Advances in Sample Preparation 16 (2025) 100207

Contents lists available at ScienceDirect

Advances in Sample Preparation

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

Paper as an ecodesigned and smart material for sample preparation integrated with electrochemical (bio)sensors

Vincenzo Mazzaracchio [a,1], Christian Gosti [a,1], Laura Belcastro [a], Fabiana Arduini [a,b,*]

[a] Department of Chemical Science and Technologies, University of Rome Tor Vergata, Via della Ricerca Scientifica 1, Rome 00133, Italy
[b] SENSE4MED s.r.l., via Bitonto 139, Rome 00133, Italy

ARTICLE INFO

Keywords:
Pump-free fluid management
Filtration
Acidification
Reagent preloading
Detection
Paper-based devices

ABSTRACT

Since 2009, paper-based electrochemical biosensors have demonstrated several additional features in analytical chemistry thanks to their capability to manage the fluids by pump-free microfluidics, easily preconcentrate the target analytes, treat the sample with a task-free approach, and detect the target analyte with high sensitivity and accuracy. Herein, we point out the use of paper as a functional material for sample preparation, addressing easily and in a sustainable way the fluid sample management, filtration, separation, centrifugation, chemical sample treatment, and reagent addition. The use of paper for sample preparation in the electrochemical (bio)sensor field is shed light for the first time in a dedicated review. The overriding goal is to highlight the advantages of these cost-effective, environmentally friendly, and easy-to-use devices for smart preparation procedures and accurate electrochemical detection of the target analytes in several fields, including biomedical, environmental, and agrifood.

1. Introduction

The concept of the first electrochemical biosensor was reported by Clark and Lyons in 1962, in which a bulk working electrode was combined with a glucose enzyme for glucose detection in biological fluids [1]. Electrochemical detection is the core of the successful glucose biosensor strip present on the market [2], due to i) its miniaturization, thanks to the screen-printing technique which is able to transform a bulky electrochemical cell with a cost of several thousands of Euro to a printed electrochemical cell with a cost of ca. 1 Euro, ii) capability of electrochemical devices to detect the target analytes in complex matrix, like capillary blood, iii) high sensitivity, selectivity, and storage/working stability of the glucose oxidase biocomponent, and iv) need for a glucose point of care for diabetes management. In this case, the sample has the correct pH for glucose enzymatic activity, thus not requiring pH adjustment. Furthermore, physiological and pathological glucose levels are within the dynamic range of the biosensor, which does not require capillary blood dilution or preconcentration. However, at the state of the art, not all electrochemical biosensors have these features, requiring the addition of reagents, adjustment of the pH of the sample, filtration, or mineralization of the sample.

Paper-based electrochemical devices have paved the way for a new concept in quantitative analytical chemistry, starting from pump-free microfluidics harnessing the capillarity of paper, through reagent-free device by preloading reagents needed for the measurement, until lab on a chip on paper capable to treat the sample, add reagents, and make the measurement in a strip of paper.

The multifarious features of paper have attracted the attention of scientists and industries for delivering cost-effective, sensitive, and easy-to-use analytical devices for various applications in different fields. After the first article on paper-based electrochemical biosensors, published by the Henry group in Analytical Chemistry journal in 2009 [3], the first relevant review was published by the same group in the same journal in 2015, focusing on recent developments in electrochemical paper-based analytical devices (e-PADs) [4]. This review provides an in-depth investigation of the key properties of paper and how they are harnessed in the development of paper-based analytical devices (PADs). It covers theoretical aspects of fluid transport, fabrication techniques, and functionalization strategies designed to enable precise fluid control and the handling of complex samples, with particular attention to analytical detection and applications of PADs. After that, many reviews have been published on paper-based electrochemical (bio)sensors focused on

* Corresponding author.
E-mail address: fabiana.arduini@uniroma2.it (F. Arduini).
[1] These authors contributed equally.

https://doi.org/10.1016/j.sampre.2025.100207
Received 6 July 2025; Received in revised form 12 August 2025; Accepted 18 August 2025
Available online 20 August 2025

manufacturing techniques, analytical applications in biomedical, agri-food, and environmental fields [5–30]. In the last 5 years, reviews with topic paper-based devices were also published in other journal topics beyond the analytical sector, demonstrating the utility of this type of device also in other fields, such as energy ones [21].

This review addresses the topic of sample treatment using the paper-based devices for the detection of the target analytes using electrochemical (bio)sensors by harnessing the several versatile features of paper, such as porosity, foldability, and cuttability, highlighting for the first time this aspect that has never been specifically addressed before in a dedicated review (Table S1, [4–30]).

In detail, we shed light on the utility of the paper for sample preparation, demonstrating how paper has been smartly harnessed to overcome limitations associated with traditional time-consuming laboratory procedures usually required for sample handling and analysis. From a future perspective, these advantages lay the groundwork for the development of fully integrated devices encompassing multianalytical steps within a single platform, ultimately promoting the use of paper as a versatile material for streamlined sample processing. The discussed examples cover various aspects of sample treatment and are organized based on the smart use of paper to address specific sample processing needs, serving as active tools facilitating pre-analytical operations in sample preparation, which normally require ex-situ processing. Indeed, smart configurations were selected exploiting unique features of paper and are described in Section 2. "Smart paper-based configurations as a comprehensive sample handler", encompassing the aspect related to sample handling with capillarity, reagents preloading within the porous paper substrate for on-site processing, strategic handling of unconventional matrices, smart microfluidic configurations, and multifarious multistep sample handling by combining these features. The use of paper for active treatments is discussed in Section 3. "Physical treatment of the sample" and Section 4. "Chemical treatment of the sample", exploring strategies for filtration, separation, centrifugation, pre-concentration, dilution, pH control, chemical addition, and precipitation, usually performed with traditional laboratory procedures. Finally, with significant efforts focused on integrating all the discussed features into a single platform to advance smart e-PADs, key studies are discussed in Section 5. "Multiple sample treatments", highlighting the effective use of paper's inherent properties to develop fully integrated devices addressing the complex requirements of sample handling.

2. Smart paper-based configurations as a comprehensive sample handler

The peculiar and inherent features of paper are highly suitable for its integration with electrochemical (bio)sensors. Among its key properties, the cellulose network creates an ideal surface to deal with various matrices, by supporting sample collection and significantly facilitating various preanalytical steps in sample preparation. To tackle these challenges, paper can actively serve as a functional material, leveraged to design diverse configurations in several procedures, spanning from sample collection to sample treatment, integrated with electrochemical (bio)sensors.

2.1. Reagent loading for on-site sample processing

Owing to its porosity and wettability, paper can act as a reservoir for reagent storage, by easily loading and subsequently releasing upon sample introduction. This enables the development of integrated reagent-free systems, reducing required steps for sample ex-situ processing before assay performance and delivering user-friendly and cost-effective detection tools at the point-of-need. For instance, this was harnessed to create both a reagent storing substrate and a sampling system for glucose detection in artificial tears [31]. Besides favoring tear sample absorption, the porous paper substrate ensured an effective glucose oxidase enzyme and Prussian Blue redox mediator preloading for on-site electrochemical measurement. Similarly, Yao et al. exploited nanobiochar-modified filter paper to immobilize antibodies via dipping-drying method, aiming at the rapid electrochemical detection of microcystin-LR in untreated tap and river water in <5 min [32].

Nevertheless, the porous filter paper network can serve as a built-in filtering layer, removing interfering compounds without any requirement for an external sieve and minimizing matrix effects. Exploiting this property, Colozza et al. integrated an e-PAD into a vertical microfluidic system to develop a reagent-free multianalytical platform for electrochemical detection of glucose and sinigrin in mustard seeds (Brassicaceae plants) [33]. Specifically, two pads were modified via drop-casting to achieve enzymatic reservoirs placed atop two office paper-based screen-printed electrodes for glucose and sinigrin detection. Glucosinolates were first extracted by boiling mustard seeds in water for 4 h, followed by centrifugation. Finally, and ca. 100 μL of the extract were dropped onto the e-PAD and rapidly analyzed (5 min) without undergoing additional treatment, such as sample dilution.

2.2. Paper-based tools assisting handling of unconventional matrices

The porous network of paper further stands out for supporting handling of unconventional matrices, i.e., gas, aerosol, or solid, usually requiring laborious on-site sample collection and processing, thereby constraining the analytical methods on a lab-scale. For instance, aerosols can be directly collected in a few seconds when e-PADs are exposed to the sample emission site, facilitating on site analysis with preloaded ready-to-use devices. Supporting this, a single user-friendly e-PAD demonstrated a 30-second optimal collection time for amperometric detection of hydrogen peroxide in nebulized aerosol, carrying out the electrochemical measurement immediately after e-PAD exposure to the sample (Fig. 1A) [34].

Colozza et al. reported a comparable sampling time (60 s) when analyzing aerosolized samples for mustard agent detection [35]. Upon aerosol sample collection, electrochemical detection was preceded with a reagent supply by folding two porous layers, used both for electrode screen-printing and reagents loading of enzyme and substrate, redissolved by addition of buffer solution for the electrochemical quantification.

As a representative example of aerosol collection and off-site detection, Fiori et al. exploited paper as a sampling tool for aerosolized simulated breath. A laser-induced carbon nanofibers electrode was placed at 1 cm from the nebulizer, to collect simulated breath over an optimized time of 5 min, enabling glucose detection after aerosol redissolution [36]. Nonetheless, self-standing placement of the sampling pad near exhaled breath site was necessary to boost the development of wearable analytical tools, integrating collection and electrochemical detection within wearable items, e.g. face masks. In contrast, this approach extended the sampling time due to a longer distance from the breath emission spot. Indeed, Maier et al. achieved a 13-minutes optimal sampling time in developing the first reported e-PAD integrated into a mask extension device combined with a respiratory mask for analysis of hydrogen peroxide in artificial breath (Fig. 1B) [37]. As a further advancement in developing wearable (bio)sensors for breath analysis, Gutierrez-Galvarez et al. directly embedded the paper-based collector into an N95 face mask [38]. The collector was fabricated by stacking 30 layers of office paper, to effectively collect breath during respiration. The lack of outer extensions resulted in a slightly shorter 12-minute collection time, testing three different sampling zones, using real exhaled breath from four volunteers. Positioning the sampler close to the mouth provided higher sensitivity with respect to the nasal position, owing to the major oral ventilation, successfully distinguishing between one positive COVID-19 patient and three negative subjects, through an amperometric magneto-immunoassay.

To cope with the straightforward preparation of solid samples (i.e., concrete), typically involving time-consuming procedures and use of eco-harmful chemicals, the electrochemical detection can be directly

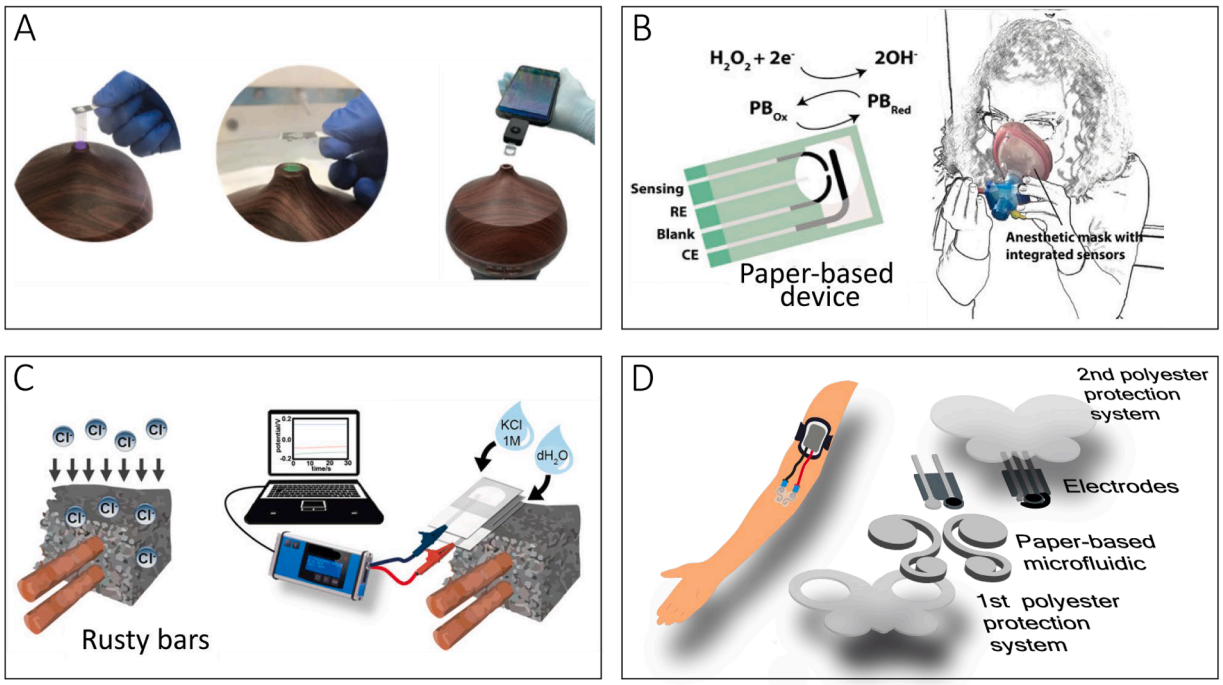

Fig. 1. A) Paper-based electrochemical sensor as sampler for nebulized hydrogen peroxide detection, using a smartphone assisted potentiostat. Reprinted with permission from [34]. Copyright © 2021 Elsevier. B) Wearable paper-based electrochemical sensor combined with a respiratory mask for direct sampling and real-time hydrogen peroxide measurements in simulated breath. Reprinted from [37] under the terms of the Creative Commons license CC BY. Copyright © 2019 American Chemical Society. C) Vertical-flow paper-based electrochemical sensor for on-site chloride contamination assessment in concrete sample. Reprinted with permission from [41]. Copyright © 2021 American Chemical Society. D) Butterfly-like microfluidic wearable electrochemical device for continuous pH and Na^+ monitoring in sweat. Reprinted from [44] under the terms of the Creative Commons license CC BY.

carried out in situ, via strategic integration of the untreated concrete sample into the electrochemical cell. More detailly, paper plays a key role in ensuring the electrochemical contact between the electrode and the solid matrix through the incorporation of electrolyte solutions within a porous pad.

Following this principle, Colozza et al. reported three paper-based detection systems to assess the corrosion of reinforced concrete structures [39–41].

As first engineered strategy, KCl electrolyte solution was loaded onto the concrete detection area, where the reinforcing bar serves as the working electrode, and herein retained by paper porosity for the potential measurement [39]. The developed sensor was tested in the laboratory-scale using small-sized reinforced concrete samples and then applied to real outdoor artwork Music Collection Session by Arman (Milan, Italy).

Further implementation of the system allowed for pH evaluation [40] and chloride contamination monitoring (Fig. 1C) [41] of concrete samples. For pH detection, KCl electrolyte was preloaded into a paper-based envelope where the plastic pH-sensor was inserted and the device was directly placed on the concrete surface for potentiometric pH measurement [40]. For corrosion evaluation, a vertical configuration was designed, including two Ag/AgCl screen-printed electrodes interfaced by a junction pad in a sandwich-like configuration. Specifically, filter paper allowed for measuring the electrochemical potential between a solution containing a known chloride concentration and the unknown concentration in concrete. To deal with the direct measure in the solid matrix, 10 μL of distilled water were drop-cast on the working layer in contact with the concrete surface. Thanks to the ease of handling and the very fast response (i.e., 30 s), the developed devices were used directly on concrete structures at the Giacomo Manzù Museum (Ardea, Italy) for the evaluation of pH and chloride contamination caused by the proximity to the seaside (Fig. 1C) [41].

2.3. *Smart lateral-flow microfluidic devices for fluid sample management*

Leveraging the straightforward technique of wax printing for defining hydrophobic barriers along with customizable fluidic channel dimensions, diverse geometries can be engineered for tailored microfluidic configurations. This innovative approach capitalizes on lateral flow dynamics to effectively steer fluid matrices toward multiple detection zones, enabling the simultaneous multi-analyte detection within a single sample. This not only streamlines the sample collection process but also minimizes waste, making it an environmentally friendly solution. Capillary forces drive the workflow, replacing external pumps while rationally directing the microfluidic pathway from simple linear configurations to more engineered branched ones.

By harnessing these principles, Ruan et al. reported a custom-designed lateral flow immunoassay test strip integrated with screen-printed electrodes for quantitative detection of atrazine and acetochlor in river waters [42]. In this configuration, aqueous samples were added onto a sample pad and transported by capillarity to two separate test strips. At the test zone, the strips were cut and dropped into a cell where the screen-printed electrodes enabled the detection of the target analytes, yielding a recovery range of 90.8 %−117 %.

Differently, Henry research group scaled up to a branched microfluidic design using Whatman filter paper integrated with plastic screen-printed electrodes for multiplexed analysis of trace metal content in airborne particulate [43]. Paper was exploited to steer the fluid sample and to preload acidic solutions, anti-interference agents, and chelating agents needed for the metals detection, treating the sample while flowing through the channels. The proposed platform demonstrated simultaneous detection of Cd(II), Pb(II), Cu(II), Fe(II), and Ni(II) in real samples, obtaining good agreement with traditional Inductively Coupled Plasma Mass Spectrometry analyses.

Continuous monitoring of key biomarkers in sweat is a primary concern in developing wearable devices. Recent strategies address this

by integrating inlet microfluidic channels and sensing areas with a final reservoir or evaporation zone to continuously renew the analyzed fluid reaching the sensing zone. One example is a butterfly-like paper-based system integrated into a wearable platform, designed to monitor pH and Na^+ during stationary biking activity (Fig. 1D) [44]. In detail, circular-shaped sampling zones made of Scottex® tissue paper were placed in contact with the epidermis for sweat sampling. Once collected, the sweat was instantly analyzed by screen-printed electrodes placed in contact with the sampling zones. The unique microfluidic design enabled pump-free transport of the analyzed sweat to waste zones via connection channel, where an adsorbent pad served both as a passive pump and waste reservoir. The whole sensing platform allowed for real-time monitoring of Na^+ and pH during the physical activity of three volunteers.

Combining features of paper with the use of hydrogels provides a straightforward approach to integrate an effective fluid management system with a simple extraction procedure. In this regard, Saha et al. conceived a wearable platform for lactate monitoring based on sweat extraction at rest, without the need for exercise or chemical stimulation [45]. Specifically, the hydrogel loaded onto the paper facilitated sweat extraction through an osmotic mechanism, while the paper microfluidic channels transported the sweat to an evaporation pad via capillary forces, once the lactate detection was carried out. This setup, paired with a custom-designed wireless potentiostat, allowed for long-term sensing of fresh sweat, lasting up to two hours.

2.4. *Origami configurations for multiple purposes*

Besides lateral flow designs, several research groups exploited the foldability of paper to fabricate origami configurations for wearable platform development. Using this approach, multiple layers are folded and stuck, enabling real-time continuous analysis of sweat. Each paper layer has its specific function: i) the collection and flow thanks to capillary forces, ii) the sensing, using screen-printed electrodes, and iii) the final evaporation, avoiding sweat accumulation in the device [46–48].

Cao et al. applied these principles by creating a five-stack paper layer, including a sweat collector, a vertical channel, a transverse channel, an electrode layer, and a final sweat evaporator (Fig. 2A) [46]. The sweat was first sampled and then driven upwards by capillary forces, reaching the electrode layer and the evaporator area. The sensing platform successfully monitored glucose during physical activity on a cycle ergometer, using a benchtop electrochemical workstation. Consecutively, the same authors upgraded the platform by a multi-parametric detection electrode for K^+ and Na^+ monitoring with a custom-fabricated smartwatch reader to deliver a wearable sensing tool [47].

The incorporation of an iontophoretic electrode is a well-known strategy to enhance sweat perspiration, significantly increasing the fluid volume. Recently, these concepts were applied to obtain higher volume with the aim of delivering a multianalyte detection of creatinine, glucose, and uric acid detection [48]. The origami wearable sensor was utilized to assess the metabolic status in healthy participants after dietary intake and during physical exercise, as well as dialysis efficacy during hemodialysis in patients with end-stage renal disease.

Beyond the use of origami structures in wearable (bio)sensors development, paper has been explored to create multifunctional, customizable origami-based configurations for other sample treatment purposes. Indeed, these systems enable rational control of sample addition and reaction steps during the analytical process. As a representative case, foldable configurations can be designed to prevent electrochemical cell contamination throughout the sample management process, especially when processing complex matrices that may cause (bio)fouling phenomena, thus resulting in poorly reproducible electrochemical detection due to the unfavorable adsorption onto the electrodes. Shi et al. leveraged this approach to develop an origami paper-

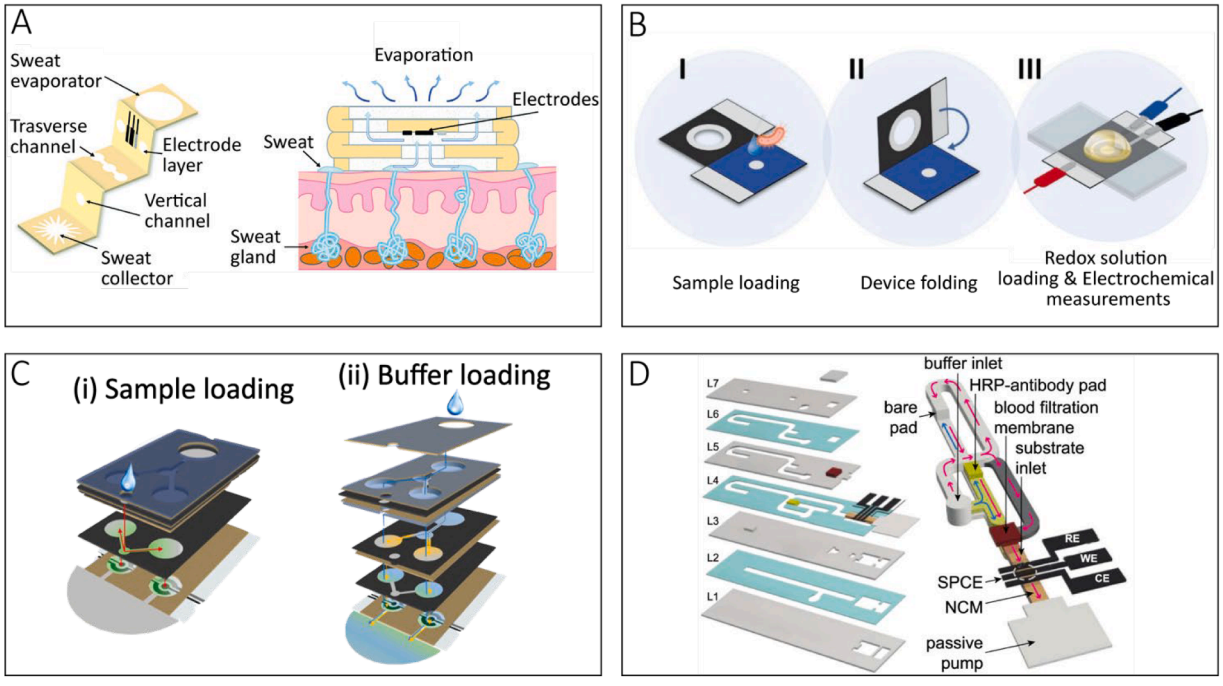

Fig. 2. A) Wearable paper-based microfluidic device for electrochemical glucose detection with integrated sweat collector. The 3D flow channel is formed by folding the device to help the sweat flow from skin into the device and thus refresh the sweat under the electrodes. Reprinted from [46] under the terms of the Creative Commons license CC BY. B) Cellulose nanocrystal-modified paper-based device for label-free electrochemical detection of *Salmonella typhimurium* in food samples. Reprinted with permission from [50]. Copyright © 2025 Elsevier. (C) Paper-based platform for automated and simultaneous electrochemical detection of hepatitis B and hepatitis C virus core antigens engineering fast-flow/delayed microfluidic channels. Reprinted with permission from [52]. Copyright © 2021 Elsevier D) Electrochemical capillary-flow immunosensor for antibodies anti-SARS-CoV-2 nucleocapsid protein detection, encompassing on-board plasma separation. Reprinted with permission from [53]. Copyright © 2021 American Chemical Society.

based immunosensor for the monitoring of cytokine content in serum samples [49]. The e-PAD was composed of three linearly aligned zones, the reaction, the detection, and the auxiliary filter pads, folded along guiding lines to assemble the final sensing configuration. In detail, a reaction and detection chamber were printed onto the reaction pad, allowing for sequential serum loading and analysis. On the opposite side, counter and reference electrodes were printed onto auxiliary pad to avoid fouling during the immunocomplex formation. Consequently, the whole electrochemical cell was created by multilayer folding of the three different pads for fouling-free measurement.

Similarly, Jampasa et al. developed an aptasensor for the detection of *Salmonella typhimurium* in several food products, relying on filter paper to technologically overcome the contamination issue of the electrodes when monitoring a bacterial target (Fig. 2B) [50]. The reference and counter electrodes were designed on the backside of the pad, while the working electrode was integrated beneath the conjugation area. Therein, 1 g of food products (i.e., eggs, chicken, pork, and shrimp) was dissolved in 2 mL of phosphate buffer saline (PBS) and then homogenized by 10-minutes centrifugation. Then, 50 μL of the supernatant were subsequently deposited on the aptamer-modified biocapture zone for 30 min, followed by the device folding on the pad and electrode clipping. Owing to the proposed configuration preventing contamination phenomena, accurate and precise pathogen quantification was achieved, while minimizing the food product consumption (1 g compared to 100 g of conventional methods) and shortening the analysis time down to 30 min.

2.5. Multifarious platforms for multi-step handling

Combining folding and stacking of paper, multilayered configurations serving as active architecture to cope with sample manipulation can be engineered, obtaining fully paper-based or heterogeneous multifarious platforms for multi-step handling.

For instance, Parrilla et al. merged filter paper pad with 3D-printed hollow microneedles array to assist sampling procedure of the apoplast fluid collection from plant leaves [51]. The 3D-printed needle array acts as an extractor, facilitating the sample uptake by punching the plant leaf. Conversely, the pad represents an efficient collector and sieve layer of the fluid via capillarity-driven absorption from the extractor array below. Interestingly, this collector pad showcased a satisfactory fluid uptake (15 μL) in 30 s from 4 different plants, with minimal user intervention consisting of active thumb pressing on the leaf, collecting the fluid for the electrochemical detection using a paper-functionalized screen-printed electrode.

Alternatively, the stacking of fully paper-based layers is a valid strategy to couple vertical ~~flow~~ with lateral flow. With the aim of accomplishing the multifarious steps of the immunological assay, and to autonomously direct the biological sample to the detection zones, multilayered dual flow channel (Fig. 2C) [52] and multi-materials microfluidics circuit were designed (Fig. 2D) [53]. These engineered systems automate washing and reagent steps, directing fluids through pathway channels encountering the pre-dried reagents and buffer washing solutions. The biological fluids are finally delivered to the detection areas to quantify Hepatitis B virus and Hepatitis C virus core antigen in only 15 μL of serum [52] and antibodies anti-SARS-CoV-2 nucleocapsid protein in 10 μL of blood in <20 min [53]. Furthermore, in the latter case, a plasma-separation membrane was integrated with the device, fostering the on-board blood sample pretreatment step.

3. Physical treatment of the sample

Sample preparation is a critical challenge when conducting analyses involving complex matrices. In this scenario, the physical treatment of the sample is often required to satisfy the analytical prerequisites needed for the target analyte quantification. Time-consuming and costly laboratory-based procedures might be easily integrated into a straightforward lab-on-paper device, thereby paving the way for decentralized, user-friendly, and cost-effective sensing platforms. For this purpose, e-PADs can be tailored to perform separation and mixing operations by functionally employing their advantageous properties, such as capillarity and porosity, combined with smart designs to mimic laboratory procedures of physical treatment of the samples.

3.1. Filtration

The filtration of complex samples can be performed by using filter paper, harnessing the porosity of cellulose networks to retain gross impurities and facilitate sample handling. This can be achieved by both lateral and vertical flow approach. In the first case, the system was applied for on-the-spot wastewater treatment, aiming at paracetamol detection [54]. Whatman chromatographic paper was exploited to print the electrodes, preload the KCl electrolyte, and assist the filtering process. Once loaded on the paper strip, wastewater was filtered by the porous network through the microfluidic channel and directed to the sensing area for electrochemical detection of paracetamol. Exploiting vertical filtration, due to the presence of pores, Jemmeli et al. reported an alternative approach to treat river and drinking water within the e-PAD [55]. Indeed, the samples were dropped on the backside of a printed sensor for the detection of bisphenol A, providing a treatment-free sensor. Additionally, to deliver a reagent-free device, a buffer was preloaded and dried in the same backside zone, ready to be dissolved in the sample to maintain the working pH.

Complex biofluid management emerges as a crucial concern to speed up and support the outcomes of medical diagnosis. Among the clinically relevant matrices, whole blood is one of the most representative samples, pointing out a broad range of diseases. However, it is a highly complex biological fluid composed of red and white blood cells, platelets, proteins, lipids, immunoglobulins, and small molecules such as glucose, lactate, drugs, and hormones, as well as clotting factors and other components. Consequently, its complexity often necessitates pretreatment before analysis, involving a centrifugation step using laboratory equipment to separate plasma from the cellular components. The use of paper features for sample pretreatment is a valid strategy to integrate the pre-analytical process into lab-on-paper devices. To detect small molecules in blood, Gautam et al. designed a dumbbell-shaped lateral flow microfluidic configuration, incorporating an LF1 membrane able to retain red blood cells thanks to small-diameter pores [56]. Two lateral separation membrane pads, both for sample loading and treatment, were overlapped to a central Whatman cellulose paper pad, placed above a paper-based screen-printed electrode. A small volume of whole blood (80 μL) from five human donors was injected from both external sides, and separated plasma laterally flowed to the central detection pad for the voltammetric detection of ascorbic acid. Plasma separation membranes can be successfully integrated within a fit-for-purpose e-PAD, eliminating the need for tedious lab-dependent centrifugation, by efficiently coupling lateral and vertical microfluidics to support fluid management toward the separation membrane. Specifically, vertical flow facilitates efficient separation (e.g., plasma from whole blood), while subsequent lateral flow enables directed transport of the processed fluid to downstream analytical zones. According to the literature, the membrane can be directly exposed to blood or incorporated into an inner layer of the e-PAD design, resulting in different separation efficiencies.

Leveraging vertical microfluidics, Sun et al. exploited a plasma separation membrane (GR vivid Plasma Separation Membrane) to filter capillary blood from a finger prick in a self-driven origami device (Fig. 3A) [57]. Using a low volume of capillary blood (ca. 80 μL), the first layer of the e-PAD consisting of separation membrane allowed for efficient separation, with plasma yield higher than 80 %. Separated plasma reached the second Whatman grade 1 filter paper layer, where two wax-patterned lateral flow channels divided it towards two separated detection zones, allowing for dual voltammetric detection of C-reactive

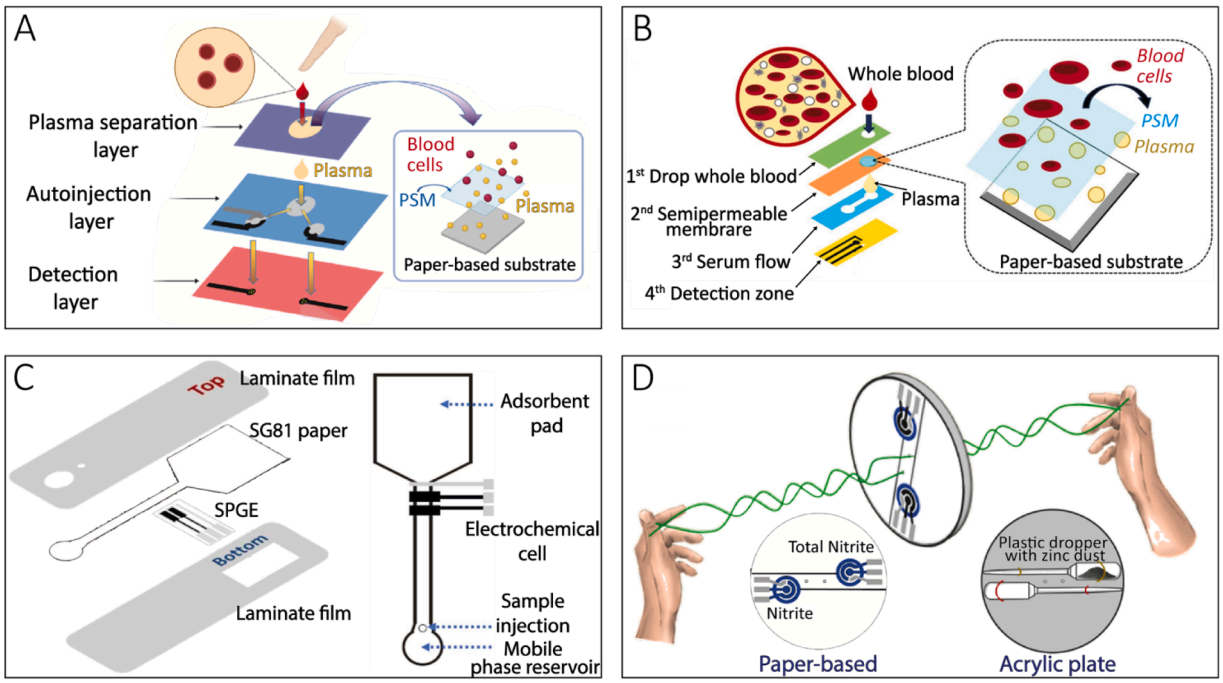

Fig. 3. A) Multifunctional self-driven origami paper-based microfluidic device directly in contact with plasma separation membrane for C-reactive protein and prealbumin detection in whole blood. Reprinted with permission from [57]. Copyright © 2022 Elsevier. B) 3D paper-based microfluidic electrochemical device integrating a plasma separation membrane for glucose detection in whole blood. Reprinted with permission from [58]. Copyright © 2025 Elsevier. C) Chromatographic paper-based electrochemical device for integrated separation and simultaneous detection of carbofuran and carbaryl pesticides in pond water, cucumber, and cabbage samples. Reprinted with permission from [59]. Copyright © 2022 Elsevier. D) Manually rotated paper-based analytical device for on-board centrifugation and following electrochemical detection for the determination of nitrite and nitrate in industrially processed meats. Reprinted with permission from [62]. Copyright © 2025 Elsevier.

protein and prealbumin in 3 volunteers, with a filtration time of 75 s.

Differently, Somsiri et al. integrated a GX vivid Plasma Separation Membrane into the second layer of a 4-layered chromatographic paper-based origami 3D microfluidic electrochemical device (Fig. 3B) [58]. The e-PAD was able to filter at least 20 μL of the whole blood with 60 % efficiency, favoring plasma flow from the top sampling layer to the third one. There, the sample laterally flowed toward the detection zone in the bottom layer, enabling amperometric quantification of glucose in satisfactory agreement with the standard method and the commercial glucometer. As emerging from a critical comparison of the mentioned approaches, plasma separation efficiency depended on the processing grade of the whole blood matrix. By specifically placing the membrane into an inner layer of the e-PAD configuration, the whole blood sample underwent a preliminary filtration by means of the top filter paper layer. This design resulted in a lower yield of separated plasma, likely ascribed to a partial retention of the fluid within the cellulose network. Conversely, the placement of the separation membrane onto the first top layer of the e-PAD led to a more efficient plasma separation, due to direct contact with preliminarily unfiltered whole blood.

3.2. Separation

Coupling fibrous chromatographic paper with an electrochemical detection system is a valid approach to deliver lab-on-chip devices encompassing smart separation procedures for rapid on-site sample preparation and prompt detection. This efficient strategy dramatically reduces the volume required for the treatment with respect to common benchtop chromatographic separations by using a few μL of mobile phase. Herein, the capillarity force of the paper drives the sample flow, while eluting it by means of the chromatographic fibrous structure, supplying pump-free systems. Thus, miniaturized separative tools are delivered and efficiently applied where either the selective analysis of multiple targets or the analyte extraction from complex matrices is strongly demanded. For instance, the detection of carbofuran and carbaryl pesticides in agrifood and environmental fields may be unfavored due to the similarity in their electrochemical profile. Nevertheless, the selective quantification of targeted pesticides in various matrices (i.e., pond water, cucumber, and cabbage) was obtained by simply injecting onto the inlet zone 2 μL of liquid samples alongside 50 μL of dropped mobile phase (40 % v/v n-hexane, 10 % v/v 0.25 M NaOH, and 50 % v/v 0.01 PBS) [59]. In this system, the lateral flow microfluidic specifically supported the separation and electrochemical detection of carbofuran and carbaryl in a selective manner, with 25 and 48 min as retention times, respectively (Fig. 3C). This approach further revealed successful improvements in the sample processing aimed at the separation of the targeted analytes from the interfering compounds within the matrix. As illustrative cases, cannabis oil and extra-virgin olive oil are challenging samples for selective quantifications, as they comprise numerous cannabinoids and o-diphenol antioxidants, respectively. Therefore, a prior sample treatment is required to set the target molecules apart from interfering ones. Upon this requirement, two feasible strategies were followed. On one hand, the sample can be preloaded onto the paper strip, subsequently dipped into the mobile phase, behaving as thin-layer chromatography. By exploiting the mentioned procedure, tetrahydrocannabinol and cannabidiol were selectively separated and detected [60]. In detail, 1 μL of cannabis oil sample was dropped onto the sampling zone of a chromatographic strip, then placed into a plastic tube containing the mobile phase (i.e. 500 μL of 20 % methanol in hexane). Waited for the 20 min-elution time, the detection parts were cut and electrochemically analyzed by dissolving the analytes in 30 μL of 0.1 M PBS at pH 7. On the other hand, both sample and mobile phase may be preloaded together onto a sampling zone drop spot. As opposed to the thin-layer approach, this method was used to monitor the antioxidant capacity in extra-virgin olive oil via the separation and quantification of hydroxytyrosol and oleuropein o-diphenols [61]. Upon loading 4 μL of sample and 5 μL of dimethyl sulfoxide

(DMSO) mobile phase, the combined microfluidics determined the extraction of the o-diphenols, moved from the lipidic matrix to the organic solvent, and ultimately transferred to the paper-strip channel. Thanks to this smart paper use, a treatment-free electrochemical analysis of extra-virgin olive oil was carried out accurately in only 2 min, as revealed by the valid recoveries ($\geq$ 90 %).

3.3. Centrifugation

Among typical laboratory procedures, centrifugation step enables the density-driven isolation of different phases in the analyzed sample. Despite providing a well-defined extraction of the analyte, bulky instrumentation constrains the approach on a lab-dependent scale. In this regard, smart paper design can be customized in a functional pattern mimicking the rotational mechanism. Indeed, circular configurations can replace the centrifugal force of traditional laboratory centrifuges by functionally rotating to extract and mix untreated samples. Besides the paper-based design, the trigger of the rotating mechanism should be further integrated to carry out the sample preparation on the same site harnessing the detection system.

As an example of food sample preparation, Kusonpan et al. merged a rotational paper-based configuration with a rope to extract and mix nitrite and nitrate ions from industrially processed meats (Fig. 3D) [62]. Therein, the ion analytes required a functional extraction before being detected. Upon this key challenge, the extraction of the ions was significantly simplified and subsequently followed by on-site quantification of both ions. The presented device resulted in a strategical assembly, featured by a front disk embedding screen-printed electrochemical sensors to detect the analytes, a back disk accommodating 4 plastic microcentrifuge tubes to centrifuge, and a double acrylic plate to cover the whole architecture. The effective functionality was demonstrated by analyzing 0.1 g of four different meat products. Upon adding 1 mL of 60 °C water to each sample and manually rotating through the rope for 15 min, the extractant was electrochemically quantified by adding 0.1 M phosphate buffer at pH 7. The device led to enhanced accuracy (93 % −106 %; 96 %−112 %; for nitrite and nitrate, respectively) and reproducibility (relative standard deviation RSD % lower than 6 %) with respect to manual shaking and capillary flow, due to a favored analyte diffusion and reaction uniformity.

3.4. Preconcentration and dilution

As a result of trace analyte content, real sample analyses may need a preconcentration step assisting in the quantitative measurements. For instance, aiming at trace elements detection in water samples (namely drinking, tap, pond, and wastewater), Ninwong et al. coupled a preconcentration strategy with the dual-mode multiplexed detection system, further integrating the reagents preloaded on the test zone of the microfluidic channel [63]. The paper strip comprises two circular-shaped areas connected by a channel: a sample zone to load the sample and a heating zone containing two miniaturized triangular test zones, placed above a heating system, which enables the preconcentration of the metals by a resistive heater. The test zones, preloaded with the necessary reagents, allowed for the colorimetric quantification of Fe^{3+} and Ni^{2+}. The heating zone was then placed onto the electrode for the electrochemical detection of Pb^{2+} and Cd^{2+} by anodic stripping-square wave voltammetry. The overall procedure endorsed the sample to evaporate, thus concentrating the metals and increasing the signal, obtaining remarkable limits of detection equal to 0.97 ppb and 2.33 ppb for Pb^{2+} and Cd^{2+}, respectively.

Following this preconcentration approach, Costa-Rama et al. employed a combination of drop-casting and evaporation cycles to effectively perform preconcentration steps by dropping and drying the sample onto a paper-based carbon working electrode [64]. This enabled the detection of trace amounts of diclofenac anti-inflammatory drugs in tap water without sample pre-treatment, showing recoveries in the range of 94–100 %.

Addressing this purpose, Amor-Gutiérrez et al. designed a T-shaped glass-fiber paper-based multifunctional device to strike sample collection, lateral flow steering, and reagents preloading for the enzymatic detection of high-content glucose in beverages [65]. In detail, high-content glucose in beverages, such as cola and orange juices, required a preliminary dilution step to lie within the linear range of quantification. Thus, the engineered T-shaped design can both collect and dilute the samples by simply dipping two combined strips (i.e. collector strip and dilutor strip) into degassed sample solution 1.5 mL-container and the diluting buffer solution (0.1 M Tris-HNO_3 pH 7) 1.5 mL-container, respectively. Aiming to develop a reagent-free device, 20 μL of invertase enzyme solution were drop-cast in the middle of the collector strip and kept drying for 30 min. The preloaded enzyme was then dissolved by the solutions flowing (in both cases around 50 μL cm^{-2}) along the strips to the common detection area, where a paper-based electrode was located. Upon clipping the upper part of the "T" with the electrode and gold-plated connectors, efficient ionic contact between the fluid and the electrode was ensured for the amperometric detection of glucose and sucrose.

4. Chemical treatment of the sample

The ability to load chemical reagents, such as buffer salts, acid solutions, or reactive agents, to perform the reaction has been widely reported in the literature [66–75]. Indeed, paper porosity offers the advantage of dropping reagents in the same electrode printing area or on separate filter pads, delivering a reagent-free sensor device. As a result, this not only can activate detection components like capture antibodies loaded in porous structure, but also chemical reagents that aid the environment of the analytical assay for a more sensitive and accurate detection.

4.1. pH control

Aiming at tuning the operational condition, a controlled pH is typically needed to meet the electrochemical requirements.

Leveraging paper to both print the electrode and load the reagent needed for the measurement, the reagent-free detection of essential oils as antimicrobial compounds, including thymol, eugenol, and carvacrol, was performed in seawater samples [66]. The paper-based working area was herein loaded with acetate buffer solution, and 5 μL of spiked seawater were directly dropped, enabling the redissolution of the buffer to achieve the working pH conditions. The accuracy of the developed sensing device was assessed by a comparison with the Gas Chromatography-Mass Spectrometry reference method, obtaining a bias % in a satisfactory range of ±5 %.

Acidic conditions are usually required for metal detection, for this reason the Henry research group directly loaded acetate buffer on the sample pad of a microfluidic platform for the Cu(II) detection in airborne particulate matter [67]. Specifically, the paper device consisted of a central sample inlet and two external detection zones, one colorimetric and the other electrochemical, for detecting reactive oxygen species and Cu(II), respectively. A volume as low as 100 μL was dropped into the sampling zone for the final detection of Cu(II) in 10 different aerosol samples, with recovery values ranging from 80.5 % to 105.3 %. Nevertheless, this sensing platform relied on the use of a commercial device to sample the air and a pretreatment by acidic digestion to give a liquid phase sample.

Conversely, with the aim of obtaining an alkaline environment for metal-assisted detection of glucose, Li et al. designed an origami e-PAD consisting of three folding pads (Fig. 4A) [68]. The origami device encompassed a screen-printed electrochemical cell on paper, NaOH preloaded sample pad for alkaline environment, and an adsorbing pad. Upon serum sample dilution and centrifugation, the obtained supernatant was dropped on the sample pad and the origami was folded for the

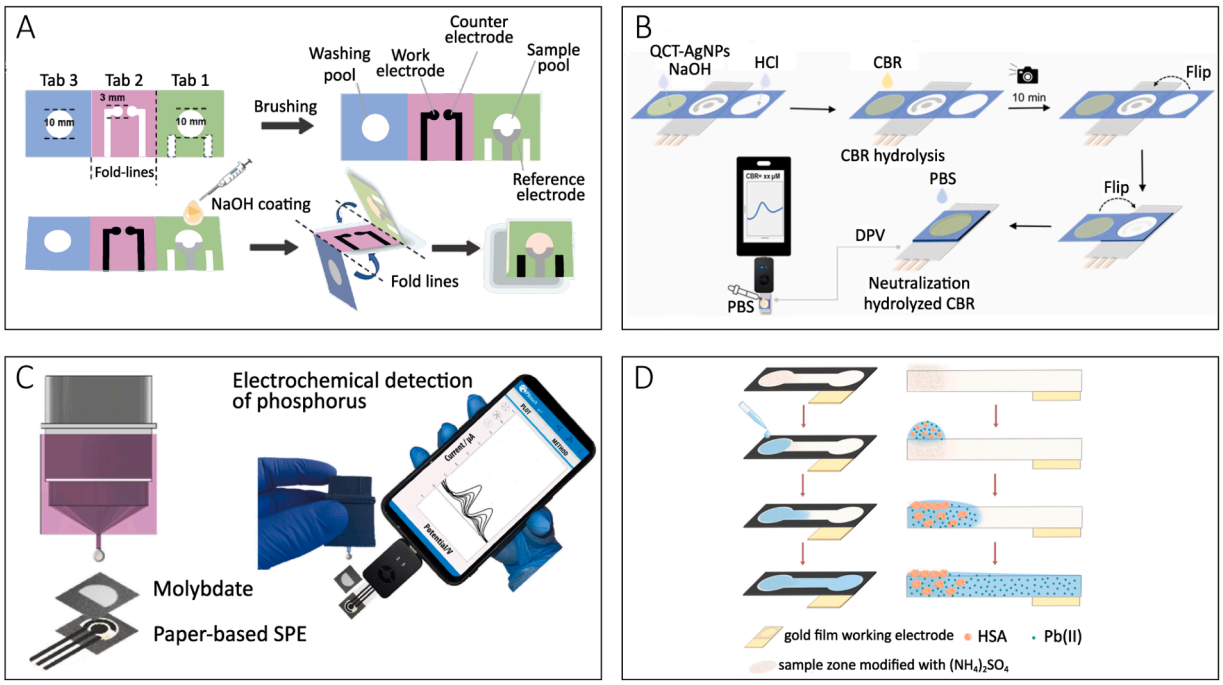

Fig. 4. A) Microfluidic origami paper-based device delivering on-site pH adjustment for non-enzymatic glucose detection. Reprinted with permission from [68]. Copyright © 2024 Elsevier. B) Origami paper-based analytical device for alkalinization and neutralization steps allowing for dual colorimetric/electrochemical detection of carbaryl in fruit samples. Reprinted with permission from [69]. Copyright © 2024 Elsevier. C) Paper-based screen-printed electrochemical sensor for phosphate detection in Antarctic lacustrine sediments harnessing a 3D printed extracting cartridge and a paper-based reagent reservoir. Reprinted from [72] under the terms of the Creative Commons license CC BY. D) Electrochemical paper-based microfluidic device for smart protein precipitation using an acidic-impregnated paper strip for direct detection of lead in urine. Reprinted with permission from [75]. Copyright © 2021 Elsevier.

electrochemical detection of glucose. Finally, the determination of glucose in three human serum samples showed close agreement with the commercial method.

Stronger pretreatment conditions are usually needed when dealing with complex matrices, i.e., oranges (Fig. 4B) [69]. Upon analyte extraction of carbaryl pesticide, the electrochemical quantification of the analyte still required pH adjustments to produce the electroactive 1-Naphtol *via* alkalinization followed by neutralization step. To overcome the limitations of the chemicals, three circular-shaped consecutive pads were integrated into a double-detection foldable configuration to carry out easy sample preparation and accurate detection directly on the same device.

4.2. Chemical addition

Addressing the introduction of the reagents needed for the analyses, paper may serve as a reagents' supplier, equipped with all the requirements to perform electrochemical detection. In this regard, filter paper porosity stands out for fostering field-deployable reagent-free analyses, providing significant advancements in analytical procedures.

The reagent introduction in the analytical platform streamlines the sample treatment specifically for *ad hoc* determination of the targeted analyte. For instance, total bilirubin content in serum requires independent quantification of conjugated and non-conjugated bilirubin. In this regard, splitting the fluid into two microfluidic pathways is an efficient strategy. The first channel did not require any additional chemicals for the quantification of conjugated bilirubin, while the second channel was preloaded with sodium caffeine benzoate to release the non-conjugated bilirubin. This configuration allowed the detection of both conjugated and non-conjugated bilirubin using a small volume of 160 μL of serum samples [70].

Harnessing specific reactions of a preloaded reagent with the target analyte to give a final quantifiable electroactive compound is a highly valuable process to facilitate the sample treatment. This solution was successfully applied to environmental sample control. Among the others, phosphate is a well-exploited compound connected with the human impact on global pollution. The usual strategy for phosphate detection was to exploit the reaction with molybdate-based compounds to deliver the electroactive phosphomolybdate complex. The integration of this molybdic compound into the analytical procedure is crucial to reduce the pretreatment of the sample under analysis.

Preloading the paper-based electrochemical cell was the simplest procedure to incorporate the molybdic compound into the detection step [71]. When the sample containing the phosphate analyte was added, namely tap, runoff, and lake water, the electroactive phosphomolybdate complex was formed, and the final electrochemical detection was carried out.

Another strategy is to let the phosphomolybdate formation happen after the phosphate extraction from solid sample, namely costal lake sediment sample from Antartica (Fig. 4C) [72]. In this case, after the acidic extraction, the sample was dropped onto filter paper pad preloaded with molybdic compound, to create the electroactive phosphomolybdate. Applying this approach, satisfactory results with the colorimetric reference methods were obtained, with a correlation coefficient equal to 0.86.

Finally, to cope with complex matrices while minimizing time and reagent usage, a double reagent system was used to avoid lengthy extraction protocols. For this purpose, Mehlich-3 reagent and molybdate ions were immobilized into filter paper, serving as the phosphate extraction and main reaction compound, respectively [73]. Soil sample was diluted and mixed for 5 min with the filter paper impregnated with the extraction/reaction reagent. The subsequent solution containing the extracted target analyte was then placed onto the screen-printed electrode for the electrochemical detection using cyclic voltammetry. Soil samples from 22 sites were tested, and the results were correlated with the inductively coupled plasma optical emission spectroscopy performed after using the reference extraction procedure. The reported analytical process recorded good correlation with the reference

methods, lowering the assay time and the generated waste.

4.3. Precipitation

Beyond reactants preloading to enable the electrochemical assay, paper porosity can be leveraged to preload specific chemicals for selective removal of interfering compounds within the sample via precipitation. This pretreatment-oriented role improves the analytical robustness of PADs, especially when dealing with complex matrices.

As an example, proteins represent unfavorable interference for the quantification of lactose and total sugars content in milk samples. For this purpose, acidic treated filter paper tools can effectively precipitate the proteins, enhancing the selectivity of the method, as reported by Dortez et al. [74]. Therein, the hydrophilic area at the beginning of a 5 cm-length microfluidic channel was impregnated with 20 μL of 10 M HCl and left to dry. Conversely, the end of the linear channel harnessed an electrochemical detection area consisting of a screen-printed electrode, where the microfluidic flow was directed. Upon loading 100 μL milk sample diluted in phosphate buffer, the interfering proteins were precipitated at the beginning of the channel, while the purified solution was analyzed in the detection zone at the end. A supporting electrolyte solution was used to wet the sensing area and conduct the electrochemical measurement, as soon as the microfluidic flow reached the detection zone in 2 min. Interestingly, the precipitation of proteins optimally reduced unfavorable fouling phenomena, leading to high precision as indicated by the low RSD % ($\leq$ 2 %).

A lateral-flow approach using an acidic-impregnated microfluidic device was further applied to successfully precipitate proteins in biological matrix, namely urine samples. In detail, Wang et al. capitalized on 1.2 cm-length Whatman chromatography paper channel to preload 5 μL of 0.7 g/mL ammonium sulphate, allowing for proteins precipitation and retain into the porous network of the paper substrate (Fig. 4D) [75]. The precipitating agent, specifically preloaded onto the sampling zone (at the beginning of the microfluidic channel), effectively provoked the salting-out of proteins contained in 50 μL of loaded human urine sample. Meanwhile, purified urine was driven by means of the lateral flow microfluidic to the detection zone pad attached atop a gold-plated plastic electrode for voltammetric detection of lead. The ability of paper to entrap proteins led to an accurate lead quantification in three urine samples, which avoided sensor fouling.

5. Multiple sample treatments

Tackling the multiple sample processing steps required to satisfy different pre-analytical requisites, multifunctional e-PADs have been conceived. The integration of several functionalities, combining the previously described features within a multirole e-PAD, facilitates the full management of the sample through the synergy of collection, treatment, and detection. These advanced PADs demonstrate how unified designs can streamline analytical workflows and enhance overall device performance.

Following the multifunctional lateral flow approach, Fiore et al. designed a wearable paper-based microfluidic platform for cortisol detection with a competitive magneto-immunoassay (Fig. 5A) [76]. Sweat was managed by paper configuration, combining the collection during a stationary cycling activity, the flowing to the sensing zone, and the preloading of the reagents needed for the immunological competitive assay, occurring through the microfluidic channel pathway. A final adsorbent pad located after the detection zones ensured the flow, acting as a passive pump. Finally, the reliability of this analytical platform was demonstrated by successfully quantifying cortisol levels in the sweat of volunteers during cycling activities.

When moving to more complex matrices, vertical configurations capitalize on gravity to assist fluid transport, enabling efficient handling by folded and stacked designs. For this purpose, Caratelli et al. developed an origami paper-based electrochemical device for cholinesterase inhibitors detection in whole finger prick blood, namely physostigmine, rivastigmine, and donepezil [77]. Vivid Plasma Separation membrane was used to separate the blood sample, avoiding sample pretreatment, while filter paper was used for preloading the reagent, i.e., the

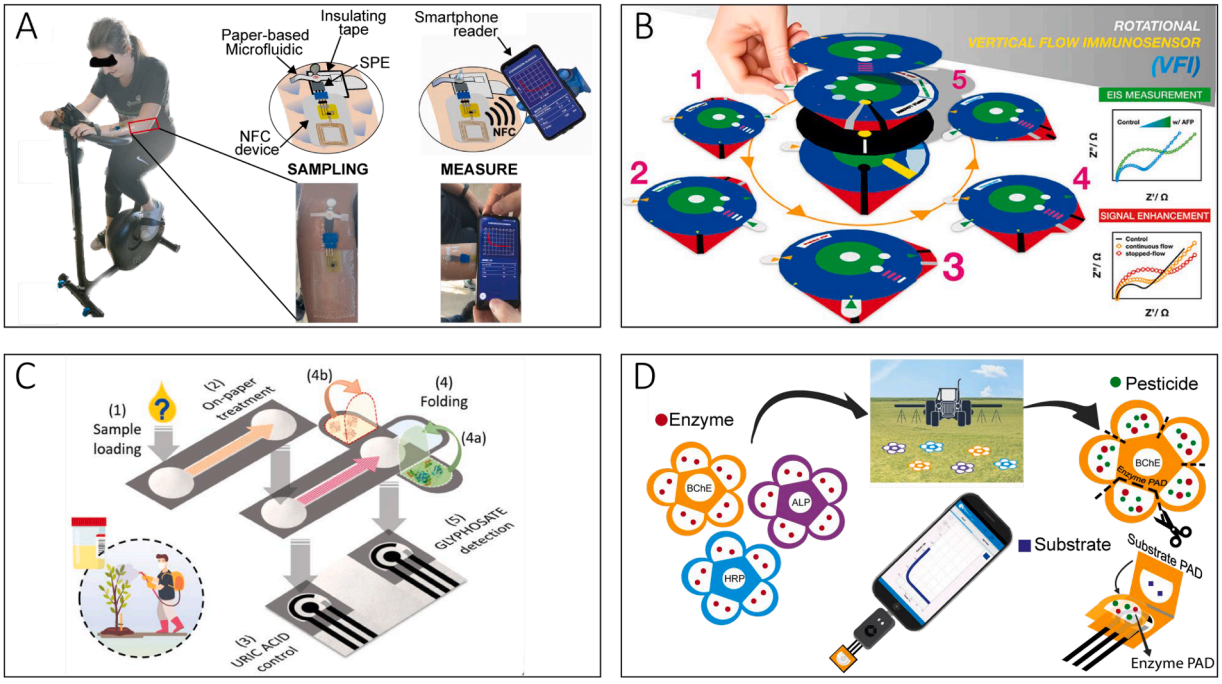

Fig. 5. A) Wearable microfluidic paper-based electrochemical biosensor for cortisol detection in sweat. Reprinted with permission from [76]. Copyright © 2022 Elsevier. B) Hand-rotating paper-based immunosensor leveraging vertical-flow microfluidics for impedimetric detection of α-Fetoprotein. Reprinted with permission from [78]. Copyright © 2022 American Chemical Society. C) Paper-based electrochemical device incorporating on-board treatment of human urine for glyphosate detection. Reprinted from [79] under the terms of the Creative Commons license CC BY. D) Flower-like origami paper-based biosensors for pesticide detection in the aerosol phase. Reprinted with permission from [81]. Copyright © 2022 Elsevier.

butyrylthiocholine enzymatic substrate, delivering a reagent-free, ready-to-use point-of-care device. The sample was managed through the first filtration to obtain plasma, then it reached the underlying porous paper to re-dissolve the preloaded reagents, finally leading to the paper-based screen-printed electrode layer. A low volume of capillary and venous blood sample was tested, namely 20 μL.

Paper's versatility fosters the design of dynamic vertical flow configurations when sequential analytical steps are required, going beyond the static stacking of different layers. By configuring a rotating system, Yakoh et al. developed an electrochemical hand-operated rotational device for immunological detection of α-Fetoprotein in serum (Fig. 5B) [78]. Consecutive steps of injection, transfer, switch, and stopped-flow needed for the immunoassay and detection were carried out using 4 disks of wax-patterned circular Whatman grade 4 chromatographic paper. The stacked configuration regulates the analytical steps as follows: sample loading, sample injection, washing step, and analysis step. After loading the serum sample in the first disk for 3 min, manual rotation of the second disk enabled the controlled sample transfer to the third and fourth disk, for the binding/washing step and detection, respectively. The rotational configuration enables a stopped-flow working mode of the device, revealing a notable dynamic linear range in a 9-minutes overall assay.

Building upon the combination of lateral and vertical microfluidic, Moro et al. developed an origami paper-based device, comprising a treatment strip, a detection strip, and two paper-based screen-printed electrodes for glyphosate detection in urine samples (Fig. 5C) [79]. Microfluidic channels were patterned on a filter paper substrate using wax printing, and three layers were stacked in an origami configuration. In the treatment strip (top layer), an acidic solution was loaded to enable ascorbic acid precipitation after commercial 60 μL urine sample loading. Through vertical microfluidics, the purified sample reached the detection strip for uric acid detection. Additionally, for glyphosate detection, the sample went through lateral microfluidic channels to reach a second detection zone. Here, upon folding the preloaded pads, the reagents were mixed, allowing enzymatic reaction and amperometric quantification of enzymatic inhibition by the underlying screen-printed electrode.

When several sequential treatments are demanded to make the untreated sample meet the electroanalytical requirements, multi-foldable origami 3D designs can be leveraged to perform sequential chemical manipulations. The study of Fiori et al. perfectly reflected the efficiency of merging a functional architecture with chemical pre-treatments to design a 3D pop-up (3D-PP) device for carbaryl pesticide quantification in 5 different types of grain (i.e., soft wheat 1, soft wheat 2, durum wheat, Kamut, and Barley) [80]. The proposed method only requires 10 min of extraction adding 5 g of grain sample in 10 mL of methanol under stirring and following centrifugation. To handle efficiently the extracts, a chromatography paper-based 3D scaffold was designed as follows: lateral arms to provide self-standing structural stability, sampling zone trimmed and folded outwards to introduce the sample extract and touch the working area after folding, interlocking tabs to facilitate the architecture closure during the folding process, and pocket aimed at the sensor holding. The proposed structure functionally aligns with the sequential preloading, addressing in respective order the carbaryl pre-concentration, its alkaline hydrolysis and pH neutralization. Therefore, the analyte pre-concentration was preliminary carried out by drop-casting onto the outward sampling zone 75 μL of extract solution in five consecutive aliquots. Subsequently, 10 μL of 60 mM NaOH were loaded on the same site and neutralized with 40 μL of 30 mM NaH_2PO_4 on the sampling window (opposite side), upon 5 min-completion of hydrolysis and 3D-PP folding that enables the overlap between the sampling zone and the detection zone. After a 1-minute neutralization, the electrochemical quantification was performed, allowing for accurate carbaryl pesticide quantification in ca. 15 min.

Similarly, Caratelli et al. exploited filter paper suitability towards the development of smart origami motifs to handle comprehensively a more challenging matrix, namely the aerosol phase. In detail, three filter paper PADs preloaded with enzymatic reagents (i.e., one enzyme-pad for the blank; two enzymatic substrate-pads, one for the blank and one for the pesticide detection) and a flower-like aerosol collector were used to enable the reagent and sample treatment-free amperometric detection of 2,4-dichlorophenoxyacetic acid, glyphosate, and paraoxon pesticides (Fig. 5D) [81]. Each origami sampler, harnessing five petals featured by preloaded enzymes (i.e., butyrylcholinesterase, alkaline phosphatase, and horseradish peroxidase), allowed for the uptake of the aerosol phase presenting the corresponding pesticide as enzymatic inhibitor. Specifically, to achieve an effective collection of the matrix, the flower-like sampler only needs 60 s as optimal exposure time toward the aerosolized pesticide solution. After collecting the sample, the petals were cut and analyzed using a paper-based electrochemical sensor integrated with the specific enzymatic substrate-pad (i.e. butyrylthiocholine, 1-naphthyl phosphate, and 3,3′,5,5′-tetramethylbenzidine). More detailly, the contaminated collector pad (petal exposed to the pesticide) was slipped between the sensor and the substrate pad, then folded upon it. Eventually, the user-friendly enzymatic inhibition of the pesticide was measured by simply adding 20 μL of distilled water onto the electrochemical cell.

6. Conclusions

In 2010, the Whiteside research group highlighted the utility of colorimetric paper-based devices for diagnostic use in developing countries, following the ASSURED criteria established by the World Health Organisation, where ASSURED means Affordable, Sensitive, Specific, User-friendly, Rapid/Robust, Equipment-free, and Deliverable to those who need it [82]. Since then, the field of paper-based analytical devices has undergone a rapid and transformative evolution. More recently, ASSURED criteria have been updated to RE-ASSURED ones, where real-time connectivity for digital healthcare and ease of specimen collection have been added, underlining the growing importance of robust sample collection, handling and preparation in modern diagnostics [83]. After the first published article focused on paper-based electrochemical biosensor reported in 2009 by the Henry research group [3], the scientific community has embraced the potential of electrochemical paper-based analytical devices across a wide range of applications, as demonstrated by a huge number of paper-based electrochemical (bio)sensors that have been designed and developed. Thanks to their versatility, electrochemical paper-based analytical devices have been applied across several fields, including biomedical, environmental, and agrifood, addressing the detection of target analytes in diverse samples, varying in physicochemical properties but sharing common requirements aiming at sample preparation, handling, and analysis. While most reviews have traditionally centered on the detection strategies and analytical performances of these devices, this review uniquely focuses on the important role of sample treatment in electrochemical paper-based analytical devices. Specifically, we highlighted the versatility of paper as an active component able to facilitate complex sample preparation steps typically confined to benchtop procedures. The inherent properties of paper, namely capillarity, porosity, and foldability, have smartly been employed in sample preparation, being the paper directly involved in i) management of fluid sample, harnessing the capillarity, ii) filtration of sample and separation of analyte from the matrix, by leveraging the porosity, iii) storage of reagents for sample treatment and chemical detection, by exploiting the 3D structure, and iv) serving as engineering tool for multifunctional platform, harnessing combined properties. Despite these advantages, significant challenges and limitations remain, constraining the full potential and broad implementation of paper-based systems. Main issues include the limited control over fluid dynamics, which hampers the precision required for complex multistep analyses and the variability and lack of standardization in paper substrates, which require batch control before use. These limitations must be critically addressed to transition paper-based

devices from proof-of-concept applications to reliable, field-deployable analytical platforms.

In summary, even if paper-based analytical devices are usually focused on target analyte detection, herein, we demonstrated that paper plays a relevant role in the sample preparation, further enlarging its prominent role in the analytical chemistry field. By turning paper into a comprehensive sample handler, research is paving the way for fully integrated, autonomous analytical platforms.

CRediT authorship contribution statement

Vincenzo Mazzaracchio: Writing – original draft, Writing – review & editing. **Christian Gosti:** Writing – original draft, Writing – review & editing. **Laura Belcastro:** Writing – original draft, Writing – review & editing. **Fabiana Arduini:** Conceptualization, Writing – original draft, Writing – review & editing, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sampre.2025.100207.

Data availability

No data was used for the research described in the article.

References

[1] L.C. Clark, C. Lyons, Electrode systems for continuous monitoring in cardiovascular surgery, Ann. N. Y. Acad. Sci. 102 (1962) 29–45, https://doi.org/10.1111/j.1749-6632.1962.tb13623.x.

[2] A.P.F. Turner, Biosensors–sense and sensitivity, Science 290 (2000) 1315–1317, https://doi.org/10.1126/science.290.5495.1315.

[3] W. Dungchai, O. Chailapakul, C.S. Henry, Electrochemical detection for paper-based microfluidics, Anal. Chem. 81 (2009) 5821–5826, https://doi.org/10.1021/ac9007573.

[4] D.M. Cate, J.A. Adkins, J. Mettakoonpitak, C.S. Henry, Recent developments in paper-based microfluidic devices, Anal. Chem. 87 (2015) 19–41, https://doi.org/10.1021/ac503968p.

[5] J. Adkins, K. Boehle, C. Henry, Electrochemical paper-based microfluidic devices, Electrophoresis 36 (2015) 1811–1824, https://doi.org/10.1002/elps.201500084.

[6] N.A. Meredith, C. Quinn, D.M. Cate, T.H. Reilly, J. Volckens, C.S. Henry, Paper-based analytical devices for environmental analysis, Analyst 141 (2016) 1874–1887, https://doi.org/10.1039/C5AN02572A.

[7] J. Mettakoonpitak, K. Boehle, S. Nantaphol, P. Teengam, J.A. Adkins, M. Srisa-Art, C.S. Henry, Electrochemistry on paper-based Analytical devices: a review, Electroanalysis 28 (2016) 1420–1436, https://doi.org/10.1002/elan.201501143.

[8] V.B.C. Lee, N.F. Mohd-Naim, E. Tamiya, M.U. Ahmed, Trends in paper-based electrochemical biosensors: from design to application, Anal. Sci. 34 (2018) 7–18, https://doi.org/10.2116/analsci.34.7.

[9] R. Tortorich, H. Shamkhalichenar, J.-W. Choi, Inkjet-printed and paper-based electrochemical sensors, Appl. Sci. 8 (2018) 288, https://doi.org/10.3390/app8020288.

[10] T. Gebretsadik, T. Belayneh, S. Gebremichael, W. Linert, M. Thomas, T. Berhanu, Recent advances in and potential utilities of paper-based electrochemical sensors: beyond qualitative analysis, Analyst 144 (2019) 2467–2479, https://doi.org/10.1039/C8AN02463D.

[11] E. Noviana, C.S. Henry, Simultaneous electrochemical detection in paper-based analytical devices, Curr. Opin. Electrochem. 23 (2020) 1–6, https://doi.org/10.1016/j.coelec.2020.02.013.

[12] E. Noviana, C.P. McCord, K.M. Clark, I. Jang, C.S. Henry, Electrochemical paper-based devices: sensing approaches and progress toward practical applications, Lab Chip 20 (2020) 9–34, https://doi.org/10.1039/C9LC00903E.

[13] E. Solhi, M. Hasanzadeh, P. Babaie, Electrochemical paper-based analytical devices (ePADs) toward biosensing: recent advances and challenges in bioanalysis, Anal. Methods 12 (2020) 1398–1414, https://doi.org/10.1039/D0AY00117A.

[14] M. Baharfar, M. Rahbar, M. Tajik, G. Liu, Engineering strategies for enhancing the performance of electrochemical paper-based analytical devices, Biosens. Bioelectron. 167 (2020) 112506, https://doi.org/10.1016/j.bios.2020.112506.

[15] E. Noviana, D.B. Carrão, R. Pratiwi, C.S. Henry, Emerging applications of paper-based analytical devices for drug analysis: a review, Anal. Chim. Acta 1116 (2020) 70–90, https://doi.org/10.1016/j.aca.2020.03.013.

[16] V.N. Ataide, L.F. Mendes, L.I.L.M. Gama, W.R. De Araujo, T.R.L.C. Paixão, Electrochemical paper-based analytical devices: ten years of development, Anal. Methods 12 (2020) 1030–1054, https://doi.org/10.1039/C9AY02350J.

[17] W. Mazurkiewicz, M. Podrażka, E. Jarosińska, K. Kappalakandy Valapil, M. Wiloch, M. Jönsson-Niedziółka, E. Witkowska Nery, Paper-based electrochemical sensors and how to make them (Work), ChemElectroChem 7 (2020) 2939–2956, https://doi.org/10.1002/celc.202000512.

[18] E. Noviana, T. Ozer, C.S. Carrell, J.S. Link, C. McMahon, I. Jang, C.S. Henry, Microfluidic paper-based Analytical devices: from design to applications, Chem. Rev. 121 (2021) 11835–11885, https://doi.org/10.1021/acs.chemrev.0c01335.

[19] R. Tang, M.Y. Xie, M. Li, L. Cao, S. Feng, Z. Li, F. Xu, Nitrocellulose membrane for paper-based biosensor, Appl. Mater. Today 26 (2022) 101305, https://doi.org/10.1016/j.apmt.2021.101305.

[20] M.S. Khan, S.A. Shadman, Md.M.R. Khandaker, Advances and current trend of bioactive papers and paper diagnostics for health and biotechnological applications, Curr. Opin. Chem. Eng. 35 (2022) 100733, https://doi.org/10.1016/j.coche.2021.100733.

[21] A. Thakur, P. Devi, Paper-based flexible devices for energy harvesting, conversion and storage applications: a review, Nano Energy 94 (2022) 106927, https://doi.org/10.1016/j.nanoen.2022.106927.

[22] V. Caratelli, E. Di Meo, N. Colozza, L. Fabiani, L. Fiore, D. Moscone, F. Arduini, Nanomaterials and paper-based electrochemical devices: merging strategies for fostering sustainable detection of biomarkers, J. Mater. Chem. B 10 (2022) 9021–9039, https://doi.org/10.1039/D2TB00387B.

[23] F. Arduini, Electrochemical paper-based devices: when the simple replacement of the support to print ecodesigned electrodes radically improves the features of the electrochemical devices, Curr. Opin. Electrochem. 35 (2022) 101090, https://doi.org/10.1016/j.coelec.2022.101090.

[24] M.B. Kulkarni, N.H. Ayachit, T.M. Aminabhavi, B.W. Pogue, Recent advances in microfluidics-based paper analytical devices (μPADs) for biochemical sensors: from fabrication to detection techniques, Biochem. Eng. J. 198 (2023) 109027, https://doi.org/10.1016/j.bej.2023.109027.

[25] J. Du Plooy, N. Jahed, E. Iwuoha, K. Pokpas, Advances in paper-based electrochemical immunosensors: review of fabrication strategies and biomedical applications, R. Soc. Open Sci. 10 (2023) 230940, https://doi.org/10.1098/rsos.230940.

[26] P.B. Deroco, D.W. Junior, L.T.Kubota, Paper-based Wearable Electrochemical Sensors: A New Generation of Analytical Devices, Electroanalysis 35 (2023) e20220017, doi:10.1002/elan.202200177.

[27] H.A. Silva-Neto, L.F. De Lima, D.S. Rocha, V.N. Ataide, G.N. Meloni, G. Moro, A. Raucci, S. Cinti, T.R.L.C. Paixão, W.R. De Araujo, W.K.T. Coltro, Recent achievements of greenness metrics on paper-based electrochemical (bio) sensors for environmental and clinical analysis, TrAC Trends Anal. Chem. 174 (2024) 117675, https://doi.org/10.1016/j.trac.2024.117675.

[28] L. Bezinge, C. Shih, D.A. Richards, A.J. deMello, Electrochemical paper-based microfluidics: harnessing capillary flow for advanced diagnostics, Small (2024) 2401148, https://doi.org/10.1002/smll.202401148.

[29] P. Aryal, C.S. Henry, Advancements and challenges in microfluidic paper-based analytical devices: design, manufacturing, sustainability, and field applications, Front. Lab. Chip. Technol. 3 (2024) 1467423, https://doi.org/10.3389/frlct.2024.1467423.

[30] R. Manikandan, H.-G. Jang, C.-S. Kim, J.-H. Yoon, J. Lee, H. Paik, S.-C. Chang, Nano-engineered paper-based electrochemical biosensors: versatile diagnostic tools for biomarker detection, Coord. Chem. Rev. 523 (2025) 216261, https://doi.org/10.1016/j.ccr.2024.216261.

[31] L. Fiore, A. Sinha, N. Seddaoui, J. Di Biasio, F. Ricci, G.M. Stojanovic, F. Arduini, Paper card-like electrochemical platform as a smart point-of-care device for reagent-free glucose measurement in tears, Chem. Commun. 59 (2023) 4300–4303, https://doi.org/10.1039/D2CC06561D.

[32] L. Yao, L. He, Y. Yang, Y. Zhang, Z. Liu, L. Liang, Y. Piao, Nanobiochar paper based electrochemical immunosensor for fast and ultrasensitive detection of microcystin-LR, Sci. Total Environ. 750 (2021) 141692, https://doi.org/10.1016/j.scitotenv.2020.141692.

[33] N. Colozza, E. Di Meo, A. Mucaria, D. Moscone, F. Arduini, An origami paper-based electrochemical biosensing platform for quality control of agri-food waste in the valorization strategy, Microchim. Acta 189 (2022) 311, https://doi.org/10.1007/s00604-022-05392-5.

[34] L. Fiore, V. Mazzaracchio, P. Galloni, F. Sabuzi, S. Pezzola, G. Matteucci, D. Moscone, F. Arduini, A paper-based electrochemical sensor for H_2O_2 detection in aerosol phase: measure of H2O2 nebulized by a reconverted ultrasonic aroma diffuser as a case of study, Microchem. J. 166 (2021) 106249, https://doi.org/10.1016/j.microc.2021.106249.

[35] N. Colozza, K. Kehe, G. Dionisi, T. Popp, A. Tsoutsoulopoulos, D. Steinritz, D. Moscone, F. Arduini, A wearable origami-like paper-based electrochemical biosensor for sulfur mustard detection, Biosens. Bioelectron. 129 (2019) 15–23, https://doi.org/10.1016/j.bios.2019.01.002.

[36] S. Fiori, C. Bruckschlegel, K. Weiss, K. Su, M. Foedlmeier, F. Della Pelle, A. Scroccarello, D. Compagnone, A.J. Baeumner, N. Wongkaew, Laser-induced carbon nanofibers as permeable nonenzymatic sensor for biomarker detection in

breath aerosol, Anal. Chem. 97 (2025) 4293–4298, https://doi.org/10.1021/acs.analchem.4c06580.
[37] D. Maier, E. Laubender, A. Basavanna, S. Schumann, F. Güder, G.A. Urban, C. Dincer, Toward continuous monitoring of breath biochemistry: a paper-based wearable sensor for real-time hydrogen peroxide measurement in simulated breath, ACS Sens 4 (2019) 2945–2951, https://doi.org/10.1021/acssensors.9b01403.
[38] L. Gutiérrez-Gálvez, N. Seddaoui, L. Fiore, L. Fabiani, T. García-Mendiola, E. Lorenzo, F. Arduini, Functionalized N95 face mask with a chemical-free paper-based collector for exhaled breath analysis: sARS-CoV-2 detection with a printed immunosensor as a case study, ACS Sens 9 (2024) 4047–4057, https://doi.org/10.1021/acssensors.4c00981.
[39] N. Colozza, A. Sassolini, L. Agosta, A. Bonfanti, K. Hermansson, F. Arduini, A paper-based potentiometric sensor for solid samples: corrosion evaluation of reinforcements embedded in concrete structures as a case study, ChemElectroChem 7 (2020) 2274–2282, https://doi.org/10.1002/celc.202000330.
[40] N. Colozza, S. Tazzioli, A. Sassolini, L. Agosta, M.G. Di Monte, K. Hermansson, F. Arduini, Multiparametric analysis by paper-assisted potentiometric sensors for diagnostic and monitoring of reinforced concrete structures, Sens. Actuators B: Chem. 345 (2021) 130352, https://doi.org/10.1016/j.snb.2021.130352.
[41] N. Colozza, S. Tazzioli, A. Sassolini, L. Agosta, M.G. Di Monte, K. Hermansson, F. Arduini, Vertical-flow paper sensor for on-site and prompt evaluation of chloride contamination in concrete structures, Anal. Chem. 93 (2021) 14369–14374, https://doi.org/10.1021/acs.analchem.1c03363.
[42] X. Ruan, Y. Wang, E.Y. Kwon, L. Wang, N. Cheng, X. Niu, S. Ding, B.J. Van Wie, Y. Lin, D. Du, Nanomaterial-enhanced 3D-printed sensor platform for simultaneous detection of atrazine and acetochlor, Biosens. Bioelectron. 184 (2021) 113238, https://doi.org/10.1016/j.bios.2021.113238.
[43] J. Mettakoonpitak, J. Volckens, C.S. Henry, Janus Electrochemical paper-based analytical devices for metals detection in aerosol samples, Anal. Chem. 92 (2020) 1439–1446, https://doi.org/10.1021/acs.analchem.9b04632.
[44] L. Fiore, V. Mazzaracchio, A. Antinucci, R. Ferrara, T. Sciarra, F. Lista, A.Q. Shen, F. Arduini, Wearable electrochemical device based on butterfly-like paper-based microfluidics for pH and Na+ monitoring in sweat, Microchim. Acta 191 (2024) 580, https://doi.org/10.1007/s00604-024-06564-1.
[45] T. Saha, T. Songkakul, C.T. Knisely, M.A. Yokus, M.A. Daniele, M.D. Dickey, A. Bozkurt, O.D. Velev, Wireless wearable electrochemical sensing platform with zero-power osmotic sweat extraction for continuous lactate monitoring, ACS Sens. 7 (2022) 2037–2048, https://doi.org/10.1021/acssensors.2c00830.
[46] Q. Cao, B. Liang, T. Tu, J. Wei, L. Fang, X. Ye, Three-dimensional paper-based microfluidic electrochemical integrated devices (3D-PMED) for wearable electrochemical glucose detection, RSC Adv 9 (2019) 5674–5681, https://doi.org/10.1039/C8RA09157A.
[47] Q. Cao, B. Liang, X. Mao, J. Wei, T. Tu, L. Fang, X. Ye, A smartwatch integrated with a paper-based microfluidic patch for sweat electrolytes monitoring, Electroanalysis 33 (2021) 643–651, https://doi.org/10.1002/elan.202060025.
[48] S. Zhang, H. Wang, Y. Zheng, Y. Yao, T. Li, Y. Ma, Y. Zhou, Z. Chen, Y. Wei, L. Fang, X. Chen, X. Ye, J. Zhou, B. Liang, An integrated paper-based patch for wearable detection of diabetic nephropathy biomarkers in sweat, Adv. Funct. Mater. (2025) 2501970, https://doi.org/10.1002/adfm.202501970.
[49] D. Shi, C. Zhang, X. Li, J. Yuan, An electrochemical paper-based hydrogel immunosensor to monitor serum cytokine for predicting the severity of COVID-19 patients, Biosens. Bioelectron. 220 (2023) 114898, https://doi.org/10.1016/j.bios.2022.114898.
[50] S. Jampasa, N. Sangthong, T. Ozer, W. Panphut, S. Naorungroj, N. Ngamrojanavanich, T. Kaneta, O. Chailapakul, W. Wonsawat, Label-free electrochemical aptasensor based on cellulose nanocrystal-modified paper-based device for *Salmonella typhimurium* detection in food samples, Microchem. J. 211 (2025) 113144, https://doi.org/10.1016/j.microc.2025.113144.
[51] M. Parrilla, A. Sena-Torralba, A. Steijlen, S. Morais, Á. Maquieira, K. De Wael, A 3D-printed hollow microneedle-based electrochemical sensing device for in situ plant health monitoring, Biosens. Bioelectron. 251 (2024) 116131, https://doi.org/10.1016/j.bios.2024.116131.
[52] S. Boonkaew, A. Yakoh, N. Chuaypen, P. Tangkijvanich, S. Rengpipat, W. Siangproh, O. Chailapakul, An automated fast-flow/delayed paper-based platform for the simultaneous electrochemical detection of hepatitis B virus and hepatitis C virus core antigen, Biosens. and Bioelectron. 193 (2021) 113543, https://doi.org/10.1016/j.bios.2021.113543.
[53] I.C. Samper, A. Sánchez-Cano, W. Khamcharoen, I. Jang, W. Siangproh, E. Baldrich, B.J. Geiss, D.S. Dandy, C.S. Henry, Electrochemical capillary-flow immunoassay for detecting anti-SARS-CoV-2 nucleocapsid protein antibodies at the point of care, ACS Sens 6 (2021) 4067–4075, https://doi.org/10.1021/acssensors.1c01527.
[54] A. Miglione, A. Raucci, F. Cristiano, M. Mancini, V. Gioia, A. Frugis, S. Cinti, Paper-based 2D configuration for the electrochemical and facile detection of paracetamol in wastewaters, Electrochim. Acta 488 (2024) 144255, https://doi.org/10.1016/j.electacta.2024.144255.
[55] D. Jemmeli, E. Marcoccio, D. Moscone, C. Dridi, F. Arduini, Highly sensitive paper-based electrochemical sensor for reagent free detection of bisphenol A, Talanta 216 (2020) 120924, https://doi.org/10.1016/j.talanta.2020.120924.
[56] N. Gautam, R. Verma, R. Ram, J. Singh, A. Sarkar, Development of a biodegradable microfluidic paper-based device for blood-plasma separation integrated with non-enzymatic electrochemical detection of ascorbic acid, Talanta 266 (2024) 125019, https://doi.org/10.1016/j.talanta.2023.125019.
[57] S. Sun, J. Luo, Y. Zhu, F. Kong, G. Mao, T. Ming, Y. Xing, J. Liu, Y. Dai, S. Yan, Y. Yang, X. Cai, Multifunctional self-driven origami paper-based integrated microfluidic chip to detect CRP and PAB in whole blood, Biosens. and Bioelectron. 208 (2022) 114225, https://doi.org/10.1016/j.bios.2022.114225.
[58] S. Somsiri, C. Kaewjangwad, N. Malarat, S. Wangchuk, J. Saichanapan, K. Samoson, K. Promsuwan, K. Saisahas, A. Soleh, T. Phairatana, W. Limbut, Portable NFC potentiostat integrated with a 3D paper-based microfluidic electrochemical device for glucose detection in whole blood using PEDOT:pSS/DMSO/GOx sensitive film, Microchem. J. 213 (2025) 113623, https://doi.org/10.1016/j.microc.2025.113623.
[59] K. Kunpatee, K. Kalcher, O. Chailapakul, S. Chaiyo, A. Samphao, A paper chromatographic-based electrochemical analytical device for the separation and simultaneous detection of carbofuran and carbaryl pesticides, Sens. Actuators B: Chem. 377 (2023) 133116, https://doi.org/10.1016/j.snb.2022.133116.
[60] T. Pholsiri, A. Lomae, K. Pungjunun, S. Vimolmangkang, W. Siangproh, O. Chailapakul, A chromatographic paper-based electrochemical device to determine Δ^9-tetrahydrocannabinol and cannabidiol in cannabis oil, Sens. Actuators B: Chem. 355 (2022) 131353, https://doi.org/10.1016/j.snb.2021.131353.
[61] F. Silveri, A. Scroccarello, F. Della Pelle, M. Del Carlo, D. Compagnone, Rapid pretreatment-free evaluation of antioxidant capacity in extra virgin olive oil using a laser-nanodecorated electrochemical lab-on-strip, Food Chem 420 (2023) 136112, https://doi.org/10.1016/j.foodchem.2023.136112.
[62] P. Kusonpan, K. Kunpatee, O. Chailapakul, K. Kalcher, A. Ortner, S. Chaiyo, A. Samphao, A simple manually rotated paper-based analytical device with electrochemical sensors for the determination of nitrite and nitrate, Talanta 292 (2025) 127919, https://doi.org/10.1016/j.talanta.2025.127919.
[63] B. Ninwong, N. Ratnarathorn, C.S. Henry, C.R. Mace, W. Dungchai, Dual sample preconcentration for simultaneous quantification of metal ions using electrochemical and colorimetric assays, ACS Sens 5 (2020) 3999–4008, https://doi.org/10.1021/acssensors.0c01793.
[64] E. Costa-Rama, H.P.A. Nouws, C. Delerue-Matos, M.C. Blanco-López, M. T. Fernández-Abedul, Preconcentration and sensitive determination of the anti-inflammatory drug diclofenac on a paper-based electroanalytical platform, Anal. Chim. Acta 1074 (2019) 89–97, https://doi.org/10.1016/j.aca.2019.05.016.
[65] O. Amor-Gutiérrez, E. Costa-Rama, M.T. Fernández-Abedul, Fully integrated sampler and dilutor in an electrochemical paper-based device for glucose sensing, Microchim. Acta 188 (2021) 302, https://doi.org/10.1007/s00604-021-04946-3.
[66] L. Fiore, A. Antinucci, G. Leotta, L. Fabiani, A. Iannini, P. Galloni, R. De Santis, A. Ciammaruconi, G. Grilli, E. Recchia, F. Lista, F. Arduini, An ecodesigned reagent-free paper-based electrochemical sensor modified with carbon black for the detection of essential oils, Green Anal. Chem. 12 (2025) 100217, https://doi.org/10.1016/j.greeac.2025.100217.
[67] J. Mettakoonpitak, N. Sawatdichai, D. Thepnuan, A. Siripinyanond, C.S. Henry, S. Chantara, Microfluidic paper-based analytical devices for simultaneous detection of oxidative potential and copper in aerosol samples, Microchim. Acta 190 (2023) 241, https://doi.org/10.1007/s00604-023-05819-7.
[68] Y. Li, H. Chen, R. Huang, D. Deng, X. Yan, L. Luo, An origami microfluidic paper device based on core-shell Cu@Cu2S@N-doped carbon hollow nanocubes, Anal. Chim. Acta 1316 (2024) 342828, https://doi.org/10.1016/j.aca.2024.342828.
[69] N. Suk-in, K. Thongpim, W. Phamonpon, J. Yukird, S. Ummartyotin, N. Rodthongkum, Dual colorimetric/electrochemical sensor of carbaryl in fruits on microfluidic paper-based analytical device connected with smartphone readout, J. Food Compos. Anal. 133 (2024) 106445, https://doi.org/10.1016/j.jfca.2024.106445.
[70] V. Ong, M.A. Mohamed, H. Ma, A. Al-Shami, S. Khazaee Nejad, F. Amirghasemi, A. Tabassum, M.J. Lee, A. Rohleder, C. Zhu, C. Tam, P. Nowlen, M.P.S. Mousavi, Bilisense: an affordable sensor for on-site diagnosis of jaundice and prevention of kernicterus, Biosens. Bioelectron. 280 (2025) 117386, https://doi.org/10.1016/j.bios.2025.117386.
[71] M. Zhang, Y. Lu, Q. Lan, Y. Fang, T. Ma, S. He, B. Liu, X. Liang, Paper-based electrochemical approach for highly rapid and sensitive quantification of inorganic phosphate in freshwater, ACS EST Water 5 (2025) 1745–1754, https://doi.org/10.1021/acsestwater.4c01111.
[72] N. Seddaoui, C. Di Gregorio, L. Gullo, E. Argiriadis, F. Arduini, A paper-based screen-printed electrochemical sensor combined with a 3D printed extracting cartridge for analysis of phosphorus in Antarctic lacustrine sediments, Talanta 289 (2025) 127749, https://doi.org/10.1016/j.talanta.2025.127749.
[73] R. Zeitoun, V. Adamchuk, A. Biswas, A novel paper-based reagentless dual functional soil test to instantly detect phosphate infield, Sensors 22 (2022) 8803, https://doi.org/10.3390/s22228803.
[74] S. Dortez, A.G. Crevillen, A. Escarpa, S. Cinti, Electroanalytical paper-based device for reliable detection and quantification of sugars in milk, Sens. Actuators B: Chem. 398 (2024) 134704, https://doi.org/10.1016/j.snb.2023.134704.
[75] W. Wang, S. Ding, Z. Wang, Q. Lv, Q. Zhang, Electrochemical paper-based microfluidic device for on-line isolation of proteins and direct detection of lead in urine, Biosens. Bioelectron. 187 (2021) 113310, https://doi.org/10.1016/j.bios.2021.113310.
[76] L. Fiore, V. Mazzaracchio, A. Serani, G. Fabiani, L. Fabiani, G. Volpe, D. Moscone, G.M. Bianco, C. Occhiuzzi, G. Marrocco, F. Arduini, Microfluidic paper-based wearable electrochemical biosensor for reliable cortisol detection in sweat, Sens. Actuators B: Chem. 379 (2023) 133258, https://doi.org/10.1016/j.snb.2022.133258.
[77] V. Caratelli, A. Ciampaglia, J. Guiducci, G. Sancesario, D. Moscone, F. Arduini, Precision medicine in Alzheimer's disease: an origami paper-based electrochemical device for cholinesterase inhibitors, Biosens. Bioelectron. 165 (2020) 112411, https://doi.org/10.1016/j.bios.2020.112411.

[78] A. Yakoh, E. Mehmeti, K. Kalcher, S. Chaiyo, Hand-operated, paper-based rotational vertical-flow immunosensor for the impedimetric detection of α-fetoprotein, Anal. Chem. 94 (2022) 5893–5900, https://doi.org/10.1021/acs.analchem.2c00079.

[79] G. Moro, F. Fama, N. Colozza, A. Gambaro, M. Bassanello, F. Arduini, C. Zanardi, A paper-based device for glyphosate electrochemical detection in human urine: a case study to demonstrate how the properties of the paper can solve analytical issues, Green Anal. Chem. 7 (2023) 100076, https://doi.org/10.1016/j.greeac.2023.100076.

[80] S. Fiori, A. Scroccarello, F. Della Pelle, M. Del Carlo, D. Compagnone, Integrated paper/graphene 3D pop-up device for the quantitative sensing of carbaryl, Sens. Actuators B: Chem. 399 (2024) 134768, https://doi.org/10.1016/j.snb.2023.134768.

[81] V. Caratelli, G. Fegatelli, D. Moscone, F. Arduini, A paper-based electrochemical device for the detection of pesticides in aerosol phase inspired by nature: a flower-like origami biosensor for precision agriculture, Biosens. Bioelectron. 205 (2022) 114119, https://doi.org/10.1016/j.bios.2022.114119.

[82] A.W. Martinez, S.T. Phillips, G.M. Whitesides, E. Carrilho, Diagnostics for the developing world: microfluidic paper-based Analytical devices, Anal. Chem. 82 (2010) 3–10, https://doi.org/10.1021/ac9013989.

[83] K.J. Land, D.I. Boeras, X.-S. Chen, A.R. Ramsay, R.W. Peeling, REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes, Nat. Microbiol. 4 (2018) 46–54, https://doi.org/10.1038/s41564-018-0295-3.