablets across both environmental and biomedical contexts.

The combinatorial use of multiple sugars has also emerged as a strategy to improve reagent stabilization.⁶⁴ For instance, pullulan–trehalose blends were recently employed to encapsulate bacteriophages (phages) for biological screening.⁶⁵ In this assay, a bacterial suspension was added to each tablet, and effective phage-induced lysis released ATP, which was detected by luminescence within 30–120 minutes. The tablets maintained stability for up to four weeks at room temperature under vacuum conditions, underscoring their potential for long-term storage. In another study, a tablet-based reverse titration system was introduced for the rapid, point-of-use measurement of water hardness, addressing major limitations of conventional EDTA titration. In this system, reagents were encapsulated in pullulan matrices to form two separate tablets: one containing Eriochrome Black T (EBT) and N-cyclohexyl-3-aminopropanesulfonic acid buffer and the other containing EDTA disodium salt dihydrate.⁶⁶

Together, these advances demonstrate the potential of polysaccharide-encapsulated tablets not only to improve reagent stability but also to simplify complex analytical workflows, transforming traditional assays into compact, user-friendly, and high-throughput diagnostic formats.

2.4 Electrochemical Sensor

As the field of microscale sensor technology continues to expand rapidly, there is a growing demand for detection methods that are fully compatible with miniaturized analytical systems. Among various approaches, electrochemical detection has gained significant attention due to its suitability for integration within compact and portable devices. Electrochemical biosensors are highly attractive because they enable the direct conversion of biochemical events into electrical signals, offering sensitive, selective, and real-time monitoring of complex biological samples. Electrochemical (EC) techniques hold great promise for such microsystems owing to their

exceptional sensitivity, comparable to that of fluorescence-based methods, along with several practical advantages, including ease of miniaturization, independence from optical path length or sample turbidity, low power consumption, and low cost. Furthermore, EC systems are inherently compatible with advanced micromachining and microfabrication technologies, facilitating seamless integration into lab-on-a-chip or POC devices. Complete and self-contained microanalytical systems can be achieved by incorporating on-chip potentiostatic control circuitry, allowing real-time signal acquisition and processing within a compact footprint. Unlike optical detection methods, where sensitivity often decreases in microchannels due to reduced optical path length, the miniaturization of electrochemical working electrodes can enhance detection performance by increasing mass transport and flux toward the electrode surface. As a result, electrochemical biosensors can achieve remarkably high sensitivity in low-volume devices, making them ideally suited for next-generation microanalytical and diagnostic platforms.^{27–30} A bioelectrochemical sensor functions as an analytical platform that combines a biological recognition system with an electrochemical transducer to identify a specific target molecule.⁷¹ As depicted in Figure 1, the operation of such a device relies on the coordinated performance of the following five essential elements:

- (a) Bioreceptor: This is the sensing component that selectively binds the analyte of interest while showing minimal response to other species. Depending on the application, the bioreceptor can be an enzyme, nucleic acid, antibody, or microorganism, each chosen for its specificity and affinity toward the target.
- (b) Transducer: Once the recognition event occurs, the transducer converts the resulting physicochemical changes, such as shifts in current, potential, or charge—into an electrical output that can be quantified.
- (c) Amplifier: The electrical signals generated by the transducer are captured and strengthened using an amplifier before being transmitted to the signal processor for further analysis.
- (d) Signal Processor: The amplified electrical signal is then transformed into a form that can be interpreted by the system, producing outputs such as numerical readings, voltammograms, or graphical traces.

(e) Display Unit: Finally, the processed data are presented through a user interface, displaying the analytical result in a readable and interpretable format for end users.

2.4.1 Electrochemical Measurement Configurations

Electrochemical measurements are typically conducted using one of two configurations: the two-electrode system or the three-electrode system.

In a two-electrode setup, the cell consists of a working electrode (also referred to as the indicator electrode) and a reference electrode, as shown in Figure 5A. The potential of the reference electrode is maintained at a fixed and stable value, while the potential difference between the working and reference electrodes is monitored during measurement.⁷²

In the three-electrode configuration, the electrochemical cell consists of a working electrode, a reference electrode, and an additional counter (or auxiliary) electrode. In this setup Figure 5B, the potential applied to the working electrode is regulated with respect to the reference electrode, while the resulting current generated by the electrochemical reaction flows between the working and counter electrodes. This arrangement minimizes polarization effects at the reference electrode and ensures more accurate potential control, which is why it remains the preferred configuration for electrochemical biosensing applications.

The working electrode functions as the core transduction element, where the biochemical interaction occurs. To achieve selective analyte detection, its surface is typically modified with biomolecules such as enzymes, antibodies, or nucleic acids, providing specific molecular recognition capabilities. During electrochemical reactions, electrons are exchanged between the analyte and the electrode surface. The direction of electron flow is determined by the applied potential: a positive potential promotes oxidation (electron transfer from analyte to electrode), while a negative potential drives reduction (electron transfer from electrode to analyte). By convention, oxidation takes place at the anode, whereas reduction occurs at the cathode.⁷³

Common materials used for working electrodes include gold, silver, platinum, carbon, and screen-printed electrodes. Carbon-paste electrodes are also frequently employed; they are prepared by mixing conductive carbon particles with a suitable binder to create a uniform, moldable surface that is both renewable and easily modified.^{72,74,75}

The counter electrode, often fabricated from inert materials such as platinum wire or mesh, serves to complete the electrical circuit. Although it does not directly participate in the redox reaction, it must have a larger surface area than the working electrode to prevent current limitation and ensure stable operation. In experimental setups, the reference electrode is positioned as close as possible to the working electrode to minimize uncompensated solution resistance, thereby improving measurement precision and potential stability.

Among the available configurations, three-electrode systems, especially carbon-paste electrodes are extensively employed in electrochemical biosensing. (Figure 5C) Their architecture enables precise potential control, and can be fabricated on various substrates, enabling adaptation to numerous analytical scenarios. Building on this versatility, numerous studies have investigated biosensors based on the three-electrode configuration across a wide range of application areas.

For example, Noiphung et al. developed a paper-based screen-printed three-electrode device for the detection of glucose in whole blood.⁷⁶ Similarly, Wongkaew et al. fabricated a three-electrode system on polymethyl methacrylate (PMMA) for on-chip electrochemical detection.⁷⁷ Srikanth et al. reported a droplet-based microfluidic device with an inkjet-printed three-electrode configuration for ascorbic acid detection, highlighting the potential of miniaturized electrochemical platforms.⁷⁸ More recently, 3D-printed and laser-induced graphene electrodes have been employed in three-electrode system for portable electrochemical sensing, including applications such as glucose monitoring and ciprofloxacin detection in milk samples.^{79,80} These examples underscore the flexibility and scalability of the three-electrode design, making it a cornerstone of modern electrochemical biosensor technology. Collectively, these studies highlight the versatility and adaptability of the three-electrode configuration, reaffirming its central role in the design and development of modern electrochemical biosensors.

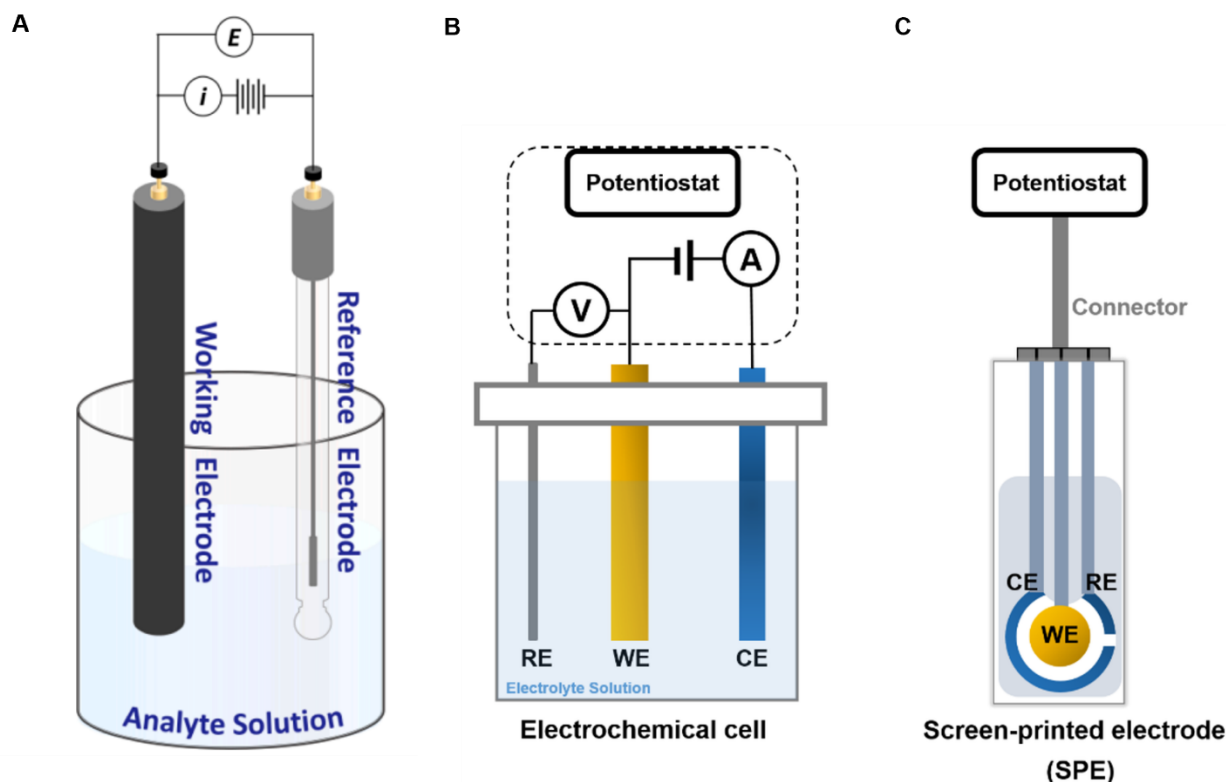


Figure 5. Schematic illustration of typical electrochemical measurement systems: (A) two-electrode cell, (B) three-electrode electrochemical cell controlled by a potentiostat, and (C) compact screen-printed electrode (SPE) integrating working (WE), reference (RE). (adopted from Ref. [72])

2.4.2 Electrochemical Detection Techniques in Biosensing

Electrochemical transducers can generate analytical responses through several distinct detection modes. The most common is the amperometric approach, in which electrochemical reactions are monitored by measuring the current produced during oxidation or reduction processes. Potentiometric sensors instead detect changes in electrical potential or charge accumulation at the electrode surface, while conductometric sensors measure variations in the conductivity of the solution resulting from the formation or consumption of charged species. Additional methods include electrochemical impedance spectroscopy (EIS), which evaluates the impedance of the electrode–electrolyte interface, and field-effect–based transducers, which employ transistor-type configurations to record current changes induced by potential variations.⁸¹ In most biosensors developed to date, the most commonly employed electrochemical detection techniques include

amperometric, voltammetric, impedimetric, and potentiometric methods, each utilized in different configurations depending on the sensing mechanism and target analyte.^{82,83} Each of these detection modes operate through different transduction principles and will be described in greater detail in the following section.

Amperometric biosensors are one of the most widely used types of electrochemical biosensors that quantify analytes by measuring the electrical current generated during redox reactions at an electrode surface. When a fixed potential is applied between the working and reference electrodes, electroactive species undergo oxidation or reduction, producing a Faradaic current proportional to their concentration. This current, which originates from electron transfer at the electrode–electrolyte interface, provides a direct and quantitative measure of analyte levels in the sample.^{72,84}

The underlying process follows Faraday’s law, expressed as:

$$\text{Equation 1: } i = nFAj$$

i : current,

n : number of electrons exchanged

F : Faraday constant,

A : electrode area

j : flux of analyte molecules reaching the electrode.

Since the flux ($j = k^0 c_0$) depends on the interfacial analyte concentration (c_0) and the heterogeneous electron-transfer rate constant (k^0), the current directly reflects analyte concentration. The applied potential determines the reaction direction: oxidation occurs at positive potentials (electrons flow from analyte to electrode), while reduction occurs at negative potentials (electrons flow from electrode to analyte).

Amperometric systems can operate under constant or varying potential. When a fixed potential is applied across the working electrode, the corresponding current–time relationship is recorded in chronoamperometry. If the potential is varied linearly or cyclically, the current–potential relationship obtained is referred to as voltammetry, which includes several sub-techniques such as

cyclic voltammetry (CV), linear sweep voltammetry (LSV), differential pulse voltammetry (DPV), and square-wave voltammetry (SWV). All these methods are governed by Faradaic processes, which involve electron transfer. Cyclic voltammetry and chronoamperometry are two widely used electrochemical techniques used in electrochemical biosensor.⁸⁵ In the past decade, amperometric biosensors have been extensively applied in a wide range of fields, including environmental monitoring, food safety, and biomedical diagnostics.

In environmental applications, *Gumpu et al.* developed an amperometric biosensor for the simultaneous detection of lead (Pb^{2+}) and mercury (Hg^{2+}) ions in water, demonstrating its potential for real-time water quality assessment.⁸⁶ Similarly, *Guerrieri et al.* designed a disposable amperometric biosensor capable of rapid screening of anticholinesterase activity in soil extracts, providing an efficient tool for monitoring pesticide contamination.⁸⁷

In the food industry, *Gajdár et al.* introduced an amperometric sensor for on-site determination of free sulfite in wines, enabling selective and reliable quality control in beverage processing.⁸⁸

Amperometric detection has also shown remarkable promise in biomedical diagnostics. *Yan et al.* reported a tear-based amperometric glucose biosensor, offering a non-invasive approach for glucose monitoring,⁸⁹ while *Romero et al.* developed an amperometric system for lactate detection in blood, demonstrating excellent analytical performance and clinical relevance.⁹⁰

All together, these studies highlight the versatility and reliability of amperometric sensing platforms, establishing them as one of the most widely used electrochemical detection techniques for chemical and biological analytes.

Cyclic Voltammetry:

Cyclic voltammetry is a powerful and popular electrochemical technique commonly employed to investigate the reduction and oxidation processes of molecular species. CV is also invaluable to study electron transfer-initiated chemical reactions, which includes catalysis.⁹¹ In this method, the potential of the working electrode is swept linearly relative to a reference electrode, first in one direction (anodic scan) and then reversed (cathodic scan), while the corresponding current is continuously measured Figure 6A The resulting plot of current versus potential is known as a

cyclic voltammogram, which provides valuable information about the oxidation–reduction behavior, reversibility, and kinetics of the analyte.⁹²

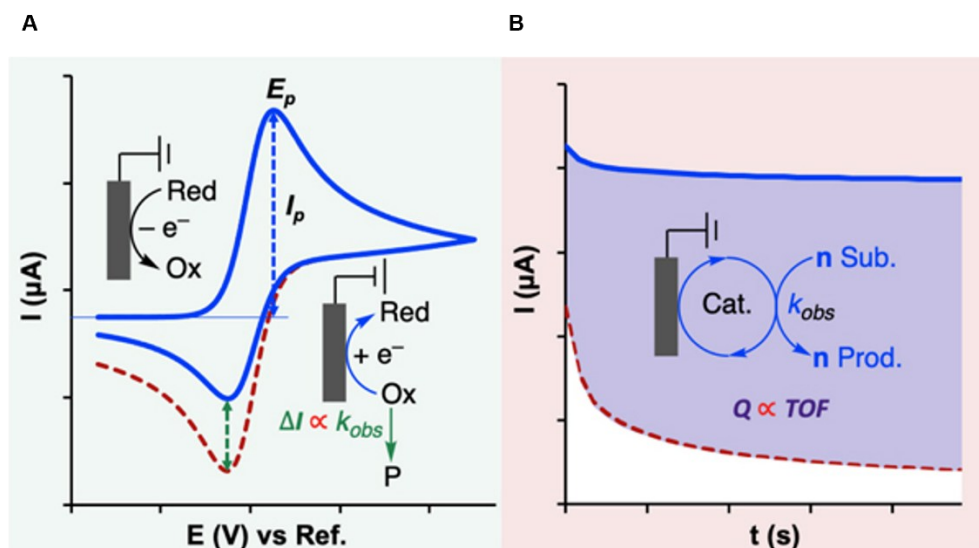


Figure 6. Schematic illustration of electrochemical signal behavior. (A) Cyclic voltammetry (CV) showing oxidation and reduction peaks, Chronoamperometric response depicting catalytic reaction dynamics over time (t). (adopted from Ref. [64]).

When the potential is scanned toward more positive values, the reduced species (Red) in solution is oxidized to its oxidized form (Ox) once the potential exceeds its redox potential. Upon reversing the scan, the generated oxidized form is reduced back to its reduced state. This continuous interconversion of Red and Ox produces the characteristic “duck-shaped” voltammogram, featuring distinct anodic and cathodic peaks.⁹¹

The equilibrium between the oxidized and reduced species at the electrode interface is described by the Nernst equation, which relates the electrode potential (E) to the ratio of their concentrations:

$$\text{Equation 2: } E = E^0 + \frac{RT}{nF} \ln \frac{[Ox]}{[Red]}$$

where E^0 is the standard redox potential, R is the universal gas constant, T is temperature, n is the number of electrons transferred, and F is Faraday’s constant. In practical applications, activities are replaced by concentration.^{91,92}

For example, in the classical ferricyanide/ferrocyanide ($\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$) redox couple, scanning the potential in the negative direction leads to the reduction of ferricyanide to ferrocyanide, whereas reversing the scan oxidizes ferrocyanide back to ferricyanide. At the formal potential, the surface concentrations of both species become equal, and the system achieves electrochemical equilibrium.

The peak current (I_p) observed in a CV experiment is directly influenced by several parameters, including the number of electrons transferred (n), electrode area (A), analyte concentration (C), diffusion coefficient (D), and the potential scan rate (ν). This relationship is defined by the Randles–Ševčík equation ⁹³ (at 25 °C):

$$\text{Equation 3: } I_p = 2.69 \times 10^5 n^{\frac{3}{2}} A D^{\frac{1}{2}} C \nu^{\frac{1}{2}}$$

According to this equation, the peak current is proportional to the square root of the scan rate, meaning that faster scans produce higher peak currents. Normalizing CV currents by $\nu^{1/2}$ allows direct comparison between experiments at different scan rates and helps identify whether the electrochemical process is diffusion-controlled or surface-controlled.⁹²

The CV shape and peak separation provide critical insights into the reversibility of the redox system. A small peak-to-peak potential difference ($\Delta E_p \approx 59/\text{nmV}$ at room temperature) indicates a reversible, diffusion-limited one-electron transfer, while larger separations suggest slower or quasi-reversible kinetics.

Chronoamperometry:

Chronoamperometry is one of the simplest and most fundamental amperometric electrochemical techniques, in which a constant potential is applied to the working electrode while the resulting current is recorded as a function of time Figure 6B. In a typical single potential-step experiment, the electrode potential is switched from an initial value, where no significant faradaic reactions occur—to a potential sufficiently positive or negative to induce oxidation or reduction of the target analyte (for example, from 0 V to +0.5 V).^{94,95}

Immediately after the potential step, a sharp increase in current is observed due to the rapid electron transfer reaction at the electrode surface. As the reaction proceeds, the analyte near the electrode is consumed, creating a concentration gradient that drives diffusion from the bulk solution toward the electrode. Consequently, the current decreases gradually over time as diffusion becomes the rate-limiting process and equilibrium is approached.⁹⁶

This behavior is described quantitatively by the Cottrell equation,⁹⁷ which defines the time-dependent decay of current under diffusion control at a planar electrode:

$$\text{Equation 4: } I_t = nFAC_0 \sqrt{\frac{D}{\pi t}}$$

where I_t is the current at time t , n is the number of electrons transferred per molecule, F is Faraday's constant, A is the electrode area, C_0 is the bulk analyte concentration, and D is the diffusion coefficient. According to this relationship, current is inversely proportional to the square root of time ($I_t \propto t^{-1/2}$), reflecting the rate at which analyte molecules diffuse from the bulk to the electrode surface.

The underlying concept of the diffusion layer, first introduced by Nernst, is essential to understanding CA behavior. This thin stationary layer of solution adjacent to the electrode surface has a lower analyte concentration than the bulk solution. During electrolysis, the analyte concentration at the electrode approaches zero, and diffusion becomes the dominant transport mechanism for replenishing reactants at the surface.⁹⁵

Chronoamperometry provides several advantages over other electrochemical techniques, notably a superior signal-to-noise ratio, particularly when experiments are conducted using thin fluid layers rather than large solution volumes.⁹⁸

2.4.3 Electrochemical Enzyme Based Biosensor

An enzyme can be combined with an electrochemical sensor to produce an electrode that will measure the substrate for that enzyme. Such electrodes, known as enzyme electrodes, have enabled assay of many compounds of biochemical interest.⁹⁹ Electrochemical enzyme-based biosensors are one of the most advanced and commercially successful bioanalytical devices because of a high

catalytic activity and selectivity of enzymes, as well as commercial availability of purified enzymes.³³

The modern concept of the electrochemical enzyme-based biosensor was first introduced by Leland C. Clark and Champ Lyons in 1962, who demonstrated an amperometric glucose sensor by immobilizing GOx on an oxygen electrode.³⁸ This pioneering work laid the foundation for the development of modern electrochemical biosensors and point-of-care diagnostic devices, many of which continue to rely on enzymatic reactions for their exceptional selectivity and sensitivity.

The mostly applicable enzymes that have extensively been utilized in sensing design are the oxidoreductases.³⁴ Oxidoreductases are a major class of enzymes that catalyze oxidation–reduction (redox) reactions by facilitating electron transfer between reductants (electron donors) and oxidants (electron acceptors).

In electrochemical biosensors, the function of oxidoreductase enzymes depends primarily on charge transport between the enzyme's active site and the electrode surface, which is often mediated through coenzymes (such as NAD⁺/NADH or FAD/FADH₂) or redox mediators (such as ferrocene derivatives).^{34,100} The efficiency of this electron transfer directly affects the sensor's response time, sensitivity. As mentioned in section 2.2.1, enzymes based on their catalytic function, oxidoreductases are generally categorized into five major subgroups: oxidases, dehydrogenases, peroxidases, oxygenases/hydroxylases, and reductases. Among these, oxidases, peroxidases, and oxygenases rely on oxygen or hydrogen peroxide as electron acceptors, whereas dehydrogenases and reductases operate independently of oxygen, using other cofactors instead. Each subgroup exhibits distinct catalytic activity and substrate specificity, making them suitable for different analytical applications.^{34,101}

During the past decade, oxidoreductase-based biosensors have attracted considerable attention owing to their broad applicability, high selectivity, and compatibility with electrochemical detection methods. Numerous biosensors have been developed for applications ranging from food quality control and environmental monitoring to clinical diagnostics. For example, *Morales et al.* designed an amperometric tyrosinase-based biosensor for the detection of benzoic acid in mayonnaise and cola beverages.¹⁰² *Dervisevic et al.* reported a xanthine oxidase biosensor for evaluating fish freshness,¹⁰³ while *Portaccio et al.* developed a laccase-based biosensor for the

determination of bisphenol A in tomato juice.¹⁰⁴ In biomedical applications, *Dungchai et al.* demonstrated a multi-analyte biosensor integrating GOx, lactate oxidase LOx, and uricase for simultaneous detection of glucose, lactate, and uric acid in blood samples.⁹⁸ *Alvau et al.* further expanded the scope by developing an enzyme-based electrochemical biosensor for therapeutic drug monitoring of the anticancer drug irinotecan (CPT-11), employing AChE and ChOx as biorecognition elements.¹⁰⁵ Similarly, *Pohanka et al.* proposed an AChE-based biosensor to monitor tacrine and 7-MEOTA, two cholinesterase inhibitors used in the treatment of Alzheimer's disease.¹⁰⁶

2.4.3.2 Enzyme–Electrode Electron Transfer and Generations of Biosensors

Achieving efficient electrical communication between immobilized enzymes and electrode surfaces has long been one of the greatest challenges in the development of enzyme-based electrochemical biosensors.¹⁰⁷ The rate of electron transfer is often hindered by several factors, including the burial of redox-active prosthetic groups deep within the protein structure, enzyme denaturation upon adsorption to the electrode, and unfavorable enzyme orientation that impedes direct electron exchange with the surface.³³ To overcome these limitations, biosensors have evolved through different “generations” based on how electron transfer occurs between the enzyme and the electrode Figure 7.¹⁰⁸ The Gox biosensor, arguably the most studied enzymatic system, serves as a representative model for these generations.

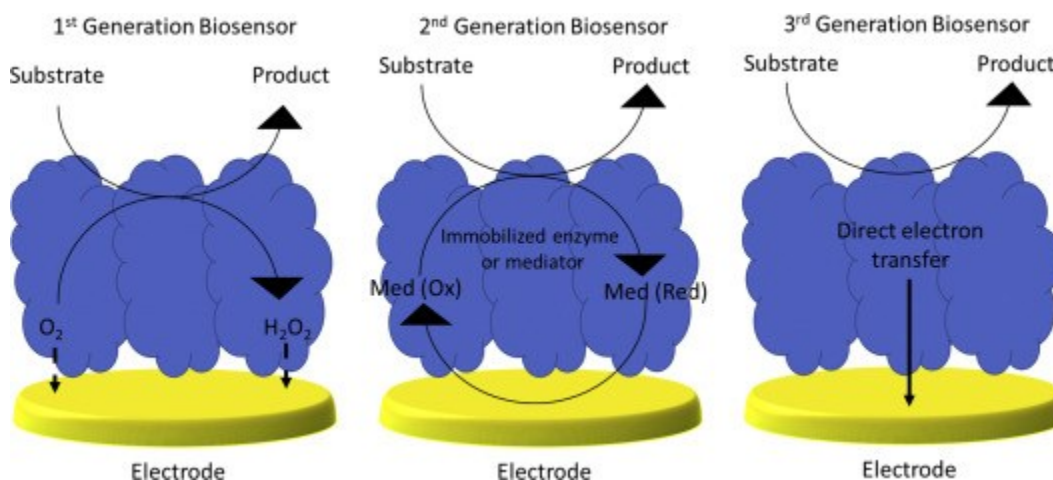
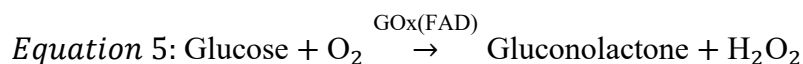


Figure 7. Schematic illustration of working principles for different generations of electrochemical glucose sensors (adopted from Ref. [107]).

First-Generation Biosensors

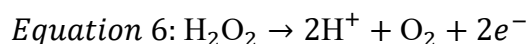
First-generation biosensors operate by using molecular oxygen (O_2) as the natural electron acceptor and detecting the electroactive product hydrogen peroxide (H_2O_2). The reactions involved are:

1. Enzymatic reaction:



where FAD is the enzyme's flavin adenine dinucleotide cofactor.

2. Electrochemical detection:



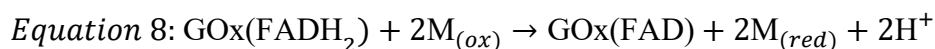
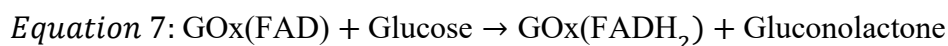
The generated current is proportional to the glucose concentration. However, this configuration suffers from oxygen dependence and electrochemical interference, since high oxidation potentials required for H_2O_2 detection can also oxidize other electroactive compounds such as uric acid and ascorbic acid.¹⁴

Second-Generation Biosensors

Second-generation biosensors introduced synthetic redox mediators to replace oxygen as the electron acceptor. These mediators shuttle electrons between the enzyme's redox center and the electrode surface, significantly improving electron-transfer efficiency and lowering the operational potential.

The reactions can be represented as follows:

1. Enzymatic reduction of mediator:



where $M_{(ox)}$ and $M_{(red)}$ are the oxidized and reduced forms of the mediator, respectively.

2. Electrochemical reoxidation of mediator at the electrode:

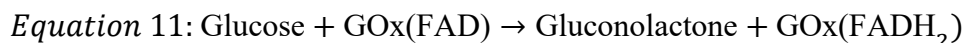
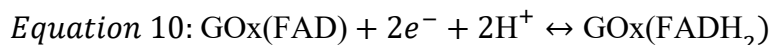


Common mediators include ferrocene derivatives, ferricyanide, quinones, and transition-metal complexes. Commercial blood glucose self-testing meters commonly are based on second-generation and employ ferricyanide or ferrocene-based mediators to facilitate electron transfer between the enzyme and the electrode. However, most vivo biomarker monitoring devices operate without mediators, as the potential leaching and toxicity of these compounds pose safety concerns.¹⁴

Third-Generation Biosensors

Third-generation biosensors achieve direct electron transfer (DET) between the enzyme's redox-active site and the electrode, eliminating the need for mediators altogether. Electron transfer occurs through conductive nanomaterials (e.g., carbon nanotubes, graphene, or metallic nanoparticles) that bring the enzyme's active site into electrical contact with the electrode surface.^{109,110}

The simplified redox process is:



In this case, electrons are transferred directly from the reduced enzyme cofactor ($FADH_2$) to the electrode surface without a diffusional mediator. This approach allows operation at low potentials, improving selectivity, sensitivity, and response speed. However, achieving efficient DET requires precise control of enzyme orientation and immobilization, since only a small fraction of enzymes may form productive electrical contact.

2.4.4 Enzyme Immobilization

The fabrication of enzyme-based electrochemical sensors requires establishing efficient electrical communication between the enzyme and the electrode surface, which remains one of the major challenges in biosensor design.¹¹¹ To construct a functional biosensor, the biological component must be properly attached to the transducer while maintaining enzyme activity. This process is known as enzyme immobilization. The main objective of immobilization is to achieve optimal bio-stability and high reaction efficiency; however, achieving optimal immobilization remains challenging.¹¹²

The chemical and thermal microenvironment surrounding the enzyme plays a crucial role in maintaining its stability and activity, directly influencing the overall sensor performance. Therefore, the selection of an immobilization strategy depends on multiple factors, including the type of biological element, nature of the transducer surface, physicochemical characteristics of the analyte, and the operating conditions under which the sensor will function. Ultimately, the chosen immobilization method should ensure that the enzyme exhibits maximum catalytic efficiency within its immobilized microenvironment.¹¹³

Over the years, numerous immobilization techniques have been developed to improve enzyme stability, reproducibility, and electron-transfer efficiency. Broadly, these techniques can be categorized into four main approaches: 1-adsorption, 2-entrapment, 3-covalent attachment and 4-cross-linking,¹¹⁴ and are demonstrated in Figure 8 below.¹¹⁵

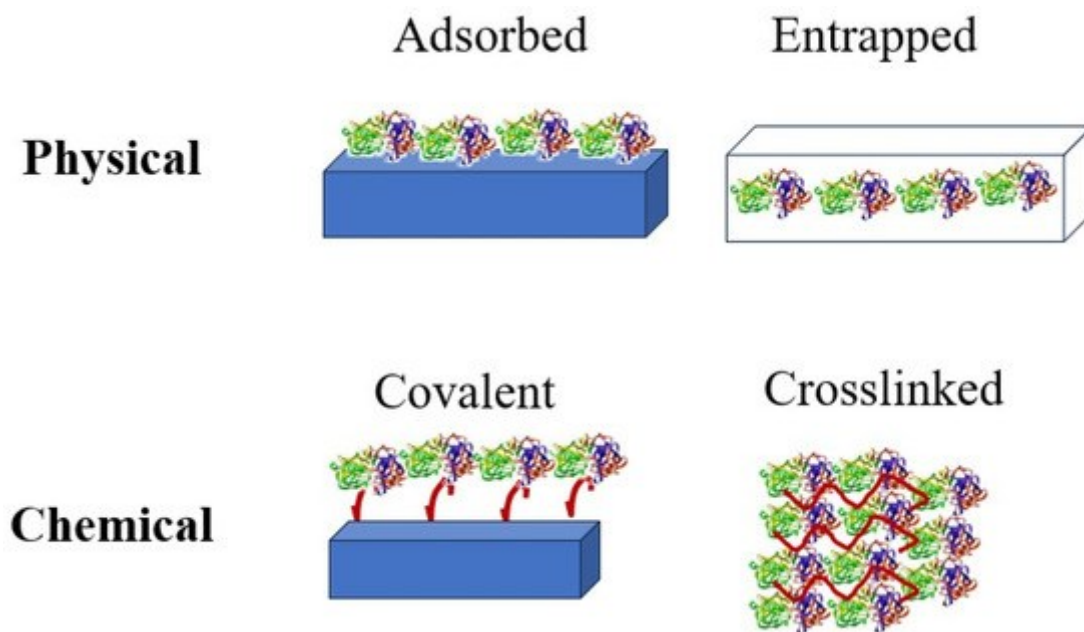


Figure 8. Representation of major enzyme immobilization strategies used in biosensor fabrication. (adopted from Ref. [115]).

2.4.4.1 Adsorption

The physical adsorption method relies on non-specific interactions between enzyme molecules and the surface of a solid support or matrix, such as glass, plastics, cellulose, or conducting polymers (Figure 9). In this technique, a concentrated enzyme solution is mixed with the matrix, allowing the enzyme molecules to adhere to the surface through weak intermolecular forces.¹¹⁶ The primary interactions involved include van der Waals forces, hydrogen bonding, and electrostatic (salt) linkages.¹¹⁷ This immobilization technique offers several advantages, including high catalytic efficiency, excellent enzyme specificity, and operation under mild conditions, making it an attractive alternative to chemical catalysts for a wide range of applications.¹¹⁸ Numerous studies have demonstrated its effectiveness in biosensor fabrication.

For example, *Chaubey et al.* co-immobilized LOx and lactate dehydrogenase (LDH) on electrochemically synthesized polyaniline (PANI) films using physical adsorption. Their results showed that substrate recycling significantly enhanced the sensor response, with a stability of approximately three weeks at 4–10 °C.¹¹⁹ Similarly, *Soylomez et al.* employed physical adsorption to immobilize ChOx onto a polymer-modified graphite electrode for cholesterol detection. The conducting polymer, poly((Z)-4-(4-(9H-carbazol-9-yl)benzylidene)-2-(4-nitrophenyl)oxazol-

5(4H)-one) [poly(CBNP)], served as an effective immobilization matrix, forming hydrogen bonds with the enzyme and enhancing both attachment strength and biosensor performance.¹²⁰

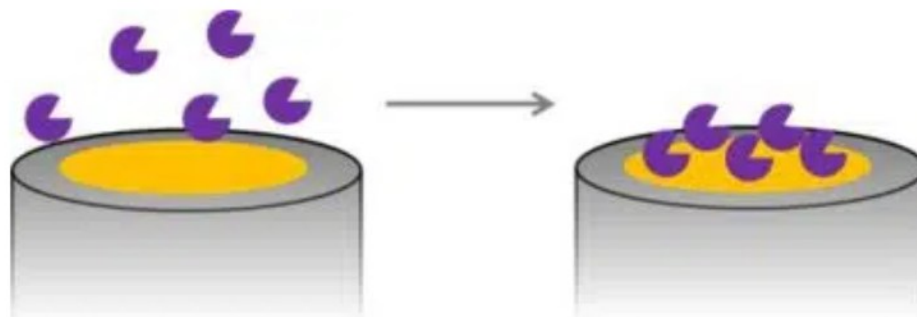


Figure 9. Schematic representation of a simple adsorption due to interaction of the enzyme with the electrode surface of major enzyme immobilization strategies used in biosensor fabrication. (adopted from Ref. [116]).

2.4.4.2 Encapsulation

Another widely used immobilization technique is encapsulation or entrapment, in which a gel-like or membrane-like material is employed to physically retain the enzyme on the electrode surface. (Figure 10) In this method, the enzyme molecules are physically confined within a three-dimensional matrix without forming covalent bonds, allowing them to remain active while being immobilized. Various materials can be used for enzyme encapsulation, including liposomes, natural or synthetic polymers, sol-gel matrices, and hydrogels, depending on the sensing conditions and desired sensor characteristics. This approach is relatively simple and effectively preserves the intrinsic catalytic properties of the enzyme, often leading to enhanced stability and extended operational lifetime of the biosensor.^{112,121}

However, a key limitation of this method is the lack of control over enzyme orientation within the matrix, which can result in inefficient electron transfer. Additionally, partial detachment of enzyme molecules from the electrode surface or from each other may reduce the overall catalytic activity of the immobilized enzyme layer. Despite these challenges, biosensors based on enzyme encapsulation offer notable advantages, including high substrate selectivity, prolonged enzyme activity, and improved operational stability. Continuous research into novel encapsulating

materials and fabrication strategies continues to enhance the performance and selectivity of encapsulated enzyme systems.¹²²

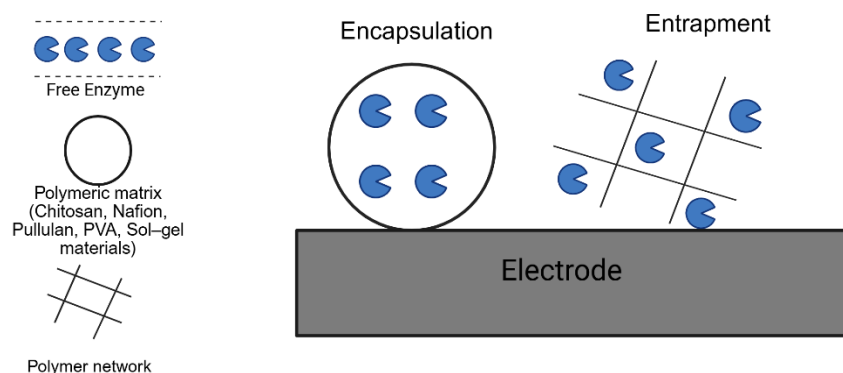


Figure 10. Schematic illustration of enzyme immobilization by encapsulation and entrapment on an electrode surface. (figure created with BioRender.com)

Several studies have demonstrated the successful application of enzyme encapsulation in biosensor fabrication using different matrix materials. Pandey et al. reported a non-mediated glucose biosensor based on GOx encapsulated within a composite sol-gel glass prepared from optimized concentrations of 3-aminopropyltriethoxysilane and 2-(3,4-epoxycyclohexyl) ethyltrimethoxysilane. The cross-linked GOx retained its enzymatic activity for more than one month at 4 °C, demonstrating excellent stability of the sol-gel encapsulation matrix.¹²³ Similarly, natural polymer-based hydrogels have been explored for their biocompatibility and conductivity. A biosensor incorporating LOx immobilized on a glassy carbon electrode modified with a laponite-chitosan hydrogel was developed for the quantification of L-lactate in alcoholic beverages and dairy products. This bioelectrode demonstrated remarkable analytical features, including high stability, strong resistance to common interferents such as ascorbic acid, and excellent sensitivity ($(0.326 \pm 0.003) \text{ A cm}^{-2} \text{ M}^{-1}$), with a response time below 5 s and a detection limit of $(3.8 \pm 0.2) \times 10^{-6} \text{ M}$.¹²⁴

2.4.4.3 Covalent

Covalent binding is one of the most common and reliable methods for enzyme immobilization. In this approach, stable covalent bonds are formed between functional groups on the enzyme surface

and reactive groups on a support matrix. (Figure 11).¹²⁵ The immobilization process generally involves two steps: (1) activation of the support surface using linker molecules such as glutaraldehyde or carbodiimide, and (2) covalent coupling of the enzyme to the activated surface. These linker agents act as bridges between the enzyme and the matrix, forming self-assembled monolayers (SAMs) that provide a uniform and stable interface for enzyme attachment.¹²⁶

Covalent immobilization offers strong and stable enzyme–matrix interactions, minimizing enzyme leaching and enhancing long-term stability. Additionally, this method allows controlled loading of enzymes on the surface. However, the process can lead to partial enzyme denaturation if reactive groups near the active site are modified, and it often requires longer reaction times and careful optimization of chemical conditions to preserve enzyme activity.¹²⁶

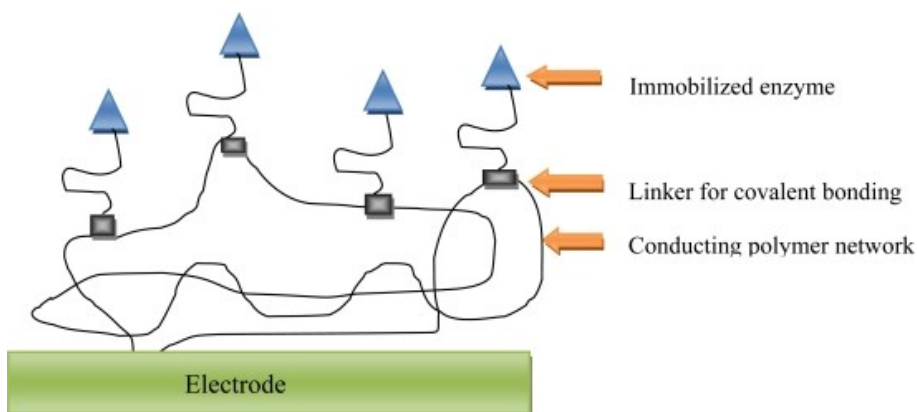


Figure 11. Schematic illustration of enzyme immobilization by covalent binding on an electrode surface. (adopted from Ref. [125]).

A representative example of covalent enzyme immobilization was reported by *Shen et al.*, who developed an amperometric cholesterol biosensor based on the covalent attachment of ChOx onto a gold working electrode using a self-assembled monolayer (SAM) approach. In this method, 3-mercaptopropionic acid (MPA) was first self-assembled onto the gold surface to introduce terminal carboxylic groups, which were then activated with EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) to form amide linkages with the amine groups of the enzyme. The resulting biosensor exhibited a linear response for cholesterol concentrations ranging from 6.5×10^{-4} M to 7.8×10^{-3} M, demonstrating the efficiency of covalent coupling for enzyme immobilization. The storage stability of the biosensor was limited, after four days the current response decreased to

approximately 20% of its initial value, likely due to gradual enzyme denaturation on the electrode surface.¹²⁷

2.4.4.3 Cross-linking

The cross-linking method is an irreversible enzyme immobilization technique that involves the formation of intermolecular covalent bonds between enzyme molecules, producing stable three-dimensional aggregates (Figure 12). This process is typically facilitated by multifunctional cross-linking agents, which act as chemical linkers connecting enzyme molecules or linking them to supporting materials. Common cross-linking agents include glutaraldehyde, glyoxal, and hexamethylenediamine, which react with functional groups (such as amino or carboxyl groups) on enzyme surfaces to form stable chemical bridges. In some cases, an inert carrier protein, such as BSA, is added during cross-linking to prevent excessive enzyme–enzyme interactions and to stabilize the resulting structure.¹²⁸

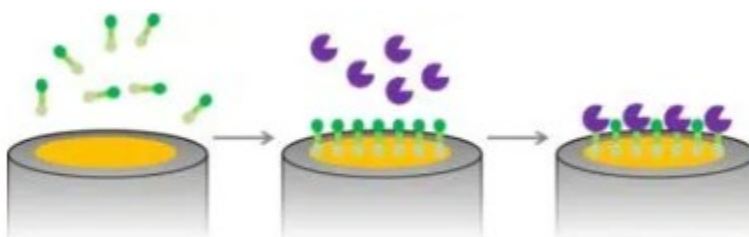


Figure 12. Schematic representation of a simple self-assembled monolayers (SAM) formation for a covalent bond to an enzyme (adopted from Ref. [116]).

A number of enzyme-based biosensors have been successfully fabricated using the cross-linking approach. *Luo et al. (2008)* developed a disposable alcohol biosensor by co-immobilizing alcohol dehydrogenase (ADH) and its cofactor NAD^+ onto a Nafion–Meldola Blue–modified SPE through glutaraldehyde-mediated cross-linking. The resulting biosensor exhibited acceptable specificity, reproducibility, storage stability, and rapid response, and was successfully applied to the determination of serum alcohol levels.¹²⁹

In another study, *Kong et al. (2009)* reported an amperometric glucose biosensor in which GOx was immobilized onto a ZnO nanotube–modified electrode using the same cross-linking technique.

The sensor showed a linear response in the glucose concentration range of 5×10^{-5} to 1.2×10^{-2} M, with a detection limit of 1×10^{-6} M. It also demonstrated good anti-interference capability and long-term stability, retaining approximately 70% of its initial activity after 60 days of storage.¹³⁰

2.5 Recent Developments in Glucose and Lactate Monitoring

2.5.1 Glucose

Given that diabetes continues to be a major global health challenge with glucose dysregulation leading to severe complications such as cardiovascular disease, nephropathy, neuropathy, and retinopathy, effective self-monitoring of blood glucose remains essential for prevention and management. The need for rapid, user-friendly, POC monitoring has therefore driven extensive research toward developing reliable glucose sensors, resulting in a large body of studies dedicated to advancing glucose-sensing technologies.¹³¹

The foundation of glucose biosensing dates back to 1962, when Clark and Lyons first introduced the concept of an enzyme-based electrochemical glucose electrode.³⁸ Since then, extensive research has focused on improving the selectivity, stability, and portability of glucose biosensors. Moreover, beyond blood analysis, non-invasive and minimally invasive glucose monitoring techniques, based on alternative biological fluids such as urine, sweat, saliva, and tears, have gained increasing attention. Wearable and continuous glucose monitoring (CGM) systems have further revolutionized diabetes management, enabling real-time feedback and improved glycemic control.

In recent years, substantial research efforts have been directed toward developing noninvasive glucose biosensors capable of detecting glucose in alternative biological fluids such as saliva, tears, sweat, and urine. Gao *et al.* developed a nonenzymatic saliva-range glucose sensor based on electrodeposited cuprous oxide (Cu_2O) nanocubes on a graphene strip. The device demonstrated high sensitivity and achieved approximately 95% accuracy in real saliva samples, ranking among the best saliva-range glucose sensors reported to date.¹³² Similarly, Iguchi *et al.* reported a flexible amperometric contact lens biosensor designed for continuous tear glucose monitoring, representing a promising step toward real-time in situ glucose detection within the eye.¹³³

Karpova *et al.* proposed a non-invasive sweat-based glucose monitoring system that continuously analyzed glucose in sweat using a flow-through biosensor configuration.¹³⁴ Building upon this,

Wiorek *et al.* developed an epidermal patch for glucose analysis in sweat, incorporating pH and temperature correction to account for dynamic fluctuations during on-body tests, thereby improving analytical reliability.¹³⁵ Another work, a flexible self-powered biosensor integrated within a diaper was reported for glucose detection in urine.¹³⁶ This system operated using an enzymatic biofuel cell (EBFC), in which glucose served as the fuel source to generate electricity. The EBFC was connected to a power management system (PMS) equipped with a capacitor for energy storage, which successfully powered a light-emitting diode (LED) as a visual signal of glucose presence.

2.5.2 Lactate

L- lactate reflects both metabolic and physiological status, since a patient's lactate level can serve as an important indicator of disease severity and assist in improving the diagnosis and treatment of various medical conditions, its continuous and accurate monitoring is of great importance. Consequently, numerous studies have focused on the development of lactate biosensors capable of detecting this metabolite in different human body fluids, employing both invasive and noninvasive approaches, and designed for single-use or continuous monitoring applications.

Several recent studies have focused on the development of innovative lactate biosensors for non-invasive or minimally invasive monitoring.

Prophet *et al.* reported a textile-based colorimetric sensor for the simultaneous detection of sweat pH and lactate, fabricated by depositing three functional layers onto a cotton substrate. This noninvasive sensor successfully measured lactate concentrations in real human sweat within the 0–25 mM range, demonstrating high flexibility and comfort. Its textile integration allows potential incorporation into sportswear or wearable accessories, enabling real-time and continuous monitoring of physiological parameters during exercise.¹³⁷

In another work, Romero *et al.* developed a single-use electrochemical lactate biosensor for blood that required only 0.2 U of LOx while maintaining nearly unchanged sensitivity over five months of storage. The device detected lactate concentrations from 2 to 1000 μM , and recovery assays from human blood samples showed a mean recovery of $(89 \pm 6)\%$, confirming its accuracy and reliability.¹³⁸

Similarly, Xuan *et al.* designed a fully integrated wearable system for continuous sweat lactate monitoring during athletic performance. The flexible skin-mounted sensor demonstrated a linear response within the 1–20 mM range, with a sensitivity of $-12.5 \pm 0.53 \text{ nA mM}^{-1}$ and a rapid response time of less than 90 s. It exhibited excellent stability, reversibility, and reproducibility, and was validated in on-body tests with elite athletes during cycling and kayaking under controlled conditions.¹³⁹

Chapter 3: Electrochemical Tablet-based Assay for Stable and Reproducible Biosensing

In this chapter, I present the research recently submitted to a journal. The chapter begins with an abstract, introducing the development of an innovative electrochemical tablet-based assay. It then details the tablet preparation and characterization procedures, followed by an evaluation of the electrochemical performance and practical applications for glucose and lactate detection in human blood plasma. Finally, the chapter discusses the analytical performance, interference studies, and stability assessments, providing a complete overview of the research process and key findings.

Electrochemical Tablet-based Assay for Stable and Reproducible Biosensing

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3.1 Abstract

Rapid, reliable, and easy-to-use point-of-care (POC) tests remain a critical need for on-site diagnostics. Tablet-based assays offer portability and long shelf life but have traditionally relied on slow, reader-dependent optical detection that is often only semi-quantitative. Electrochemical biosensors provide a faster and more sensitive alternative but often require enzyme immobilization multi-step process that can reduce activity, complicate fabrication, and limit scalability and reproducibility. Here, we introduce an electrochemical tablet platform (eTab) that combines the operational simplicity of tablet-based assays with the sensitivity and quantitative capability of electrochemical sensing. All assay reagents are encapsulated within a pullulan matrix and released upon dissolution, forming a ready-to-use electrochemical solution for rapid, reagent-free analysis. The eTabs exhibited excellent reproducibility ($RSD < 6\%$) using ferri/ferrocyanide redox probes and were successfully adapted for glucose and lactate detection, achieving detection limits of 0.11 and 0.14 mM with wide linear ranges (0–15 and 0–4 mM, respectively). The eTabs exhibited less than 10% signal deviation after 30 days of storage at room temperature, significantly outperforming equivalent solution-based reagents. Both systems operated accurately in human plasma, showing negligible interference and reliable recoveries. By merging the stability of tablet-based assays with the analytical precision of electrochemical sensors, this work establishes eTabs as a simple, scalable, and versatile platform for next-generation POC diagnostics

Keywords: Electrochemical sensing, Tablet-based assay, Enzyme immobilization, Enzyme stability, Lactate, Glucose, Human blood plasma

3.2 Introduction

Affordable and portable diagnostic platforms are central to timely disease detection and effective patient management.¹⁻³ However, many conventional assays still rely on bulky instrumentation, trained personnel, and lengthy workflows, which limit accessibility in resource-limited contexts.^{2,3} POC were developed to address these barriers by providing rapid and reliable analysis in simple, low-cost formats that can be performed directly at the site of need.^{4,5} To guide the development of POC technologies, the World Health Organization (WHO) introduced the ASSURED criteria, which were later expanded into the REST-ASSURED framework, emphasizing real-time connectivity, ease of sample collection, scalability, transferability, affordability, sensitivity, specificity, user-friendliness, rapidity and robustness, equipment-free operation, and deliverability to end-users.¹⁴⁰

Within this framework, tablet-based assays have emerged as particularly attractive platforms owing to their portability, extended shelf life, ease of use, and low production cost.^{8,9,141} The concept of reagent tablets can be traced back to the early 20th century, when methylene blue tablets were employed in Denmark to assess milk quality,⁴² followed by major breakthrough in the 1941 when the first tablet-based assay for detection of reducing substances in urine was introduced.⁴⁶ Interest in this format was revitalized in 2014, when Jahanshahi-Anbuhi et al. fabricated pullulan-encapsulated reagent tablets with simple drop casting that stabilized biomolecules and produced robust bioassays.⁹ Since then, sugar-based tablets have been expanded by our group and others to incorporate various substances including enzymes, gold nanoparticles, and bacteriophages for applications such as adenosine triphosphate, uric acid, cysteamine, and glucose detection.^{8,55,63,65,142} These tablets are inexpensive, scalable, and compatible with diverse chemical and biological reagents, while polysaccharide matrices in particular enhance stability and eliminate the need for cold-chain storage.^{1,9} Despite this progress, for more than a century, nearly all reported tablet-based systems have relied on optical detection, including colorimetric, fluorometric, and chemiluminescence.^{1,42,58,62,141} While effective under controlled conditions, optical readouts can be constrained by long assay times and susceptibility to sample turbidity or color variations. Moreover, optical methods are generally not quantitative unless specialized and expensive readers such as UV-Vis spectrometers are used.

In parallel, electrochemical biosensors have become the most widely employed class of biosensors for POC applications, valued for their rapid response, low detection limits, operational simplicity, portability, and relatively low cost.^{11,143–145} The origins of this field date back to 1962, when Clark and Lyons reported the first amperometric glucose enzyme electrode, establishing the foundation of modern biosensing.³⁸ While early approaches relied on solution-phase reactions, these systems suffered from poor storage stability in water,¹⁴⁶ subsequent advances shifted toward immobilizing recognition elements at electrode surfaces, thereby simplifying workflows and enabling compact devices.^{144,147} So far, a wide range of immobilization strategies have been developed, including adsorption, covalent coupling, cross-linking, and entrapment in polymeric or sol–gel matrices.^{148,149} However, depending on the approach, immobilization suffers from drawbacks such as enzyme leakage under variable pH or temperature, creation of diffusion barriers, reduction of reagent activity and reproducibility, and involving harsh chemistries that compromise enzyme function and stability.^{148,150} More recent innovations such as nanogels,¹⁵¹ hydrogels,¹⁵² mesoporous supports,¹⁵³ two-dimensional supports such as MXenes¹⁵⁰ and nanoparticle such as ZnO¹⁵⁴ have showed improved outcomes with respect to enzyme stability and sensor functionality, but these approaches often require multi-step chemistries,¹⁵⁵ specialized nanomaterials,¹⁵⁶ or complex electrode modifications, which limit scalability and reproducibility. Indeed, even with such advanced techniques, some groups have reported difficulty immobilizing DNazymes directly onto electrode surfaces.¹⁵⁷ Therefore, developing new approaches for the fabrication of portable, easy-to-use electrochemical sensors with prolonged stability is crucial for advancing the field, eliminating the challenges and constraints associated with immobilization methods.

Here, we introduce the first-of-its-kind electrochemical tablet-based sensor (eTab), a platform that integrates the inherent benefits of tablet-based assays such as simple fabrication, portability, and extended shelf stability, with the high sensitivity and quantitative capabilities of electrochemical detection. This design simultaneously overcomes the major limitations of tablet-based assays, such as their semi-quantitative result interpretation, and eliminates the immobilization step required in conventional electrochemical biosensors, which introduces its own set of challenges. In this strategy, all reagents are encapsulated within a dissolvable polysaccharide matrix, eliminating complex immobilization steps, while providing an all-in-one reagent tablet with pre-measured quantities of reagents that maintains consistent performance under ambient conditions. Once dissolved in the sample, the tablet produces a ready-to-use assay mixture that can be directly

applied to an electrode surface for rapid, sensitive, and user-friendly testing (Figure 1). We first optimized the tablet formulation parameters, including polysaccharide concentration and electrolyte content, to ensure robust and consistent tablet formation. We then demonstrated that the incorporation of pullulan and the tablet fabrication process do not compromise electrochemical performance, followed by an evaluation of tablet reproducibility. As proof of concept, we developed assays for glucose and lactate detection in blood plasma, two clinically relevant biomarkers of metabolic dysfunction and critical illness, and then assessed the stability of the tablets, which showed less than 10% signal deviation after one month of storage at room temperature. Together, these results position electrochemical tablet-based assays as a promising addition to the POC diagnostic toolbox, bridging the gap between tablet formats and electrochemical sensing while enabling new opportunities in biomedical diagnostics, food safety, and environmental monitoring.

3.3 Materials and Methods

3.3.1 Chemicals and Materials

Potassium chloride (KCl, Cat. No. 7447-40-7), sodium phosphate monobasic (S0751), sodium phosphate dibasic (S9763), glucose oxidase from *Aspergillus niger* (GOx, G7141), horseradish peroxidase (HRP, P8250), glucose (G8270), sodium lactate (L7022), potassium ferrocyanide trihydrate (P236), potassium ferricyanide (P232), fructose (PHR1002), ascorbic acid (PHR1008), uric acid (90381) and lactate oxidase from *Aerococcus viridans* (LOx, L9797) were obtained from Sigma-Aldrich (Canada). Pullulan (desalinated) was purchased from Polysciences, Inc. (Cat. No. 01917, Warrington, PA, USA), and carbon screen-printed electrodes (CTI, CSCL4W) with a three-electrode configuration were purchased from PalmSens Inc. Carbon steel baking trays (Betty Crocker) were purchased locally. Deionized Milli-Q water was generated using a Milli-Q system (Reference Z00QSV001). Human blood plasma samples were obtained from Biobanque IRCAM (Montreal, Canada). This research was approved by Concordia University's Institutional Review Board (Approval No. SC5823; BioPermit No. B-SJA-22-01).

3.3.2 Tablet Fabrication

Tablets were prepared as illustrated in Figure 13A. The powdered reagents (GOx, HRP, LOx, potassium ferrocyanide, KCl, and pullulan) were weighed and combined in a 50 mL Falcon tube

with potassium phosphate buffer (pH 7.4 for glucose tablets; pH 7.0 for lactate tablets). The mixture was vortexed until homogeneous, and 100 μ L aliquots were drop-cast into clean carbon steel trays using a micropipette. The trays were left to dry overnight at room temperature under a fume hood. On the following day, the dried tablets were released from the tray by gentle tapping and collected into glass vials for storage. This simple approach ensured uniform tablet composition at the laboratory scale.

To obtain solid, homogeneous tablets with good mechanical stability and no visible cracks or brittleness, different concentrations of pullulan, a water-soluble polysaccharide used as a film-forming and binding agent, were evaluated, followed by adjustment of KCl concentration, since ionic strength critically influences electrochemical performance. For tablet dissolution, each tablet was first added to 100 μ L of deionized water. To facilitate dissolution and mixing, the solution was vortex-mixed for 5 s. However, to ensure consistency across all experiments, the vortex-mixing method (5 s) was employed throughout this study.

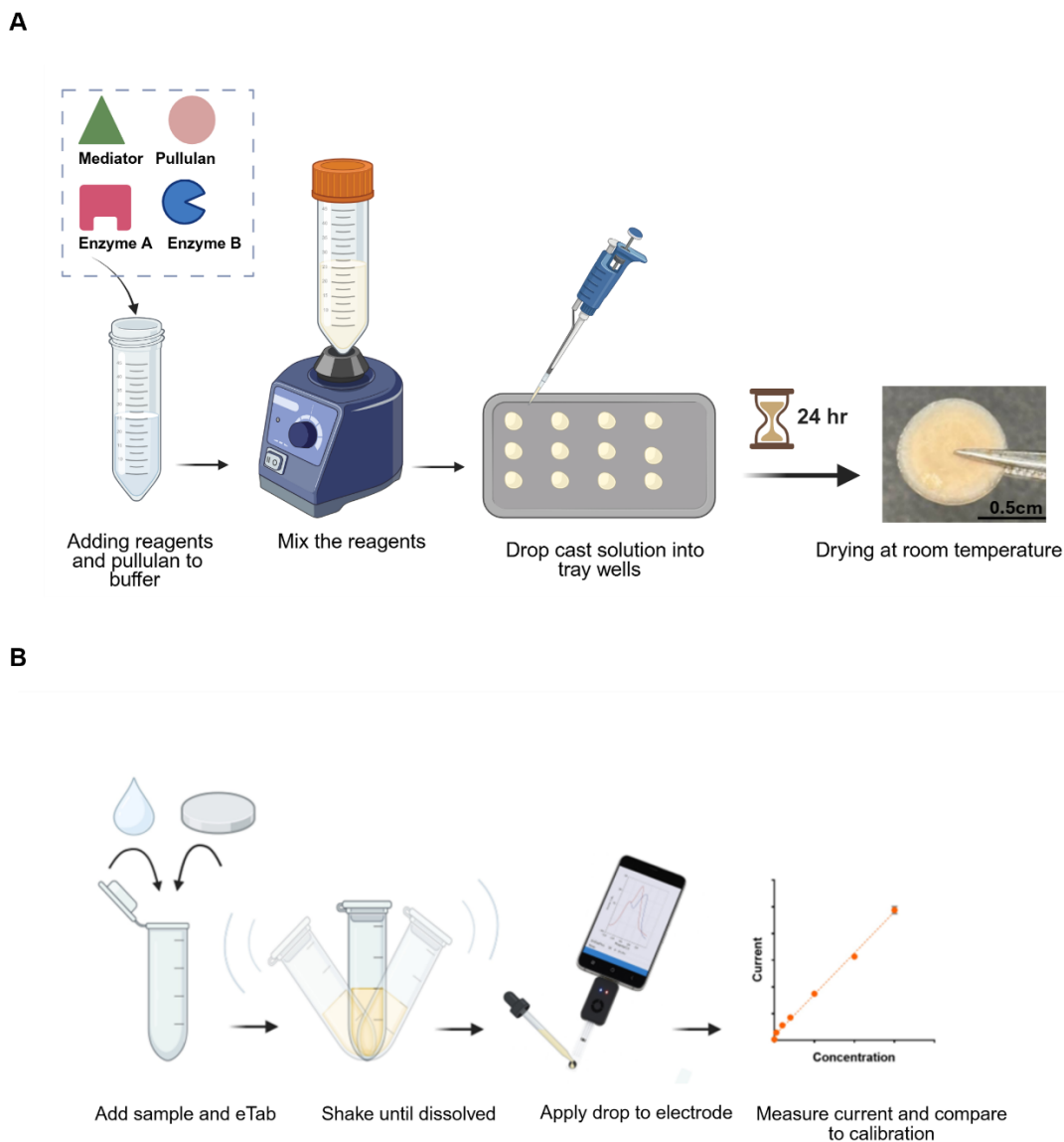


Figure 13. Fabrication and workflow of the electrochemical tablet-based biosensor. (A) To fabricate the eTabs, reagents for electrochemical sensing together with pullulan are dissolved in potassium phosphate buffer, mixed thoroughly, drop-cast into tray wells, and dried overnight at room temperature to obtain all-in-one solid tablets. (B) For analysis, the sample (analyte) and an eTab are combined in a microtube and shaken until the tablet is completely dissolved. A small droplet of the resulting solution is then applied to the electrode surface and the current measured with a portable smartphone-connected potentiostat; the analyte concentration is determined by comparison with a calibration curve (figure created with BioRender.com).

3.3.3 Electrochemical Testing

Electrochemical characterization was performed by cyclic voltammetry (CV) using tablets prepared from a solution containing 10 mM potassium ferricyanide and 10 mM potassium ferrocyanide in 200 mM KCl with 4% pullulan. Tablets were dissolved in 100 μ L of deionized water, and 20 μ L of the resulting solution was applied to the working area of the screen-printed electrode (SPE). CV measurements were recorded over a potential range of -1.0 to $+1.0$ V at a scan rate of 0.1 V/s using a portable PalmSens4 potentiostat (PalmSens Inc., The Netherlands). The oxidation peak currents were used for further analysis. All electrochemical characterizations were performed using a PalmSens4 potentiostat (PalmSens BV, The Netherlands), while validation for smartphone-based operation was conducted using the Sensit Smart potentiostat (PalmSens BV).

Commercial carbon screen-printed electrodes were characterized by CV scans using a solution of 10 mM potassium ferricyanide and 10 mM potassium ferrocyanide prepared in 200 mM KCl. A 20 μ L aliquot of this solution was directly applied to the working area of the electrode, and voltammograms were recorded from -1.0 to $+1.0$ V at a scan rate of 0.1 V/s using the PalmSens4 potentiostat. The raw detected oxidation peak currents were used for further analysis.

3.3.3.1 Glucose Tests

Glucose detection was performed using a bienzymatic system consisting of Gox, HRP with ferrocyanide as the mediator.^{158 159} Tablets (100 μ L aliquots containing 4% pullulan w/v, 100 mM ferrocyanide, 200 mM KCl, 0.1 M phosphate buffer pH 7.4) were prepared with varying enzyme loadings as an initial step in developing the glucose eTab assay. For electrochemical testing, each tablet was dissolved in 100 μ L of the sample and allowed for incubation for 2 min. Then, 20 μ L of the mixture was applied to the working area of a screen-printed electrode. Chronoamperometry was performed at -0.3 V, and currents were recorded at 45 s. Calibration curves were obtained using glucose concentrations ranging from 0 to 20 mM (0, 0.25, 0.75, 1, 2.5, 5, 10, 15, and 20 mM) in deionized water. In addition, the colorimetric change produced by the GOx/HRP/ferro system was analyzed using ImageJ (grayscale intensity) and validated with UV-vis spectroscopy details of which are provided in the Supporting Information

To compare the stability of tablet-based assays with their solution equivalents, all-in-one tablets containing GOx, HRP, and ferrocyanide were stored in glass vials at room temperature (22 °C) and tested in 5 mM glucose solution at defined intervals. In parallel, two solution-based systems were prepared and stored under the same conditions: (i) a solution containing GOx and HRP, and (ii) a solution containing GOx, HRP, and ferrocyanide in phosphate buffer (pH 7.4), to assess enzyme stability and overall reagent preservation. At each time point, the current measured on that day (e.g., day 7) was compared with the initial current (I_0) obtained on day 0. The corresponding percentage absolute change, calculated as $(|I_{\text{day}} - I_0| / I_0 \times 100 \%)$, was used to quantify the deviation from the initial response and thereby assess the stability of the sensor over time. Interference from common electroactive and sugar species was assessed by comparing responses to 5 mM glucose alone and in combination with excess fructose, lactate, ascorbic acid, uric acid in addition to a mixture of all interferents.

For real sample analysis, frozen plasma samples from Biobanque IRCM (Montreal, Canada) with known glucose concentrations were tested directly.

3.3.3.2 Lactate Test

Lactate detection was performed using a bienzymatic system consisting of LOx and HRP,¹⁶⁰ with ferrocyanide as the mediator. Tablets (100 μL aliquots containing 4% pullulan w/v, 100 mM ferrocyanide, 200 mM KCl, 0.1 M phosphate buffer pH 7.0) were formulated with a fixed HRP loading (20 U per tablet) and varying LOx concentrations to develop the lactate eTab assay.

For electrochemical measurements, microtubes were preloaded with 50 μL of deionized water, followed by the addition of 50 μL lactate standard sample solution. Each tablet was dissolved in the 100 μL final volume and allowed to incubate for 2 min. Then, 20 μL of the mixture was applied to the screen-printed electrode. Chronoamperometry was carried out at -0.3 V, and currents were recorded at 45 s. Calibration curves were generated using L-sodium lactate standards of 0–6 mM (0, 0.25, 0.75, 1, 2, 4, and 6 mM) in deionized water. The colorimetric response from the LOx/HRP/ferro reactions was quantified using ImageJ and validated against a UV–vis spectroscopy as detailed in the Supporting Information

Interference from common electroactive and sugar species was assessed by comparing responses to 2 mM Lactate alone and in combination with excess fructose, glucose, ascorbic acid, uric acid, and a mixture of all interferents.

For real sample analysis, human plasma samples (Biobanque IRCM, Montreal, Canada) were spiked and analyzed using the optimized lactate eTab protocol. Recoveries were calculated by comparing measured concentrations with reference values, as described in the results section. To compare the stability of tablet-based assays with their solution equivalents, tablets containing LOx, HRP, and ferrocyanide were stored in glass vials at room temperature (22 °C) and tested in 2 mM lactate solution at defined intervals. In parallel, two solution-based systems were prepared and stored under the same conditions: (i) a solution containing LOx and HRP and (ii) a solution containing LOx, HRP and ferrocyanide in phosphate buffer (pH 7). To assess enzyme stability and overall reagent preservation at each time point, the current measured on that day (e.g., day 7) was compared with the initial current (I_0) obtained on day 0. The corresponding percentage absolute change, calculated as $(|I_{\text{day}} - I_0| / I_0 \times 100 \%)$, was used to quantify the deviation from the initial response and thereby assess the stability of the sensor over time

3.4 Results and discussion

3.4.1 Tablet Fabrication and Characterization

The eTab fabrication involves a few simple steps, as illustrated in Figure 13A. Tablet formulation began with visual and structural assessment to identify a composition that produced a single, solid piece—uniform in shape and resistant to cracking or chipping. The concentration of pullulan, a film-forming and binding polysaccharide, was first optimized by varying its content between 1–6 % (w/v) in deionized water (Figure 14A). At 1 %, the tablets failed to solidify, whereas 2–3 % yielded cracked and non-uniform structures. Structurally uniform and mechanically robust tablets were obtained at 4–6 %, and 4 % pullulan was selected as the optimal concentration, consistent with previous reports employing 3–7 % (w/v) pullulan for tablet fabrication.. For instance, Ezeoke *et al.* used 7% pullulan for water-hardness detection tablets, whereas Al-Kassawneh *et al.* employed 3% for cysteamine detection,^{61,161} providing comparable mechanical cohesion to higher loadings while remaining cost-effective and easy to dissolve without extended mixing. Supporting electrolyte content was then evaluated to find the optimum loading capacity within pullulan matrix.

Understanding this content is important as ionic strength influences conductivity and double-layer formation, which together affect electrochemical performance.¹⁶² Tablets were prepared with 4% pullulan and 100–500 mM KCl (Figure 14B). At 100 and 200 mM, tablets remained uniform and solid, while concentrations ≥ 300 mM produced brittle and fractured structures. Based on these results, 200 mM KCl was selected to balance mechanical integrity with sufficient ionic conductivity.

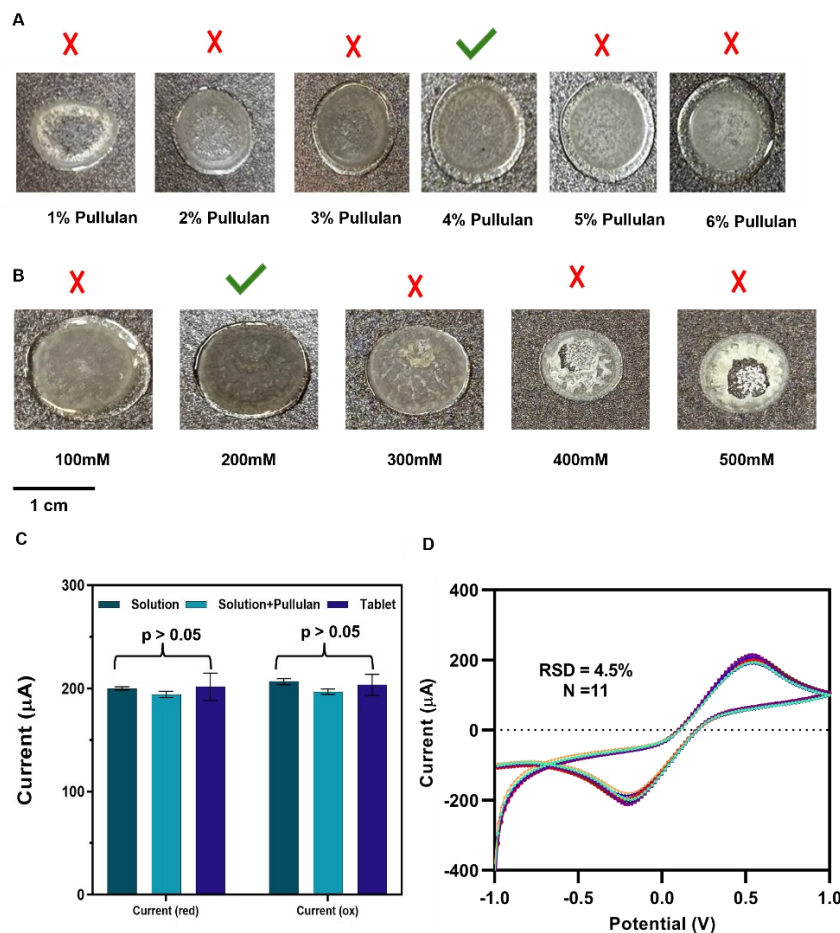


Figure 14. Characterization of tablet formulation design. (A) Formulation refinement of tablets made by 100 μL aliquots of solutions with varied pullulan concentrations of 1–6% w/v in deionized water. (B) Formulation refinement of tablets made with 4% pullulan solutions containing concentrations of potassium chloride (KCl) from 100 to 500 mM in deionized water. (C) Effect of pullulan on electrochemical sensing. Cyclic voltammetry peak currents of 10 mM ferri + 10mM ferrocyanide in 200 mM KCl were measured in solution, in solution with 4% pullulan, after tablet formation and re-dissolving in deionized water ($n = 6$ for each condition).

(D) Tablet-to-tablet reproducibility using 10 mM ferri/ferrocyanide in 200 mM KCl with 4% pullulan tablets dissolved deionized water, showing a relative standard deviation in peak currents (RSD) of 4.5% (n = 11).

The electrochemical response was characterized to assess the effect of pullulan incorporation and tablet fabrication on SPE performance. Cyclic voltammetry (CV) using 10 mM ferri/ferrocyanide in 200 mM KCl was conducted under three conditions: (i) solution only, (ii) solution containing 4 % pullulan, and (iii) the tablet format dissolved in water (Figure 14C). All experiments were performed using a single commercial screen-printed electrode, which was rinsed and reused between runs to maintain consistent conditions across all tests. The corresponding reduction peak currents were $200 \pm 2 \mu\text{A}$, $194 \pm 3 \mu\text{A}$, and $202 \pm 13 \mu\text{A}$, respectively, while oxidation peaks were $207 \pm 3 \mu\text{A}$, $197 \pm 3 \mu\text{A}$, and $203 \pm 10 \mu\text{A}$. Pairwise *p*-tests showed that the solution produced only slightly statistically different currents compared to the solution + pullulan group ($p = 0.0085$), whereas responses from the tablet were statistically comparable to both solution-based systems ($p = 0.76$ and 0.31 , respectively), indicating that pullulan incorporation and tablet fabrication did not affect the electrochemical behavior of the system. Tablet-to-tablet reproducibility was evaluated using eleven independently fabricated ferri/ferro tablets (Figure 14D). The relative standard deviation (RSD) was 4.45% for the reduction peak and 5.15% for the oxidation peak, both well below the 10% threshold for the commercialization of POC devices.¹⁶³

To confirm that variability was not introduced by the commercial electrodes themselves, five different screen-printed electrodes (SPEs) were tested independently using the same ferri/ferrocyanide solution ($n = 6$ per electrode). The oxidation peak currents ranged from 212 to 228 μA , with standard deviations of 3-10 μA , yielding RSD of 2.99 (batch-to-batch) (Figure SI1 A). In addition, the repeatability of CV measurements on the same SPE was evaluated under identical conditions, resulting in an RSD of 3.78 % (Figure SI1 B). This confirms that the eTab method has the potential to reduce overall cost to the user and reduce waste by enabling reusability of electrodes.

Overall, we found that a 4% (w/v) pullulan concentration yielded uniform and mechanically robust tablets. The incorporation of pullulan and the tablet fabrication process did not alter the electrochemical response of the system, and tablet-to-tablet measurements demonstrated

acceptable reproducibility (RSD < 10%). In the next sections, the functionality of the optimized tablet system is demonstrated for glucose and lactate detection.

3.4.2 Proof-of-Concept Demonstration Using Glucose Detection

To demonstrate the functionality of the eTab platform, we conducted a proof-of-concept assay using glucose as a representative analyte. Glucose detection was achieved via a bienzymatic reaction involving GOx and HRP, with ferrocyanide serving as the mediator¹⁵⁸. In this system, GOx catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide (H₂O₂). The H₂O₂ then oxidizes ferrocyanide to ferricyanide in the presence of HRP, and at the electrode surface ferricyanide is reduced back to ferrocyanide, generating a measurable current as shown in Figure 15A. Chronoamperometry (CA) was performed at −0.3 V, a potential confirmed by preliminary CVs showing no response in blanks and a clear reduction peak in the presence of glucose (Figure SI2 A). Current measurements were based on the current recorded at 45 s following a 2 min incubation.

Enzyme loading. Since this work represents the first demonstration of electrochemical tablet-based assays, enzyme loading was investigated to determine its effect on reaction efficiency, signal strength, and overall tablet cost. Tablets containing 4% pullulan (w/v), 100 mM ferrocyanide, 200 mM KCl, and 0.1 M phosphate buffer (pH 7.4) were prepared with varying enzyme concentrations. For HRP optimization, tablets containing 5–40 U per tablet were evaluated at 5 mM glucose with fixed amount of 10 U per tablet GOx. The current increased with HRP loading up to 20 U per tablet ($18 \pm 1 \mu\text{A}$). Although doubling the HRP content to 40 U per tablet resulted in only a ~4.9% increase in current, the corresponding rise in enzyme cost outweighed the minor gain in signal (Figure 15). Therefore, 20 U per tablet was selected as the HRP loading for subsequent tests. With HRP fixed at this value, GOx loading was varied, and the current increased up to 10 U per tablet ($18 \pm 1 \mu\text{A}$) before reaching a plateau, identifying 10 U per tablet as the optimal GOx level (Figure 15C). Higher enzyme loadings did not further enhance the signal and would unnecessarily increase cost.

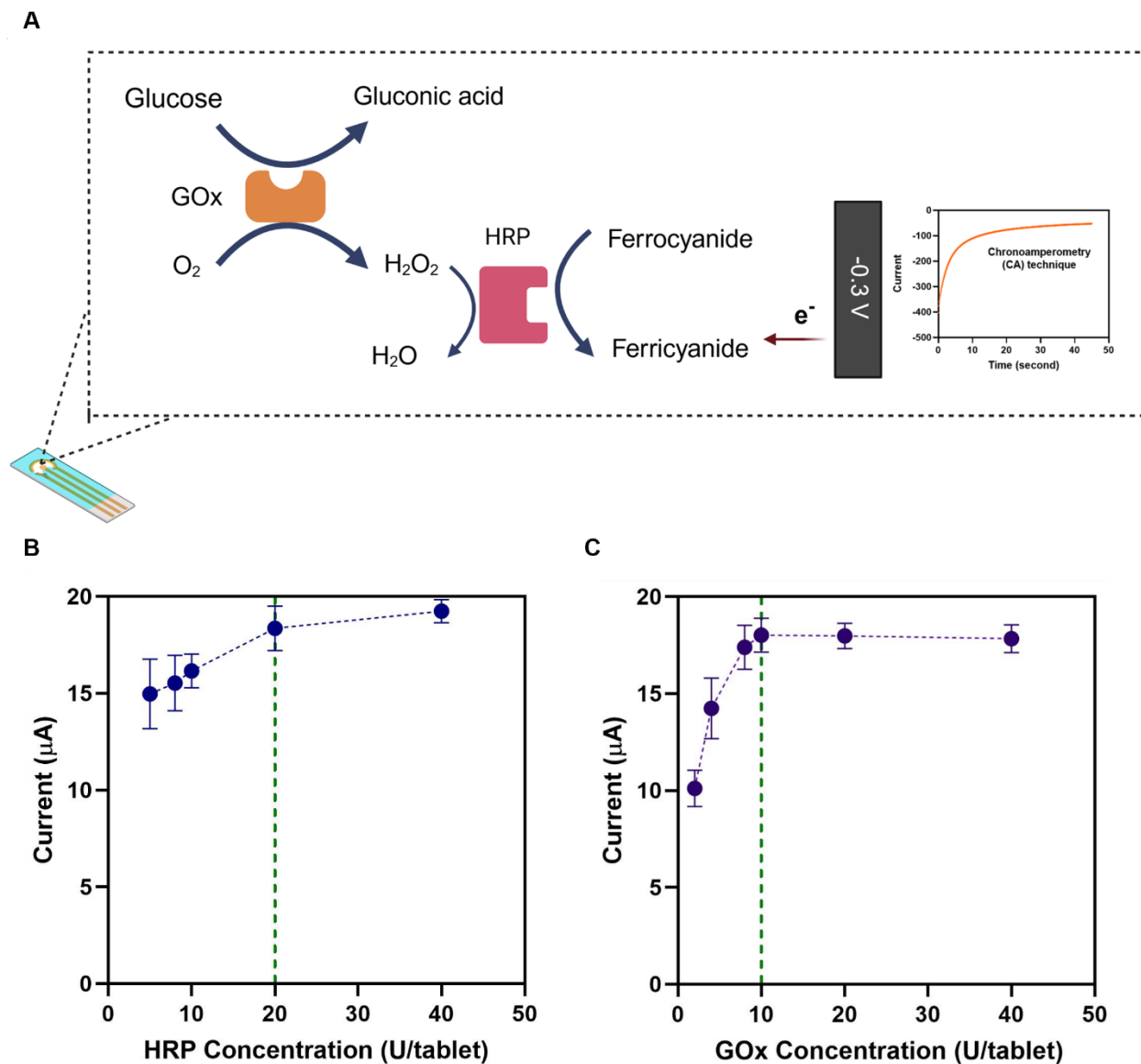


Figure 15. (A) Schematic illustration of the reaction mechanism. (figure created with BioRender.com). (B) Current response using different HRP concentrations (5, 8, 10, 20, and 40 U/tablet) with fixed 10 U/tablet GOx tested in 5 mM glucose solution ($n = 6$). (C) Current response using different GOx concentration (2, 4, 8, 10, 20, and 40 U/tablet) with fixed 20 U/tablet HRP tested in 5 mM glucose solution ($n = 6$). In both B & C graphs, the dashed green line indicates the concentration selected for subsequent analytical tests.

Analytical performance. Once the glucose eTab formulation was finalized (10 U/tablet GOx, 20 U/tablet HRP), the linear behaviour of the biosensor towards glucose was then evaluated using chronoamperometry (Figure 16A) and a calibration curve was generated with 0–15 mM glucose

(Figure 16B), with linearity decreasing above this concentration range (Figure SI3). The limit of detection (LOD) of the device was determined as the glucose concentration corresponding to the mean blank signal plus three times its standard deviation,^{164,165} yielding an LOD of 0.11 mM. The sensor exhibited a working range that fully covers the physiological glucose levels found in human blood plasma, encompassing <5.6 mM for healthy individuals and 7–11.1 mM for diabetic conditions.^{166,167} Interestingly, when the tablets were dissolved in the analyte solution, a yellow coloration proportional to analyte concentration appeared (Figure SI4), suggesting potential for a future dual electrochemical–colorimetric sensing mode.

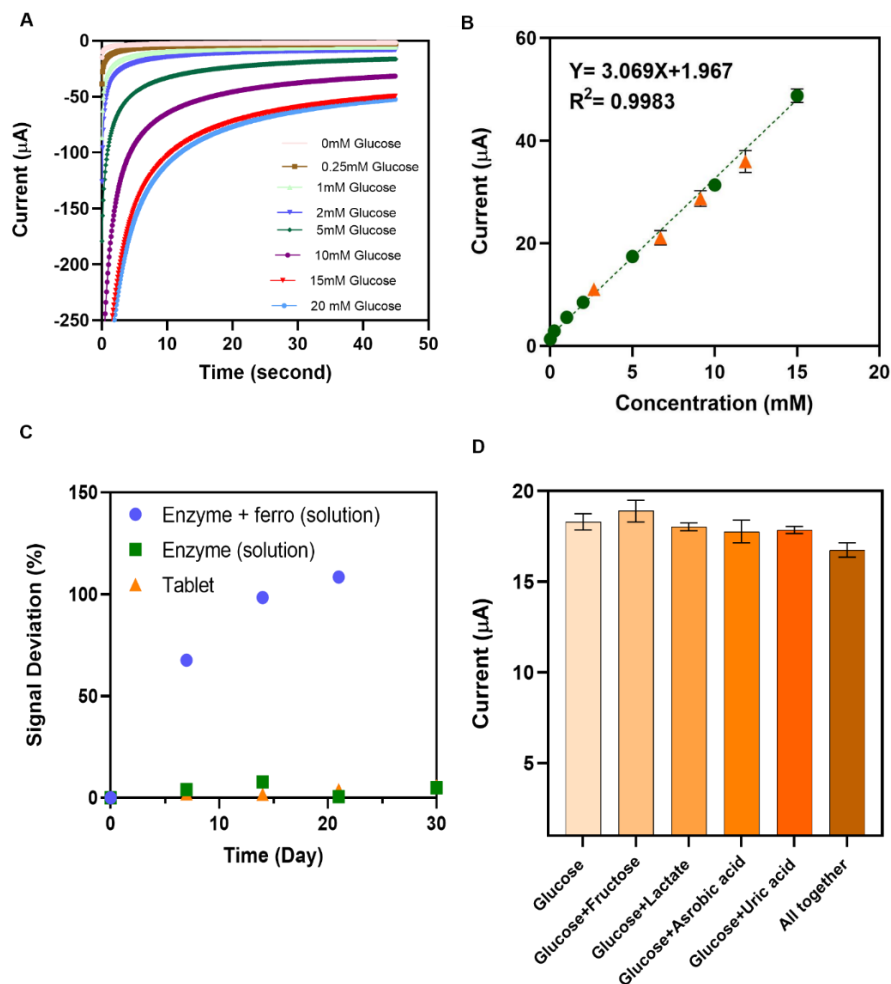


Figure 16. Performance of electrochemical tablets (eTabs) for glucose detection. (A) Chronoamperometry response for glucose detection using tablet-based sensor with commercial screen-printed electrode. (B) Calibration curve of glucose measured in DI water ($n=6$). A successfully detected glucose in real human blood plasma as shown by orange triangles (mean $\pm$ SD; $n = 5$). (C) Stability test of glucose assays stored at room temperature over 30 days, comparing enzyme solution with mediator, enzyme solution without mediator, and all-in-one tablet format ($n=3$). (D) Interference study: current response of 5 mM glucose alone, 5 mM glucose with 10 mM fructose, 10 mM lactate, 1 mM uric acid, or 1 mM ascorbic acid, and a mixed solution containing 5 mM glucose with all interferents ($n=6$). No statistically significant differences were observed for the individual interferents (all $p > 0.05$ vs. glucose alone), whereas the mixture of all interferents produced a slightly lower signal.

As summarized in Table SI1, the glucose eTab demonstrated a comparable or superior analytical performance relative to several reported POC glucose sensors. For instance, a colorimetric tablet-based device reported a detection limit of 0.3 mM with a narrow linear range of 0–2.5 mM, while a paper-based electrochemical sensor utilizing an all-carbon SPE achieved an LOD of 0.35 mM across a 0–10 mM range.

Stability. Given that stability is a fundamental requirement for any reliable sensing system, we evaluated the long-term stability of the glucose eTab sensors against their equivalent solution-based formulations over 30 days at room temperature (22 °C). Both the tablet and the enzyme-only solution exhibited minimal drift, with deviations of approximately 5 % from their initial responses after one month (Figure 16C). In contrast, the solution containing all reagents required for electrochemical sensing—enzymes together with ferrocyanide, displayed a marked instability, with current deviations exceeding 100 %. Further investigation revealed that ferrocyanide gradually underwent auto-oxidation to ferricyanide in pH 7.4 buffer, as confirmed by cyclic voltammetry and by the progressive development of a yellow color over time (Figure SI5). The tablet format effectively prevents this reaction, maintaining both electrochemical performance and reagent stability throughout the storage period, while providing a portable and easy-to-use device with pre-measured quantities for POC applications.

Interference Study. To ensure the applicability of the biosensor to real samples, potentially interfering substances commonly present in blood plasma were evaluated, namely ascorbic acid, uric acid, lactate, and fructose. Amperometric measurements were performed in water containing both glucose and an excess amount of each interferent, and the responses were compared with those obtained for 5 mM glucose under identical conditions. Concentrations of 1 mM were selected for ascorbic acid and uric acid to represent their high-end physiological levels, which are typically below 0.1 mM in blood.^{168,169} In contrast, 10 mM lactate and fructose were chosen to be approximately twice the typical glucose level, which is normally below 5.6 mM in healthy individuals.¹⁷⁰ This approach allowed assessment of whether elevated concentrations of other sugars or electroactive molecules could interfere with glucose detection under extreme conditions. As shown in Figure 16D, under the optimized experimental conditions, negligible changes in current were observed compared with glucose alone ($18.3 \pm 0.4 \mu\text{A}$). The recorded responses were fructose ($19 \pm 0.6 \mu\text{A}$), lactate ($18.0 \pm 0.2 \mu\text{A}$), ascorbic acid ($17.8 \pm 0.6 \mu\text{A}$), uric acid (17.9 ± 0.2

μA), and the combined mixture ($16.8 \pm 0.4 \mu\text{A}$). All p-values except the combined mixture are below the significance threshold ($p > 0.05$), confirming no meaningful interference. The slight difference in the mixture is small in magnitude and not practically significant, supporting the high selectivity of your glucose eTab biosensor.

Real-Sample Analysis with Human Blood Plasma. To evaluate the applicability of the biosensor in real-world analysis, the glucose eTabs were tested using human blood plasma samples obtained in collaboration with Biobanque IRCM (Montreal, Canada). Samples from both healthy and diabetic individuals with known glucose concentrations of 2.65, 6.70, 9.13, and 11.85 mM were analyzed using the glucose eTab system and lie along the calibration curve seen in Figure 4A. As shown in Table 2 and described in detail, the validation of the eTab sensor against standard clinical glucose measurements in human blood plasma demonstrated recoveries ranging from 93% to 117%, indicating high accuracy and reliable quantitative performance in complex biological samples. Altogether, glucose was successfully detected using the eTab system with high precision and accuracy in real blood plasma samples, without interference from common electroactive species. These findings demonstrate that the eTab platform is a reliable and promising tool for POC glucose sensing applications.

Table 2. Validation of eTab sensor measurements against standard clinical glucose value in human blood plasma samples from healthy and diabetic individuals.

Standard clinical measurement (mM)	eTab sensor measurements (mM) $\pm$ SD	Recovery (%) $\pm$ SD
2.65	3.1 $\pm$ 0.3	117 $\pm$ 11
6.70	6.2 $\pm$ 0.3	93 $\pm$ 5
9.13	8.7 $\pm$ 0.4	96 $\pm$ 4
11.85	11.3 $\pm$ 0.4	96 $\pm$ 4

3.4.3 Proof-of-Concept Demonstration Using Lactate Detection

To further demonstrate the versatility of the eTab platform, we performed a proof-of-concept assay using lactate as an additional representative analyte.

Lactate detection was conducted using a bienzymatic system composed of LOx and HRP, with ferrocyanide serving as the electron mediator.¹⁶⁰ (Figure 16A). In this reaction cascade, LOx catalyzes the oxidation of lactate to pyruvate, generating hydrogen peroxide, which is subsequently utilized by HRP to oxidize ferrocyanide to ferricyanide. The ferricyanide produced is then electrochemically reduced back to ferrocyanide at the electrode surface, producing a quantifiable current (Figure 17A). Chronoamperometric measurements were performed at -0.3 V, with signals recorded at 45 s following a 2 min incubation. Preliminary cyclic voltammetry verified that -0.3 V is an optimal potential for detection, exhibiting no background signal in blanks and a pronounced reduction response in the presence of lactate. (Figure SI2 B)

Enzyme Loading. Similarly to the procedure described in section 3.2, the enzyme loading and performance trade-off was investigated using HRP fixed at 20 U/tablet and LOx concentrations varying from 0.5 to 1.5 U/tablet for a concentration of 2 mM lactate in deionized water. Currents increased from 2.7 ± 0.6 μ A (0.5 U/tablet) to 10.4 ± 1 μ A (1.5 U/tablet), with diminishing returns beyond 0.75 U/tablet (8.8 ± 0.9 μ A). Based on current output, 0.75 U/tablet LOx was selected as the optimal loading (Figure 17B).

Analytical Performance. Once the ideal enzymatic loading was determined (0.75 U/tablet LOx, 20 U/tablet HRP), the linearity of the biosensor towards L-lactate was established and calibration curves were collected from 0–6 mM lactate (Figure SI6 A). Linear responses were observed from 0–4 mM (Figure 17C), with deviation at 6 mM. (Figure SI6 B) The limit of detection (LOD) was calculated as the concentration corresponding to the mean blank signal plus three times its standard deviation, the corresponding LOD concentration was determined to be 0.14 mM. The sensor exhibited a linear working range of 0–4 mM, fully covering the physiological and pathological lactate concentrations found in human blood plasma, which range from 0.5–1 mM under normal conditions and 2–4 mM in cases of hyperlactatemia.¹⁷¹

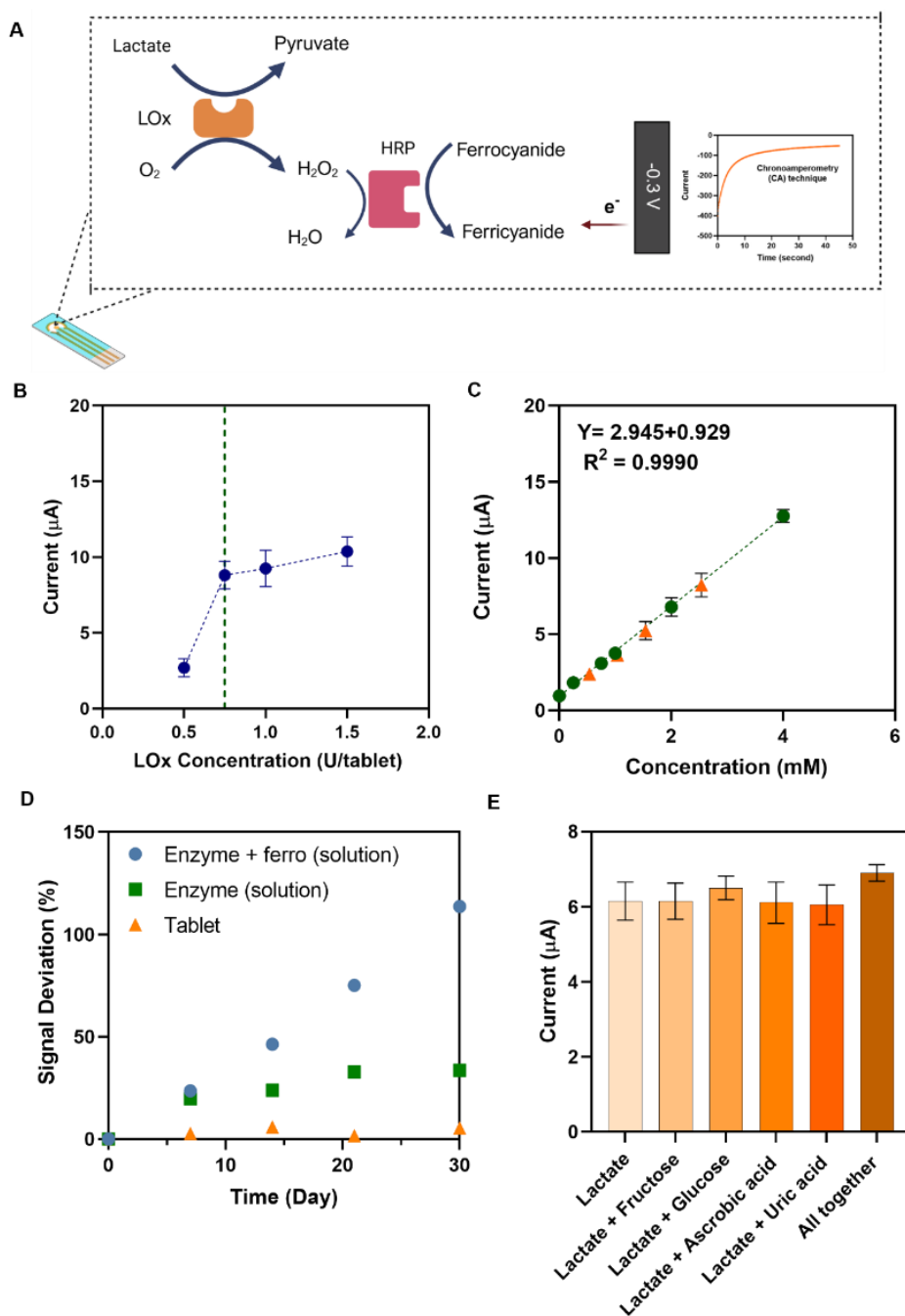


Figure 17. Performance of electrochemical tablets (eTab) for lactate detection. (A) Schematic illustration of the reaction mechanism. (B) LOx concentration (0.5, 0.75, 1, and 2.5 U/tablet) with fixed 20 U/tablet HRP tested in 2 mM lactate solution ($n = 6$). (C) Calibration curve of lactate measured in DI water ($n=6$). and successfully detected lactate in spiked real human blood plasma as shown by orange triangles (mean $\pm$ SD; $n = 5$). (D) Stability test of lactate assays stored at room temperature over 30 days, comparing enzyme solution with mediator, enzyme

solution without mediator, and tablet assay (n=3). (E) Interference study: current response of 2 mM lactate alone, 2 mM glucose with 10 mM fructose, 10 mM Glucose, 1 mM uric acid, or 1 mM ascorbic acid, and a mixed solution containing 2 mM lactate with all interferents (n=6). The individual interferents produced current responses that were statistically indistinguishable from lactate alone (all $p > 0.05$), whereas the mixture of all interferents produced a slightly higher signal.

Similar to the glucose detection tablets, dissolution of the lactate detection tablets in the analyte solution produced a yellow coloration proportional to analyte concentration (Figure SI7), indicating the potential for future development of a dual electrochemical–colorimetric sensing mode. As shown in Table SI1, the lactate eTab achieved analytical performance on par with or superior to several reported POC lactate sensors.

Stability. The stability of the lactate eTab was assessed in comparison with its equivalent solution-based formulations over 30 days of storage at room temperature (22 °C). The all-in-one tablet format exhibited excellent stability, showing only ~5% deviation from its initial current response after one month (Figure 17D). In contrast, the enzyme-only solution showed moderate degradation with ~35% signal loss, while the complete solution containing both enzymes and ferrocyanide displayed severe instability, with current drift exceeding 100%. As discussed in Section 3.2, ferrocyanide undergoes gradual auto-oxidation to ferricyanide in pH 7.0 buffer (Figure SI5), and lactate oxidase also loses activity over time in solution. The tablet configuration effectively mitigates these issues, preserving both electrochemical performance and reagent stability during storage.

As shown, lactate oxidase rapidly loses activity when stored in solution at room temperature, whereas the tablet-based format effectively preserved enzyme functionality over the same period. This enhanced stability highlights a major advantage of the eTab approach over previously reported POC platforms that rely on various enzyme immobilization techniques but exhibit limited long-term stability. For instance, sensors employing cellulose acetate (CA) membranes with Meldola’s Blue and NADP on carbon screen-printed electrodes retained activity for only 3 days¹⁷², while sol–gel films derived from MTEOS/Os-polymer on glassy carbon electrodes maintained stability for 7 days¹⁷³. Similarly, lactate sensors fabricated on PANI films over ITO-coated glass plates demonstrated stability for 21 days,¹²⁴ and Laponite–chitosan hydrogel-based systems on

GCE for 10 days.⁵¹ In contrast, the eTab system maintained electrochemical and enzymatic stability for at least 30 days at room temperature without the need for complex immobilization matrices or controlled storage conditions.

Interference Study. Similar to the procedure described in Section 3.2 for glucose eTabs, the selectivity of the lactate biosensor was evaluated to ensure its applicability to real samples. Potentially interfering substances commonly present in blood plasma were tested, namely ascorbic acid, uric acid, glucose, and fructose. Amperometric measurements were performed in water containing both lactate and an excess amount of each interferent, and the responses were compared with those obtained for 2 mM lactate under identical conditions. Concentrations of 1 mM were selected for ascorbic acid and uric acid to represent high-end physiological levels,^{168,169} while 10 mM glucose and fructose were chosen to be approximately five times higher than typical resting lactate levels (0.5–2 mM).¹¹² This approach allowed assessment of whether elevated concentrations of other sugars or electroactive species could interfere with lactate detection under extreme conditions. As shown in Figure 17E, current responses in the presence of interferents—fructose ($6.2 \pm 0.5 \mu\text{A}$), glucose ($6.5 \pm 0.3 \mu\text{A}$), uric acid ($6.1 \pm 0.5 \mu\text{A}$), and ascorbic acid ($6.1 \pm 0.5 \mu\text{A}$) produced current responses that were statistically indistinguishable from lactate alone ($6.2 \pm 0.5 \mu\text{A}$), with all comparisons yielding $p > 0.05$. In contrast, the mixed interferent solution ($7.1 \pm 0.2 \mu\text{A}$) showed a higher signal relative to lactate ($p = 0.0016$). Overall, these results confirm that individual common interferents do not affect the lactate signal, although their combined presence produces a small increase in current.

Real-Sample Analysis with Human Blood Plasma. Finally, to evaluate the applicability of the biosensor in real-world analysis, the lactate eTabs were tested using human blood plasma samples obtained in collaboration with Biobanque IRCM (Montreal, Canada). Since no prior information was available regarding the initial lactate concentration in the plasma, one sample was randomly selected, spiked with different concentrations, and analyzed using the calibration curve. As shown in Table 3, the baseline lactate concentration was determined to be 0.5 mM. The same sample was then spiked to reach final concentrations of 1.0, 1.5, and 2.5 mM and analyzed using the lactate eTab system. Table 3 summarizes the results obtained from human plasma samples fortified with known amounts of lactate. The eTab sensor achieved recoveries of 86–99%, demonstrating its strong accuracy and dependable quantification in complex biological matrices. Altogether, lactate

was successfully detected using the eTab system with high precision and accuracy in real blood samples, showing no interference from common electroactive species and excellent preservation of enzyme stability. These results demonstrate that the eTab platform is a reliable and promising tool for POC lactate sensing applications.

Table 3. Validation of eTab sensor measurements in human blood plasma spiked with known lactate concentrations

Spiked concentration (mM)	eTab sensor measurements (mM)± SD	Measured increase (mM)	Recovery (%) ± SD
Blank	0.5 ± 0.2	—	—
0.5	0.9 ± 0.3	0.5	86±5
1.0	1.5 ± 0.6	1	97±10
2.0	2.5 ± 0.8	2	99±7

Chapter 4: Conclusion and Future Work

This work introduces an electrochemical tablet-based biosensing platform that extends the capabilities of tablet assays from simple colorimetric reactions to reliable quantitative electrochemical detection. The approach addresses a major limitation in conventional electrochemical biosensors: the immobilization of bioreagents onto electrode surfaces. Immobilization often leads to reduced enzyme activity, compromised sensitivity, and batch-to-batch variability due to multistep fabrication. In contrast, the eTab format encapsulates all essential reagents, including GOx or LOx, HRP, potassium ferrocyanide, and potassium chloride, within a 6% pullulan matrix to form stable, ready-to-use tablets. Once dissolved in the analyte, the tablet produces an immediate electrochemical sensing solution compatible with commercial screen-printed electrodes and portable potentiostats, eliminating the need for surface modification or liquid reagent handling.

Evaluation using the ferri/ferrocyanide redox system confirmed that the presence of pullulan does not disrupt electron-transfer behavior, and the tablets provided highly reproducible voltammetric responses ($\text{RSD} < 5\%$). As proof of concept, tablets were developed for glucose and lactate detection at an estimated fabrication cost of \$0.80 and \$2.00 per tablet, respectively. Both systems performed effectively in human plasma, yielding recoveries between 86% and 116%. The glucose eTab enabled quantitative detection across 0–15 mM with a LOD of 0.14 mM, while the lactate eTab covered 0–4 mM with an LOD of 0.11 mM, capturing clinically relevant concentrations. Interference studies further demonstrated strong selectivity, and stability assessments showed that both systems maintain their initial performance with less than 10% deviation after one month at room temperature, contrasting sharply with significant degradation observed in equivalent solution-based formulations.

While the eTab platform significantly simplifies biosensor preparation and operation, several practical limitations must be acknowledged for realistic deployment. First, the assay requires users to introduce a specific and consistent volume of analyte into a tube containing the tablet. Variations in sample volume will directly influence reagent concentration and therefore the electrochemical signal, making user guidance and standardization important for accurate measurements.

Second, uniform tablet dissolution and complete reagent mixing are essential for reproducible sensing. In this study, either brief vortexing or manual mixing for approximately 30 seconds was required. Without adequate mixing, spatial gradients in reagent concentration may occur, leading to signal variability.

Third, fabrication and performance are sensitive to ambient humidity. Pullulan's hygroscopic nature affects tablet hardness, compression characteristics, and dissolution rate. Maintaining controlled humidity during manufacturing and using moisture-protective packaging will be necessary for large-scale production and long-term storage.

Together, these considerations outline the technical and practical constraints that must be addressed to transition the eTab biosensing platform from laboratory development to dependable point-of-care use.

Future Directions

Building on the promising results demonstrated in this work, several avenues are proposed to further expand the utility and impact of the eTab platform:

1. Expanding reagent versatility: While enzymes served as the bioreagents in this study, the same concept can be extended to a wide range of reagents and biocatalysts for which immobilization is more challenging, including nanozymes, DNAzymes, and aptamers, thereby broadening the applicability of this platform across diverse electrochemical sensing strategies.
2. Integrating multimodal detection: Combining electrochemical and colorimetric readouts could enable a versatile “drop-and-detect” format, offering both quantitative and rapid visual analyses for field applications.
3. Simplifying device operation: Adapting the eTab platform into a dip-in configuration, where electrodes are directly immersed into the tablet-dissolved solution, could further enhance user accessibility and practicality.

Overall, this work establishes electrochemical tablet-based sensors (eTabs) as a simple, reproducible, and highly stable platform for point-of-care detection. The approach holds significant potential for broad adaptation across diverse analytical and diagnostic applications.

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Appendix: Supporting Information

Appendix contains supplementary material supporting the methodology described in Chapter 3.

SI.1 Characterization of Commercial Test Strip

To evaluate the baseline performance and reproducibility of the commercial screen-printed electrodes (SPEs) used throughout this work, we first characterized their response to a standard redox system (10 mM ferro/ferricyanide in 200 mM KCl). Cyclic voltammetry (CV, -1.0 to $+1.0$ V, 50 mV/s) was performed with five independent commercial SPE batches ($n = 6$ per batch) to compare average oxidation peak currents. The mean $\pm$ SD values were 226 ± 5 μ A (SPE 1), 228 ± 10 μ A (SPE 2), 218 ± 4 μ A (SPE 3), 212 ± 4 μ A (SPE 4), and 217 ± 3 μ A (SPE 5). The overall batch-to-batch variability, calculated as the RSD of the five mean values, was 3.0%, confirming consistent electrochemical responses across commercial sources. (Figure SI1 A)

Representative CVs from a single SPE recorded over ten consecutive runs in the same solution, where the electrode was rinsed between runs and reimmersed to mimic repeated use, are shown in Figure SI1 B. The repeated tests demonstrated an RSD of 3.78% for the oxidation peak current, highlighting the reusability of the SPE sensor. Together, these results confirm that electrode variability did not confound subsequent tablet-based measurements.

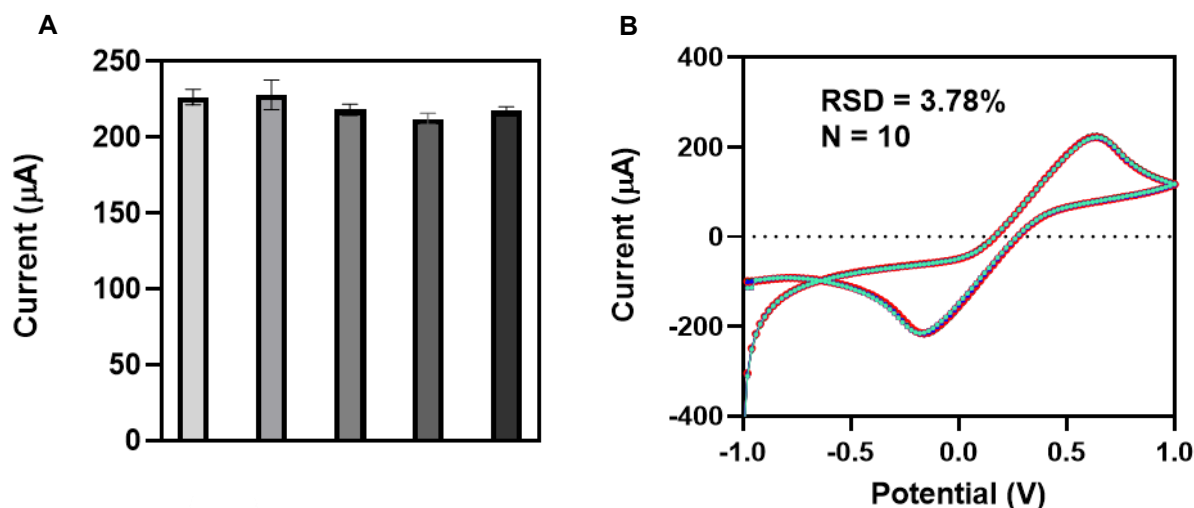


Figure SI1. (A) Oxidation peak currents of five commercial SPE batches in 10 mM ferri/ferricyanide with 200 mM KCl ($n = 6$). (B) Reproducibility of a single SPE over ten consecutive runs ($n = 10$).

SI.2 Analytical Performance

To evaluate potential interference at -0.3 V during chronoamperometry (CA), we first performed cyclic voltammetry (CV) on commercial test strip by dissolving our glucose tablet into DI water using a blank sample (Milli-Q water) and a 5 mM glucose solution in water. Scans were performed from -1 to 1 V (starting from the open-circuit potential in the backward direction) at a scan rate of 50 mV/s. cases. The results show no reduction peak up to -0.3 V in the absence of glucose (Figure SI2 A). However, in the presence of glucose, ferrocyanide was oxidized to ferricyanide, producing a clear reduction peak, confirming that -0.3 V is a suitable detection potential for CA analysis. The same test was performed for lactate activity which showed no reduction peak up to -0.3 V in the absence of lactate (Figure SI2 B). However, in the presence of lactate, ferrocyanide was oxidized to ferricyanide, producing a clear reduction peak, confirming that -0.3 V is a suitable detection potential for CA analysis.

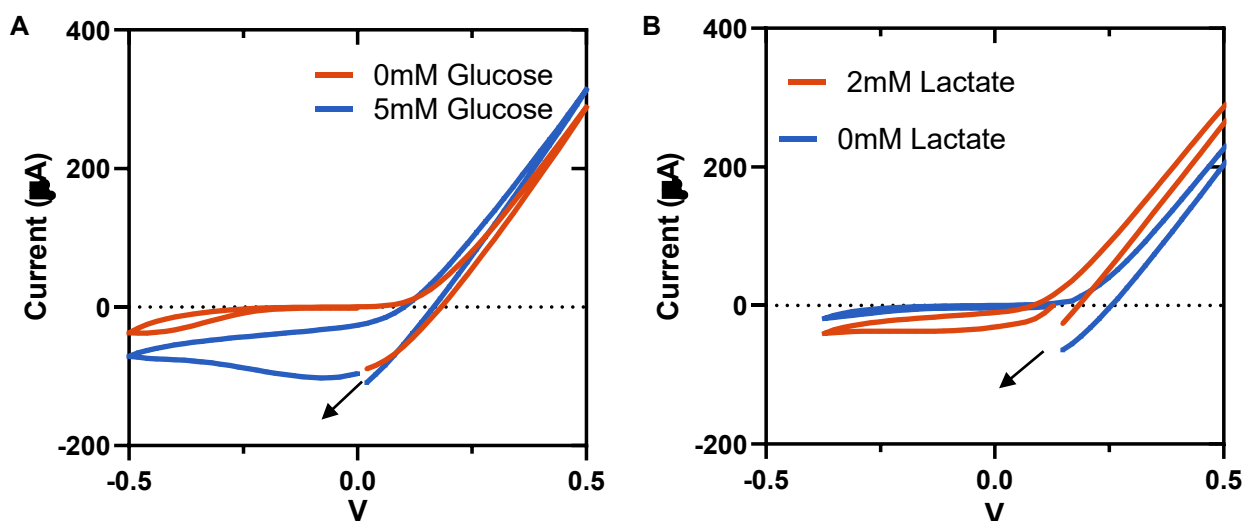


Figure SI2. (A) Comparison of CV responses of blank (Milli-Q water) and glucose (5 mM) samples with glucose e-tab. (B) Comparison of CV responses of blank (Milli-Q water) and Lactate (2 mM) samples with lactate eTab

SI.3 Glucose Assay

To generate calibration curves, glucose tests were performed using eTab with commercial test strip, at glucose concentrations of 0, 0.25, 0.75, 1, 2.5, 5, 10, 15 and 20 mM in water. The current measured at 45 seconds was used to construct the calibration curves (Figure 16B). eTab exhibited linear responses up to 15 mM, while deviations from linearity and increased variability were observed at 20 mM (Figure SI3).

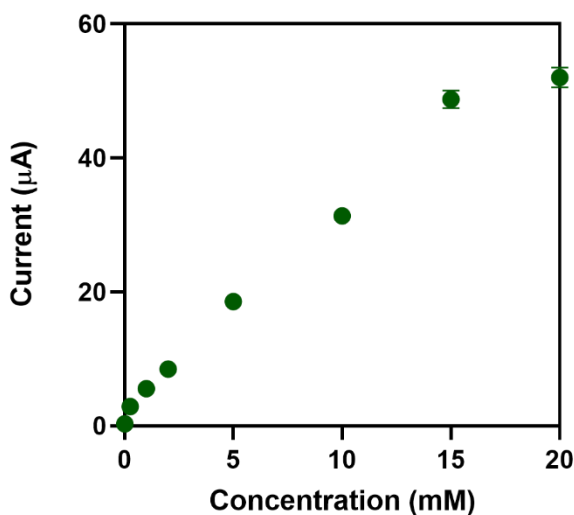


Figure SI3. Calibration curves for glucose detection using tablet-based sensor with commercial screen-printed electrode. Calibration curves based on current measured at 45 second versus glucose concentration (mean $\pm$ SD; $n = 5$)

SI.4 Dual Detection - Glucose Assay

When an eTab containing GOx/HRP/ferricyanide was dissolved in glucose solutions, a yellow color developed due to redox cycling: GOx oxidizes glucose to gluconolactone, generating H_2O_2 ; HRP then uses H_2O_2 to oxidize ferrocyanide ($\text{Fe}(\text{CN})_6^{4-}$) back to ferricyanide ($\text{Fe}(\text{CN})_6^{3-}$), deepening the yellow as glucose increases (Figure SI4 A). The colorimetric response was confirmed by UV–Vis, showing a concentration-dependent rise in absorbance at 420 nm (Figure SI4 B), and by ImageJ analysis (inverted-image intensity), which increased monotonically with glucose concentration (Figure SI4 C). However, the color signal began to saturate above ~ 10 mM glucose, and the overall sensitivity of this optical readout was relatively low. This modality was therefore not pursued further here, but it suggests a potential future direction in which eTabs could be engineered to provide complementary electrochemical and optical responses.

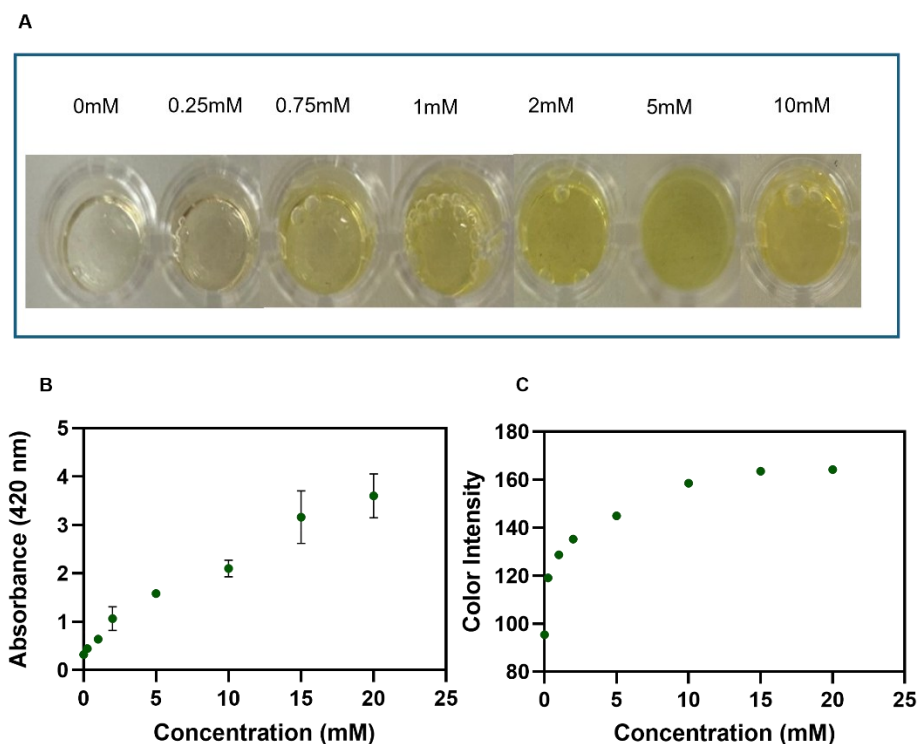


Figure SI4. (A) Representative images showing progressive color change with increasing glucose concentration, resulting from enzymatic conversion of ferricyanide to ferrocyanide. (B) UV–Vis spectra recorded at 420 nm confirmed a concentration-dependent increase in absorbance with higher glucose levels. (C) Color intensity analysis of the same solutions performed using ImageJ.

SI.5 Ferrocyanide Stability

While evaluating solution-based and tablet stability (Figures 16B and 17D), we observed that ferrocyanide-containing solutions showed increasing current responses over time and became visibly more yellow. We hypothesized that ferrocyanide ($\text{Fe}(\text{CN})_6^{4-}$) undergoes slow oxidation to ferricyanide ($\text{Fe}(\text{CN})_6^{3-}$) under neutral pH and room-temperature conditions, thereby increasing the concentration of reducible Fe^{3+} species available during measurements. To test this, we prepared 100 mM ferrocyanide + 200 mM KCl in three formats: (i) solution (buffer), (ii) solution with 4% pullulan, and (iii) tablet form. Samples were stored at room temperature and analyzed on day 0, 7, 14, 21, and 30 ($n = 3$ per condition) using: (a) chronoamperometry (CA) at -0.3 V to monitor Fe^{3+} reduction, (b) cyclic voltammetry from OCV to -0.3 V to measure the reduction peak current of ferricyanide, and (c) UV–Vis absorbance at 420 nm to track the yellow coloration.

In aerated neutral buffer at room temperature, ferrocyanide gradually oxidizes to ferricyanide, explaining the rising currents and intensified yellow color in solution controls (Figure SI5 A–C). In contrast, the tablet matrix strongly suppressed this conversion, likely by limiting oxygen and water diffusion and creating a stabilizing microenvironment within the dried pullulan matrix, thereby preserving mediator speciation over time. Interestingly, the solution containing 4% pullulan oxidized even faster than the plain solution, suggesting that dissolved pullulan does not stabilize ferrocyanide but instead may accelerate conversion in aqueous phase.

These findings demonstrate that the tablet matrix not only provides a convenient reagent format but also acts as a stabilizing environment that preserves the mediator during extended storage. Thus, the tablet-based platform offers a dual benefit: enabling point-of-care reagent delivery and enhancing the long-term stability of otherwise labile ionic mediators.

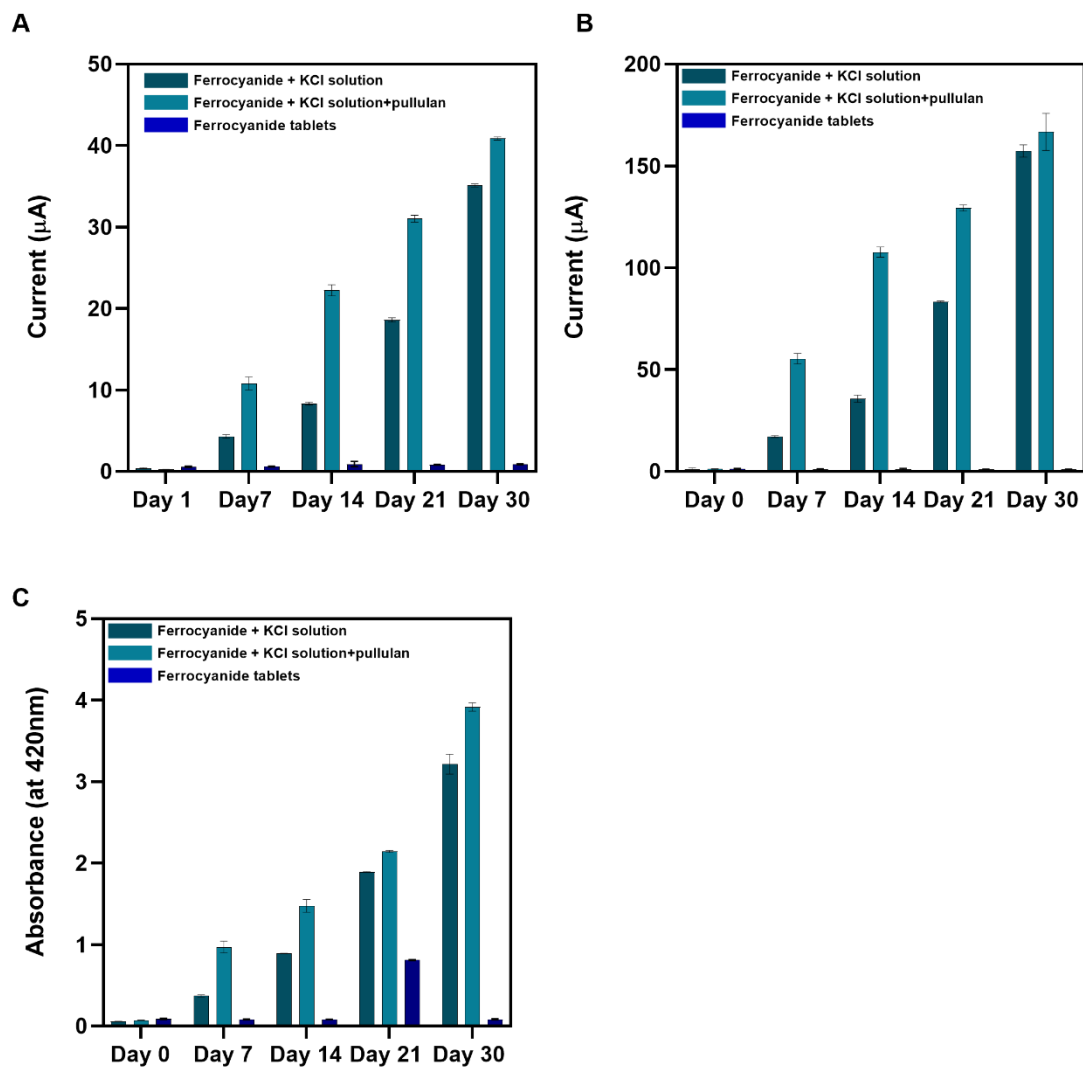


Figure SI5. Performance evaluation of ferrocyanide in solution and tablet formats. (A) Chronoamperometric current measured at -0.3 V after 45 s, comparing ferrocyanide + KCl solution, ferrocyanide + KCl with 4% pullulan solution and ferrocyanide tablets over 30 day at room temperature. (B) Reduction peak currents extracted from cyclic voltammetry (OCV to -0.3 V) for the same conditions. (C) UV-Vis absorbance at 420 nm showing the progressive yellowing of solutions compared with stable tablet formulations

SI.6 Lactate Assay

To generate calibration curves, Lactate tests were performed using e-tab with commercial test strip, at Lactate concentrations of 0, 0.25, 0.75, 1, 2, 4 and 6 mM in water. Figure SI6 A shows the chronoamperometric responses for each device. The current measured at 45 seconds was used to construct the calibration curves (Figure SI6 B). The eTab exhibited linear responses up to 4mM, while deviations from linearity and increased variability were observed at 6 mM.

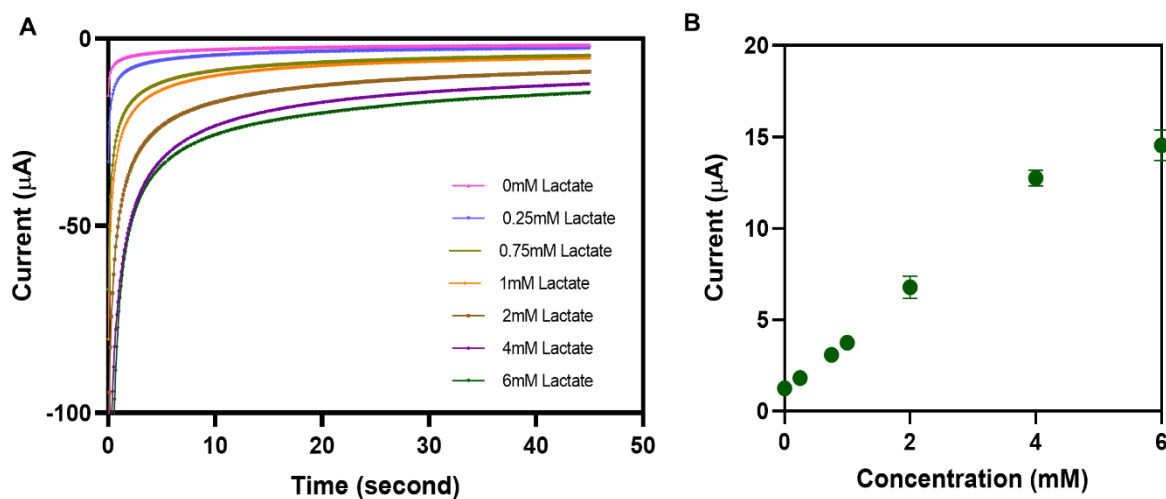


Figure SI6. Calibration curves for lactate detection using tablet-based sensor with commercial screen-printed electrode. (A) Chronoamperometric responses at varying lactate concentrations. (B) Calibration curves based on current measured at 45 seconds versus glucose concentration (mean $\pm$ SD; $n = 5$).

SI.7 Dual Detection - Lactate Assay

For lactate tablets, the same dual-detection principle described in Section SI.5 for glucose was observed. Upon dissolution in lactate solutions, the enzymatic cascade initiated by lactate oxidase (LOx) generated H_2O_2 , which was subsequently reduced by HRP, oxidizing ferrocyanide ($\text{Fe}(\text{CN})_6^{4-}$) back to ferricyanide ($\text{Fe}(\text{CN})_6^{3-}$). This redox cycling produced a yellow coloration that intensified with increasing lactate concentration (Figure SI6 A). The colorimetric response was further validated by UV–Vis spectroscopy, showing a concentration-dependent increase in absorbance at 420 nm (Figure SI6 B), and by ImageJ analysis of inverted-image intensity, which increased monotonically with lactate concentration (Figure SI6 C). As can be seen in the images, the overall sensitivity of the optical readout is low, and the differences between lactate concentrations are difficult to distinguish with the naked eye. Nonetheless, we report this observation as part of the tablet’s characterization, and it highlights a potential future direction to expanding the eTabs versatility.

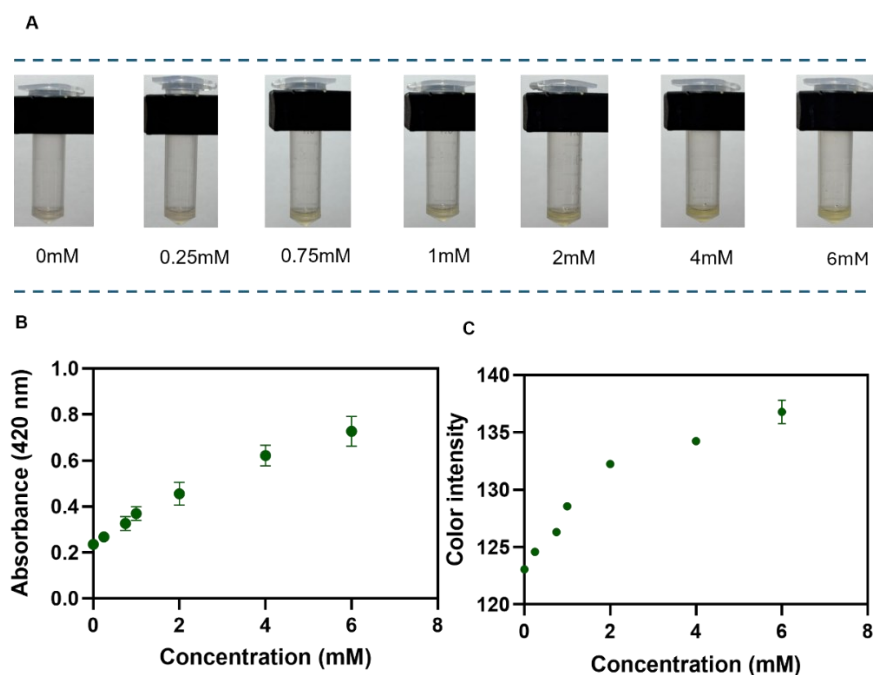


Figure SI7. (A) Representative images showing progressive color change with increasing lactate concentration, resulting from enzymatic conversion of ferricyanide to ferrocyanide. (B) UV–Vis spectra recorded at 420 nm confirmed a concentration-dependent increase in absorbance with higher lactate levels. (C) Color intensity analysis of the same solutions performed using ImageJ.

An overview of previously reported POC devices for glucose and lactate detection is provided in Table SI1, which enables a direct comparison with the analytical performance of our platform

Table SI1. Comparison of glucose and lactate detection performance with reported POC devices.

Platform	Analyte	Detection Method	LOD (mM)	Linear Range (mM)
Tablet-based ¹	Glucose	Colorimetric	0.30	0-0.25
Paper-based (All-carbon SPCE) ²	Glucose	Electrochemical	0.01	0.25-2
Tablet-based ³	Glucose	Colorimetric	0.013	0-6
Gold-Nanoelectrodes ⁴	Glucose	Electrochemical	0.3	0-20
Electrochemical Tablet-based (This Work)	Glucose	Electrochemical	0.11	0-15
Paper-based device ⁵	Lactate	Electrochemical	0.36	0-50
Paper-based device ⁶	Lactate	Colorimetric	0.26	0.3-5
Screen-printed electrode ⁷	Lactate	Electrochemical	0.01 mM	0.025–0.25
Electrochemical Tablet-based (This Work)	Lactate	Electrochemical	0.14	0-4

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