Research Article

Journal of Environmental Nanotechnology

Emerging Diagnostic Methods Using Paper-based Electrochemical Biosensors

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Received: 30.06.2024 Accepted: 30.08.2024 Published: 30.09.2024
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ABSTRACT

The development of paper-based electrochemical biosensors has brought about a paradigm shift in the diagnostics industry. These tools provide a portable, affordable, and easy-to-use platform for point-of-care (POC) testing. This has been transformed by the use of electrochemical detection techniques in paper-based analytical devices, which allow for the accurate and insightful assessment of a wide range of chemical analytes. These modified paper-based analytical gadgets have received significant recognition for their inherent benefits in the field of POC. Consequently, the sensitivity and practicality of electrochemical biosensors made of paper-based materials show great promise. They also have several other beneficial features, such as the ability to transport liquids independently, decreased resistance, low manufacturing costs, and environmental friendliness. This study examines the latest developments in paper-based electrochemical biosensing technologies and considers how they might be used in food safety, environmental monitoring, and in developing diagnostic techniques.

Keywords: Paper-based analytical devices; Electrochemical biosensors; Environmental analysis; Carcinogenic biomarkers.

1. INTRODUCTION

The need for speedy, accurate, and reasonably priced diagnostic tools has never been much significant, especially in settings with constrained resources. Traditional diagnostic methods, while reliable, often require specialized equipment and trained personnel. Paper-based electrochemical biosensors (PEBs) have become a competitive alternative because they are cost-effective and convenient with high sensitivity and specificity. Paper-based electrochemical biosensors can improve the accuracy and efficiency of disease diagnosis in poor countries. In the last two decades, paper-based analytical devices (PADs) have become a promising new way to execute POC applications. They have attracted a lot of interest as prospective low-cost disposable sensors because of their great surface-to-volume ratios, portability, affordability, and user-friendly design. Furthermore, it is important to highlight that PADs have an exceptionally high degree of sensitivity and selectivity towards a variety of analytes, making it possible to detect trace amounts of molecules (Noah *et al.* 2019). Thus, they are very suitable for a wide range of applications. Colorimetric and electrochemical methods widely employ PADs. Blood sample processing techniques have been developed in response to the fact that raw blood obscures a colorimetric response in colorimetric paper-based diagnostics, which have an equipment-free read-out. They are not appropriate for continuous monitoring and are not as sensitive as electrochemical techniques. Electrochemical techniques are expensive and require more sophisticated equipment, but they provide higher sensitivity and continuous analyte monitoring. Although colorimetric paper-based instruments are simple in design to measure and interpret, their sensitivity to interference in coloured matrices (like blood) limits their applicability (Noviana *et al.* 2021). Nevertheless, electronic PADs (ePADs) demonstrated excellent sensitivity and made it possible to identify analytes in both colourful and turbid mixtures, hence broadening the range of possible applications for ePADs. This paper evaluates the possible applications of ePADs in multiple sectors by providing a thorough overview of their design and functionality (Fig. 1).

2. PAPER DESIGN AND PRODUCTION

Paper is an excellent biosensor material due to its hydrophilic properties, affordability, accessibility, and flexibility. Its surface is easily sculpted and reshaped, and it exhibits strong biomolecule and nanoparticle adsorptive properties. The most common biopolymer is cellulose, which serves as the main ingredient in most forms of paper. It is a linear chain made up of several glucose units. Biodegradable, non-absorbent, and water-insoluble cellulose is a robust fibre (Shu *et al.* 2022). Non-toxic qualities, ease of disposal, and biodegradability are some of the advantages of paper.

One of the main advantages of cellulose is its porosity, which makes the capillary transfer of liquids easier. Cellulose can potentially function as a microfluidic pump, operating independently without the need for additional pumps. The intrinsic properties of paper, such as its porosity, flatness, and capillary forces, allow for innovative advancements in a range of applications. Paper-based analytical equipment is widely used due to its affordability, ease of fabrication, operation, portability, and disposability. As evidenced by its use in single or complicated components, lateral and vertical flow devices, and its ease of integration with other flat materials like tape, the material's versatility makes it extremely ideal for analytical decentralization. This material can also be altered by pressing, folding, cutting, or printing (Fig. 2).

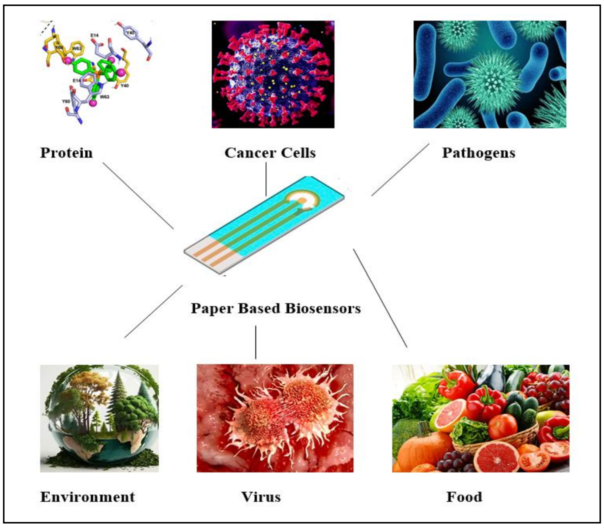

Fig. 1: Schematic illustration of applications of the paper-based biosensors

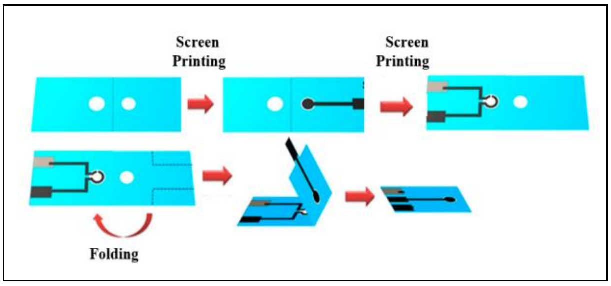

Fig. 2: Electrochemical paper-based biosensor

3. PAPER-BASED BIOSENSORS

Paper-based sensors are analytical devices that use paper as a foundation material in order to detect and quantify a variety of analytes. The sensors can be used in areas with limited resources, such as emerging markets or fieldwork due to their affordability, portability, and ease of use. By combining state-of-the-art technologies and sophisticated detection techniques, the effectiveness of PAD can be improved. Additionally, they offer benefits such as utilizing a trace amount of sample (a few μL) that can be evaluated and a quick sample analysis time. Therefore, these devices have the potential to serve as alternatives to the traditional POC devices that are now in use (Wang *et al.* 2021). Paper is utilized as the substrate material in electrophoretic ePADs, together with two or three electrodes. There are two main obstacles to the use of paper-based electrochemical sensors. Firstly, the substrate's characteristics, such as its porous structure, network, and surface can significantly affect the performance of these sensors. Furthermore, the intricate framework and particular laws governing small-scale production add to the uncertainty around accuracy and reproducibility. Secondly, the performance of paper-based sensors cannot be controlled on a large scale; instead, their surfaces must be developed and modified for selective detection. Different detection modes, including colorimetric, fluorescence, electrochemical, surface-enhanced Raman spectroscopy, and magnetic detection are compatible with the PADs. To modify the paper substrate, conductors such as gold nanoparticles or carbon nanotubes are added, which act as electrodes for the detection of analytes (Maduraiveeran and Jin, 2017). When compared to traditional electrochemical biosensors, the use of printed electrodes on PEBs offers several noteworthy advantages, including disposability, affordability, and usability. One of the most popular applications of PEBs in medical diagnostics is blood glucose detection. When everything is taken into account, PEBs appear to be a viable medium for creating portable, reasonably priced diagnostic instruments for the treatment of diabetes and other disorders needing regular blood biomarker monitoring (Ensafi, 2019).

4. ELECTROCHEMICAL BIOSENSORS

The most researched and utilized biosensors are electrochemical ones, whose principles of operation depend on the electrochemical characteristics of the transducer and analyte. High levels of sensitivity, selectivity, and detection capabilities are displayed by electrochemical biosensors (Benjamin and Júnior, 2022). This biosensor uses an electrochemical reaction between the analyte and bioreceptor on the transducer surface to provide observable electrochemical signals in terms of capacitance, voltage, current, and impedance. Based on the transduction principle, electrochemical biosensors are categorized as conductometric, voltammetric, impedimetric, potentiometric, and amperometric biosensors. Potentiometric biosensors measure the charge that builds up at the working electrode as a result of the analyte-bioreceptor interaction in relation to the reference electrode when there is no current. Ion-sensitive field-effect transistors and ion-selective electrodes are used to convert a biological reaction into a potential signal (Arduini *et al.* 2020). Two or three electrode combinations are used in amperometric biosensor operations. These sensors pick up the current produced by the electrochemical oxidation or reduction of electroactive species at the working electrode when a steady voltage is applied to it with respect to the reference electrode. The concentration of the analyte in the solution is directly correlated with the current generated on the working electrode's surface. This approach enables a sensitive, rapid, precise, and linear response, making it more suitable for mass production than potentiometric biosensors. Conductometric biosensors measure how an electrochemical response affects the conductance between two electrodes. Biosensors that measure conductance and impedance are typically employed to track metabolic activities in biological systems that are alive. When a small sinusoidal excitation pulse is provided, impedimetric biosensors evaluate the electrical impedance that results at the electrode/electrolyte contact. An impedance analyzer is used to measure the in or out-of-phase current response as a function of frequency when a low-amplitude AC voltage is applied to the sensor electrode (Surucu *et al.* 2017). Voltammetric biosensors measure the current during the regulated variation of the applied potential in order to identify the analyte. These sensors have the ability to detect numerous analytes simultaneously and offer very sensitive results. The PAD biosensors are often characterised by cyclic voltammetry (CV). The Randle-Sevcik equation-based cyclic voltammetry (CV) assessment of the sensing device is a well-validated technique to demonstrate the biosensor's repeatability and stability. PAD at CV (Fig. 3) scan speeds between 30 and 100 mV/s, the current peak outputs of a well-calibrated sensor under CV should normally show a linear relationship with the square root of scan rates when the temperature, diffusion constant, and redox event are all the same.

5. APPLICATIONS IN DIAGNOSTIC METHODS

5.1 Medical Diagnostics

Electrochemical biosensors based on paper have demonstrated significant potential for identifying biomarkers associated with illnesses like cancer, diabetes, and infectious diseases. As an illustration, consider glucose sensors for managing diabetes and HPV sensors for screening cervical cancer. With the aid of ePADs, the most pertinent biomolecules and biomarkers were identified. Since paper-based sensors are inexpensive, flexible, and have the potential to self-pump, they have emerged as a promising foundation for point-of-care testing and other rapid diagnostic procedures (Fu and Wang, 2018). An innovative electrochemical glucose sensor makes it simple and semi-quantitative to monitor urine glucose levels. The technology is made up of a paper-based, disposable sensing strip and a straightforward amplifier circuit with a visual display. On the paper strip, five electrodes that were activated by enzymes were employed. The electrodes were linked to an indicator system that, when glucose concentrations fell within a predetermined range, turned on a light-emitting diode. To detect L-cysteine, graphene and Ag-doped silica nanoporous SBA-16 electrodes have been used in ePADs. A commercial glucose meter with a linear range of 0.1 to 250 µM and a low detection limit of 0.02 µM was used for the detection. Paper-based systems have a wide range of applications, from straightforward screening to precise quantification (Badihi-Mossberg *et al.* 2007). These systems may utilize electrochemical biosensors, paper test strips, lateral flow systems, and computational techniques. In order to analyze the nasopharyngeal fluid samples, graphene/carbon electrodes were screen-printed on paper substrates and utilized as impedance sensors to detect coronavirus. Recent developments have highlighted potential uses of nanobiosensors for tracking SARS-CoV-2 in environmental settings.

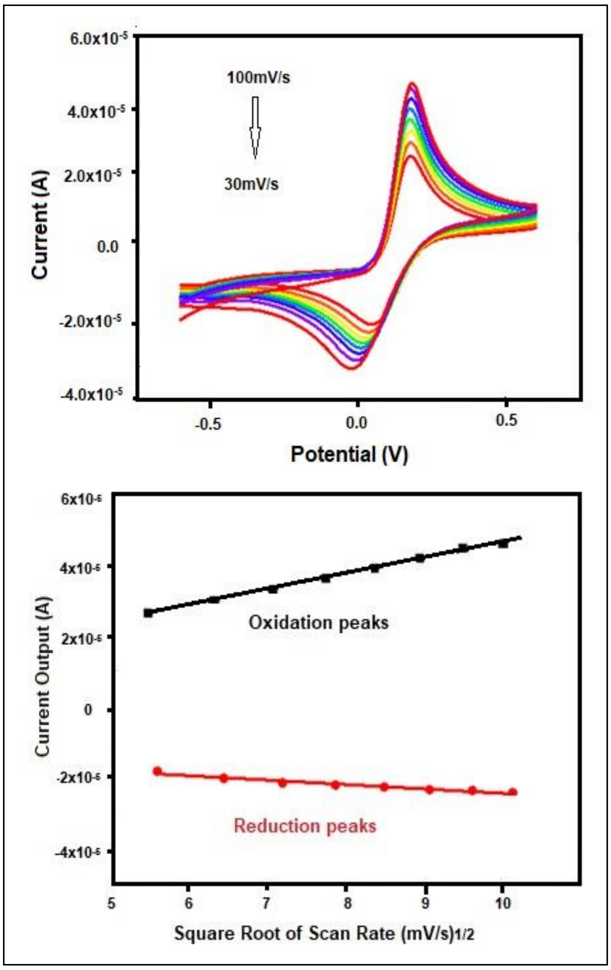

Fig. 3: CV for the electrochemical biosensor and linear calibration curve based on CV

5.2 Finding Carcinogenic Biomarkers

For colorimetric osteopontin measurement, Deroco *et al.* (2023) created a PAD modified with an aptamer (a cancer biomarker). The thiolated aptamer, a biological detection layer, was then appended to cellulose paper by chemically modifying it with (mercaptopropyl) methyl dimetoxisilane. The paper-based aptasensor's great sensitivity was demonstrated by its limit of detection (LOD), which was less than 5 ng/mL. An electrochemical aptasensor made of paper is intended to monitor neuron specific enolase (NSE) and carcinoembryonic antigen (CEA) in order to detect lung cancer early. Label-free electrochemical immunoassay technology offers the potential for more straight forward and targeted detection of certain cancer biomarkers. Microchannels and a three-electrode system were the end products of the screen and wax printing operations. The working electrodes of the aptasensor were changed, and their sensitivity was increased using nanocomposites of amino-functional graphene (NH_2-G)/thionin (THI)/gold nanoparticles (AuNPs) and Prussian blue/poly (3,4-ethylene dioxythiophene)/AuNPs. The aptasensor exhibits detection limits of 2 pg mL^{-1} and 10 pg mL^{-1}, respectively, and can identify CEA concentrations ranging from 0.01 to 500 ng mL^{-1}. Lung cancer is diagnosed in human serum at 5 and 15 ng mL^{-1}, respectively, using CEA and NSE levels (Wang *et al.* 2019).

5.3 Food Safety and the Environment

Many other types of environmental pollutants, such as heavy-metal anions, explosives, neurotoxins, pesticides, volatile organic compounds, and other small molecules, have been identified through the widespread use of ePADs. Pollutant detection has benefited from recent improvements in paper-based sensing systems. An optoelectronic nose can detect pesticide aerosols selectively based on an origami paper sensor.

5.3.1 Sensors of Humidity

When the temperature is suitable, the ideal range of humidity that produces a comfortable feeling in the human body is usually between 40 and 70%. Since exhaled breath is far more humid than swallowed air, a humidity sensor detects relative humidity (RH) of breath to track breathing patterns and offers health data in real time. Using inkjet printing, a unique printed graphene humidity and respiration sensor has been effectively created and verified on paper substrates (Justino *et al.* 2017). The sensors demonstrated excellent RH level detection performance between 10% and 70%, with a sensitivity value of 0.03 pF/RH%. This also shows the potential of built-in paper for human respiration rate monitoring, an application that could benefit tremendously from the fast reaction of paper sensors and recovery durations. The detection of aflatoxin B1 in samples of maize flour using a disposable immunological sensor based on chitosan and multi-walled carbon nanotubes film was investigated by (Boonkaew *et al.* 2021). An anti-aflatoxin B1 was used to modify the immunological sensor. The LOD of the impedimetric immunological sensor was 0.62 ng mL^{-1}, and its linear range was 1–30 ng mL^{-1}. This sensor showed remarkable sensitivity and the sensing platform demonstrated a high degree of selectivity when additional mycotoxins were present.

5.3.2 Food-borne Infections

The growing world population drives up demand for food production. As food production rises, so does the significance of food quality monitoring techniques in the fight against food-borne illnesses. There is an increasing need, especially in the food business, for inexpensive, highly responsive sensors that can identify harmful bacteria in food-borne applications. *Salmonella*, *Campylobacter*, *E. Coli* O157:H7, *S. aureus*, and *L. monocytogenes* are the most harmful foodborne bacteria. Colorimetric or fluorometric detection techniques, either qualitative or semi-quantitative, are used by the majority of PADs to identify infectious microorganisms. By applying conductive materials via printing techniques, it is possible to integrate electrochemical, electro chemiluminescent, and photoelectrochemical detection technologies into paper-based electrodes (Jiao *et al.* 2017).

5.3.3 Carbon Paper

Carbon materials are widely used in bio electrochemical applications; they include graphite, carbon paper, and carbon felt. Recently, carbon paper-based electrochemical biosensors have been developed for photosynthetic herbicide detection (Akhtar *et al.* 2020) based on thylakoid membranes. Weak non-covalent interactions (such as hydrogen bonds, stacking (π–π), and van der Waals) sustain the thylakoids, which in turn stabilize the interaction of thylakoids with carbon paper. Without redox mediators, the biosensor can produce photocurrent levels about 14 $\mu A/cm^2$. It explores the potential of carbon fibre-based composite paper as a high-surface, cost-effective framework for biosensor production. Wax printing and vacuum filtering were used to speed up the application of pyrene carboxylic acid /single-walled carbon nanotube ink onto paper, creating hydrophilic and distinct channels without the need for masks or stencils. The quantitative and selective detection of human serum albumin was demonstrated by its detection at a limit of detection of 1 pM (Yang *et al.* 2015).

6. CONCLUSION

Paper-based electrochemical biosensing, which combines cost, portability, and simplicity, is a revolutionary technique in the field of diagnostics. Paper-based electrochemical sensors show great potential for detecting a wide range of analytes. These sensors have proven effective in a variety of fields, including defence, agriculture, and medicine. One way to improve their analytical performance is by incorporating nanomaterials or bioreceptors into the electrodes mounted on paper substrates. Enhancing ePAD detection through the development of inks with superior electrochemical properties for electrode deposition is also highly desirable. New developments in ePAD technology indicate enormous promise for enhancing devices from the marketplace to the lab. The widespread acceptance of ePADs depends on their success in being portable and easy to use. Similarly, ePADs used in resource-poor environments or for point-of-care diagnostics need to be dependable and durable.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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