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Paper-Based Electrochemical (Bio)Sensors for the Detection of Target Analytes in Liquid, Aerosol, and Solid Samples

Noemi Colozza,[1,2,*] Vincenzo Mazzaracchio,[1,*] and Fabiana Arduini[1,2]

[1]Department of Chemical Science and Technologies, University of Rome Tor Vergata, Rome, Italy; email: fabiana.arduini@uniroma2.it

[2]Sense4Med S.R.L., Rome, Italy

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*These authors contributed equally to this article

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Abstract

The last decade has been incredibly fruitful in proving the multifunctionality of paper for delivering innovative electrochemical (bio)sensors. The paper material exhibits unprecedented versatility to deal with complex liquid matrices and facilitate analytical detection in aerosol and solid phases. Such remarkable capabilities are feasible by exploiting the intrinsic features of paper, including porosity, capillary forces, and its easy modification, which allow for the fine designing of a paper device. In this review, we shed light on the most relevant paper-based electrochemical (bio)sensors published in the literature so far to identify the smart functional roles that paper can play to bridge the gap between academic research and real-world applications in the biomedical, environmental, agrifood, and security fields. Our analysis aims to highlight how paper's multifarious properties can be artfully harnessed for breaking the boundaries of the most classical applications of electrochemical (bio)sensors.

1. INTRODUCTION

Sustainability is a broad concept in our society that has recently permeated different fields, from economics, to technologies, and to the environment and social sciences. The modern concept of sustainability in analytical chemistry was introduced by acquiring the vision of green chemistry, as presented, for example, in 1998 (1) and 1999 (2) by Anastas and colleagues, and by achieving the idea of green analytical chemistry, as recently rationalized in 2013 by Gałuszka et al. (3), and white analytical chemistry reported by Nowak et al. in 2021 (4). The sustainability vision in analytical chemistry relies on the use of eco-designed material, low-energy devices, reduced use of chemicals, and onsite analyses, all features present in electrochemical paper-based devices. Indeed, since the first publication of the Henry group in 2009 (5), all of these relevant features are the reasons that electrochemical paper-based devices have attracted growing interest, which increases every year in both the academic and industrial sectors. From the academic point of view, the first publication on a paper-based electrochemical device has received significant attention (5), while from the industrial point of view, significant attention is attested by the growing global paper diagnostic market, with a compound annual growth rate of 8.1% across the 2021–2031 forecast period (6). Another relevant application of paper-based devices based on low-cost diagnostic devices is highlighted in the 2010 publication by the Whitesides group entitled "Diagnostics for the developing world: microfluidic paper-based analytical devices" (7). Indeed, this type of device is a close fit with the criteria of ASSURED established by the World Health Organization. This describes an ideal diagnostic test to be used in resource-limited settings, where ASSURED means affordable, sensitive, specific, user-friendly, rapid, equipment-free, deliverable to end users. This definition was recently updated to REASSURED, highlighting the real time connectivity and ease of specimen collection (8).

Since 2020, several reviews have focused on different approaches for the construction of paper-based devices, with applications mainly in the environmental and biomedical fields, and with different configurations such as lateral flow, vertical flow, and origami (9–23). Herein, we report how the features of electrochemical paper-based devices have been used to detect various target analytes in different samples ranging from blood, serum, urine, tears, and sweat to aerosol phase and solid phase (concrete) structures.

2. LIQUID PHASE

For liquid samples, paper offers a wide range of possibilities by combining its multifarious properties and configurations, including lateral and vertical flow microfluidics, reagent preloading, and origami-like configuration, to detect target analytes in biofluids, environmental water, and agri-food liquid extracts. Among the wide range of paper-based electrochemical (bio)sensors developed for the detection of several analytes in fluids, we mainly focus on studies where the paper was used as the active material.

2.1. Biofluids

The latest trend of developing point-of-care analytical systems is leading the way for the fabrication of sensors to perform noninvasive and miniaturized analyses. As a result, point-of-care sensors reduce costs and time of hospitalization, providing real-time information about the patient's health. Depending on the biological sample type, decentralized health monitoring at home can be carried out, providing an alternative approach to laboratory-based analyses.

2.1.1. Serum and blood. A serum sample requires a laboratory setup treatment due to the centrifugation step. As a consequence, this matrix is not suitable for point-of-care analyses. Nevertheless, serum is an established sample type by clinicians, thanks to extensive studies on the

multiple biomarkers correlated with several diseases (24). Thus, it is largely used in the development of electrochemical paper-based devices for biomedical applications. Because of the liquid nature of the serum, several electrochemical paper-based devices are focused on harnessing filtering properties combined with equipment-free microfluidics, owing to the capillarity properties of the porous paper.

Owing to the porous cellulose network structure of filter and chromographic paper, Wei et al. (25) fabricated a two-layered platform to introduce the samples from a wax-patterned inlet and detect the prostate-specific antigen biomarker. As the sample is introduced through the inlet, lateral and vertical flows drive the target analyte to the DNA capture–modified electrode for final electrochemical detection. By exploiting the same flow concept, it is possible to conduct multiple detection of the same analyte (e.g., 17β-estradiol) (26) or different target analytes by designing wax-patterned channels for the capillary-driven flow to the different screenprinted working electrodes. This was demonstrated in the cases of hepatitis B and C viruses (27), carcinoembryonic antigen and neuron-specific enolase (28), and carcinoma antigen 125 and carcinoembryonic antigen (29).

The porosity of paper serves the additional function of loading the reagents for delivering a reagent-free device. By simply adding the reagents needed for the measurement and waiting for the volatilization of the solvent, the paper-based device is ready for the measurement: The addition of the liquid sample solubilizes the reagent, allowing for smart analyses. The reagents loaded also encompass, for example, acid for pH adjustment (30) or enzymatic substrate (31).

Decreasing the time-consuming multiple steps of immunological assays without losing sensitivity and specificity is an ambitious goal. Paper can potentially help in this regard by fabricating semiautonomous analytical devices owing to its easy foldability, reagent storing capacity, and wax-patterned channels.

Shi and coworkers (32) presented an origami-like platform for the detection of interleukin-6, a potential biomarker of COVID-19 progression. Exploiting paper foldability, the authors overlayered an initial wax-patterned layer comprising a reaction and a detection chamber on a working electrode layer and a final reference and counter electrode layer. While the reaction chamber was used to perform the magnetic bead–based immunological assay, the detection chamber was used to insert the enzymatic substrate for the amperometric revealing of the target analyte. The possibility to customize paper substrates by creating different shapes and sizes was accomplished by Yakoh et al. (33) to fabricate a noteworthy rotational and vertical flow immunosensor for α-fetoprotein detection (**Figure 1*a***). The main goal of the developed platform is to remove interference from the convective component of fluid motion. To perform the immunoassay, users can freely transfer, switch, and stop fluid flows needed for the assay by manually rotating the paper disk. As a result, the authors were able to enhance the analytical performances of the sensor compared to the nonrotating approach. Qi et al. (34) reported a fascinating device for molecularly imprinted polymer in situ synthesis and analyte quantification. The highly structured paper platform comprises five functional folding parts, including (*a*) a working electrode, (*b*) one circular counter or reference electrode for synthesis, (*c*) one circular counter or reference electrode for analyte detection, (*d*) a movable valve to direct the sample to the detecting zone, and (*e*) a washing channel. To confirm the applicability of the developed platform, the proposed method was applied to the detection of carcinoembryonic antigen as a model target for clinical diagnostic analysis in serum.

To develop a semiautonomous analytical system, the Chailapakul group (35) used the wax patterning of nitrocellulose paper to develop an intriguing automated, paper-based lateral flow electrochemical device for hepatitis B DNA detection (**Figure 1*b***). Wax barriers and straight channels were used to drive an Au probe and the DNA target to the peptide nucleic acid (PNA)-capturing agent situated in the detection zone. The mathematical investigation allowed

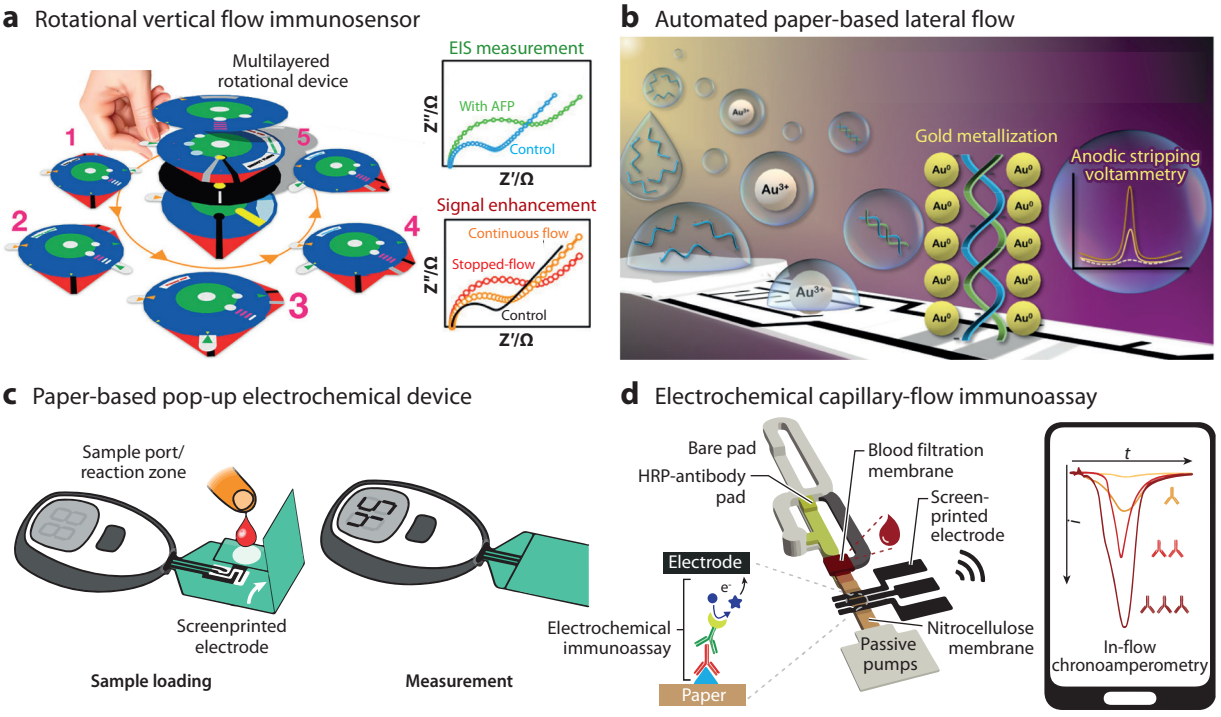


Figure 1

(*a*) Paper-based rotational vertical flow immunosensor for the impedimetric detection of AFP. Numbers in pink refer to the analysis steps: (Step 1) sample and buffer loading, (Step 2) sample injection, (Step 3) washing, (Step 4) $[Fe(CN)_6]^{3-/4-}$ probe injection, and (Step 5) EIS measurement. Panel *a* adapted with permission from Reference 33; copyright 2022 American Chemical Society. (*b*) Automated paper-based lateral flow electrochemical device for one-step detection of the hepatitis B virus. Using a printed delayed/non-delayed configuration, sequential reagent delivery is automatically enabled after the sample introduction. Once Au^{3+} ions bind the captured target DNA, the complex is electroreduced to form Au^0-metallized DNA, and anodic stripping voltammetry assay is carried out to determine the hepatitis B virus DNA concentration via the gold signal. Panel *b* adapted with permission from Reference 35; copyright 2021 American Chemical Society. (*c*) A paper-based pop-up electrochemical device for analysis of β-hydroxybutyrate in capillary blood samples. The entire sensing tool is composed by a commercially available glucometer and single sheet of paper folded into a three-dimensional device. This pop-up paper structure comprises a screenprinted sensor and sample port/reaction zone. It can change shape and fluidic and electrical connectivity by simply folding and unfolding the structure. Panel *c* adapted with permission from Reference 45; copyright 2016 American Chemical Society. (*d*) Electrochemical capillary-flow immunoassay for the detection of anti-SARS-CoV-2 nucleocapsid protein. Detection is based on the sequential delivery of sample and reagents to the nitrocellulose membrane that is modified with a recombinant SARS-CoV-2 nucleocapsid protein. Anti-N antibodies present in the sample are then captured on the nitrocellulose membrane and detected via chronoamperometry done on a screenprinted carbon electrode. Panel *d* adapted with permission from Reference 47; copyright 2021 American Chemical Society. Abbreviations: AFP, α-fetoprotein; EIS, electrochemical impedance spectroscopy; HRP, horseradish peroxidase.

the authors to create slowing barriers to allow the Au probe to arrive at the detection zone once the DNA hybridized the PNA-capturing agent. The subsequent stripping voltammetry applied to detect the Au bond to the DNA–PNA complex allows for the quantification of the target DNA in <7 min, with high specificity and low background current.

Owing to the porosity of its cellulose network, paper can be used as an effective tool to synthesize nanoparticles. Following this concept, Li et al. (36) used paper to grow gold nanoparticles (AuNPs) and develop an immunological assay for prostate-specific antigen detection. Specifically, a folding platform was designed to be composed of two parts. The first sample pad was used to (*a*) print the working electrode, (*b*) grow the AuNPs, and (*c*) build the immunological chain.

A second pad was used to print the counter and reference electrodes and to add the glucose substrate once the two pads were folded for final detection.

Li & Liu (37) developed an origami-like device comprising a first layer with counter and reference electrodes and a second working electrode layer. ZnO nanowires were hydrothermally grown on the carbon working electrode paper layer for the immunological chain construction to detect immunodeficiency virus p24 antigen.

As a multiple-property substrate, paper also plays an active and direct role in the electrochemical cell. Indeed, with the aim of directly applying paper as a working electrode substrate, three interesting approaches were used to modify paper to enhance its electronic properties. The filter paper was modified by (*a*) drop-casting multiple nanocomponents, namely Ag nanowires, MoS_2, and AuNPs, to generate a conductive and high surface area (38) for the detection of microRNA targets; (*b*) dip coating with nanostructured Fe_2O_3-poly(3,4-ethylene dioxythiophene) polystyrene sulfonate (PEDOT:PSS) and dimethyl sulfoxide (DMSO) for chronoamperometric detection of carcinoembryonic antigen (39); and (*c*) using ZnO nanorods grown on paper after graphene oxide reduction to detect three target analytes, namely human chorionic gonadotropin, prostate-specific antigen, and carcinoembryonic antigen (40).

In contrast to serum samples, blood is an appealing matrix to develop point-of-care devices owing to the possibility of its use without laboratory-based pretreatments. Nevertheless, the main issue related to the analysis of biomarkers in blood is undoubtedly the complexity of the matrix, forcing the final user to perform several preanalytical separative procedures. Paper is a valid solution to resolve this, mainly acting as a filter for large-dimension particles, thus preventing direct contact of the whole sample with the surface of the electrochemical working electrode (41, 42). In addition, by flowing through the porous paper network, a filter process always occurs, making this procedure highly appealing for blood sample analyses. For instance, Labroo & Cui (43) designed four-channel microfluidic pathways to deliver multiplexed detection of different metabolites (glucose, lactate, xanthine, and cholesterol) by using four different biosensors integrated into the same paper substrate. To confer a smarter design, the origami approach was used to exploit the vertical flow and folding capability of the paper-based devices. For instance, Li et al. (44) designed a sandwich-type origami configuration to store glucose oxidase enzyme needed for the analysis in a small sample amount and detected the target analyte in untreated blood samples. The Whitesides group (45) developed a foldable pop-up device to detect β-hydroxybutyrate, i.e., a biomarker for diabetic ketoacidosis, by loading 3-hydroxybutyrate dehydrogenase enzyme and its cofactor in the same electrode substrate, but in a different position from the electrodes (**Figure 1*c***).

To perform a superior constituent separation, commercial membranes were applied to separate plasma from the hematocrit by developing combined multilayer paper-based devices based on vertical flow configuration. Sun et al. (46) used a plasma separation membrane as a first layer of a multilayer system, and Samper et al. (47) merged the fluidic capability of nitrocellulose paper with a paper absorption pad and a blood separation membrane to create a multilayered immunoassay (**Figure 1*d***). Caratelli et al. (48) adopted a three-paper layer configuration comprising a first plasma separation membrane for whole-blood filtration, a second filter paper to preload the substrate of the enzymatic reaction, and a third office paper for a screenprinted electrochemical cell.

Besides functioning as a simple physical loading of the reagents, paper can also be directly modified to exploit the functional group present in the cellulose. Following this approach, the Henry group (49) exploited the anchoring properties of paper for the chemical immobilization of pyrrolidinyl peptide nucleic acid (acpcPNA) to carry out the label-free detection of *Mycobacterium tuberculosis*, while Cinti et al. (50) modified paper with the chemical synthesis of Prussian blue nanoparticles for the detection of glucose by using loaded glucose oxidase.

2.1.2. Urine, tears, saliva, sweat, and nasopharyngeal liquids as samples for noninvasive analyses. Despite containing lower amounts of fat, proteins, and big-shaped particles compared to blood and serum biofluid, the ménage of urine samples is more challenging than expected. This can probably be ascribed to the presence of several biological waste elements produced by catabolism processes. For this reason, the filtering property of paper is essential to deliver a reliable analytical tool. Nevertheless, a filtering or dilution pretreatment may still be needed. For instance, filtration with paper was used in the case of serotonin (51) or with membrane filters followed by acidification with concentrated HCl in the case of lead ion detection (52).

Without the need for any filtering or dilution pretreatment, Fatibello-Filho and coworkers (53) fabricated a multiplex electrochemical platform based on multichannel paper microfluidics. As proof of concept, the array of 16 electrodes was used to quantify glucose in urine samples. Because the solution is loaded in the center of the wax-patterned channels, the capillary force allows the fluid to reach the multiple detection zones, radially distributed around the injection area, for the multianalyses of the target analyte.

For the similar reason of distributing the sample into different detection zones, the Fatibello-Filho research group proposed a filter paper pad sandwiched between two polyester layers (54). The lower layer was used for counter and reference electrode printing and the top layer for the working electrode. The device configuration enables a researcher to sample and flow the sample for the codetection of uric acid and creatinine in urine samples.

Saliva, tears, and sweat have attracted research attention in the last decade owing to the easy and noninvasive sampling of these fluids. However, due to their less-complex matrices, configuration of the paper-based sensing device is not focused on the matrix treatment itself, but rather on solving the sampling issue, such as in the case of sweat for the development of wearable (bio)sensors.

In the case of tears, Fiore et al. (55) used paper as a valid platform to store reagents, i.e., glucose oxidase for the detection of glucose in tears samples (**Figure 2*a***). The paper was inserted into a plastic device, working as a new concept of a paper card-like device for the reagent-free and easy amperometric detection of the target analyte.

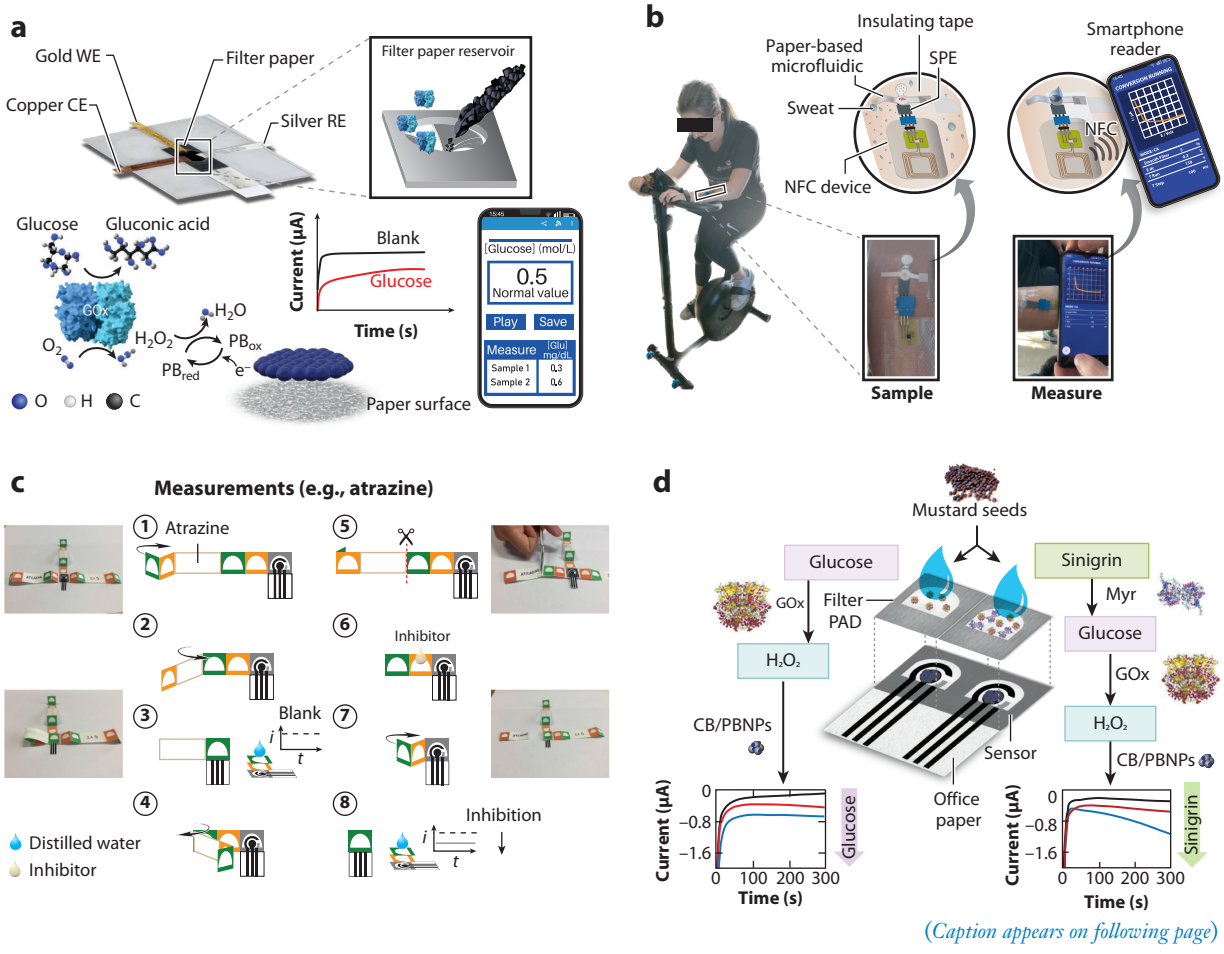
For saliva, Huang and coworkers (56) used paper to electrically connect the electrode system consisting of an indium tin oxide (ITO) working electrode, an Ag/AgCl wire reference electrode, and a Pt wire counter electrode for the electrochemical detection of uric acid in untreated saliva. Bhardwaj et al. (57) developed an immunological assay using a vertical flow paper-based impedimetric immunosensor by selecting different typologies of papers for delivering (*a*) a different pore size sample pad to filter and flow the sample, (*b*) a conjugation pad to immobilize the reagents, (*c*) a nitrocellulose pad where the electrochemical cell was printed, and (*d*) an absorption pad for H1N1 virus detection in untreated saliva samples.

The use of sweat as a matrix has expanded the application of electrochemical wearable devices (58). Among these devices, paper-based devices play an important role in sweat sample management because of the intrinsic features of paper such as flexibility and foldability, which perfectly fit wearability requirements. In particular, the capillary effect of paper-based devices could solve the collection of sweat and help the fluidic flow to the detection area, which typically is a plastic-based printed electrode. In this regard, Cao et al. (59) developed a vertical flow system for the potentiometric monitoring of sodium and potassium ions, integrating the paper-based microfluidics and ion-selective screenprinted electrodes connected to a smartwatch potentiometric device for data acquisition and visualization. Five paper layers were folded, encapsulating the screenprinted electrode in the central zone and allowing the collection (lower layer) and evaporation (top layer) of sweat from the two external layers of the platform. Exploiting the same vertical flow configuration for the sampling and evaporation process, Cheng and coworkers (60) simultaneously detect multiple parameters by integrating paper with a colorimetric and electrochemical system. A combined

sampling and evaporation paper pad platform was adopted by Li et al. (61) for the monitoring of glucose and lactate during physical exercise. As a sampler layer between the epidermal surface and the Kapton screenprinted electrode, Mazzaracchio et al. (62) used a cotton pad to absorb and collect the produced sweat during physical activity for pH monitoring.

Instead of using a vertical flow system, Fiore et al. (63) moved to a lateral flow approach. They designed a microfluidic platform to perform a competitive immunoassay for cortisol detection, integrating the system with polyester screenprinted electrodes and a near-field communication wireless module (**Figure 2*b***). Here, the cellulose network not only enabled the sweat collection and flow to the reaction zone, but it was also used to load the reagents to perform the various immunoassay steps in a semiautonomous way.

2.1.3. Nasopharyngeal liquids. The extensive spread of COVID-19 has increased the use of the nasopharyngeal matrix as an important infectious biomarker-containing sample. The application of paper to analyze this matrix was mainly focused on developing semiautonomous electrochemical platforms. Jaewjaroenwattana et al. (64) exploited the foldability of paper to perform the immunological steps to detect the receptor-binding domain spike protein of SARS-CoV-2.



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

(*a*) Paper card–like electrochemical platform as a smart point-of-care device for reagent-free glucose measurement in tears. Configuration of the sensing device integrates a polyvinyl chloride support, where a paper layer loaded with reagents is inserted, and an electrochemical cell comprising a gold WE, copper CE, and silver RE. Final detection of glucose in tears is carried out by chronoamperometric measurement of H_2O_2 as by-product of the GOx-based reaction and exploitation of the PB mediator loaded onto the filter paper surface. Panel *a* adapted with permission from Reference 55; copyright 2023 Royal Society of Chemistry. (*b*) Microfluidic paper-based wearable electrochemical biosensor for cortisol detection during stationary cycling. The paper-based microfluidic is exploited to sample the sweat, manage the flow, and load the reagents needed for cortisol detection. The chronoamperometric measurement is then made by a smartphone reader connected by NFC technology to the wearable device. Panel *b* adapted with permission from Reference 63; copyright 2023 Elsevier. (*c*) Origami multiple paper–based electrochemical biosensors for multiple pesticide detection. The origami is composed of three strips, each with two pairs of different pads; the red pads contain the enzymes and green pads the substrates separated by an empty piece of paper. In the middle, two electrochemical sensors printed on office paper allow for pesticide measurement after the origami has been cut and folded. One sensor is modified with a dispersion of CB/PBNPs to detect paraoxon as an inhibitor of butyrylcholinesterase enzyme, while the second electrode is modified with a dispersion of CB to detect atrazine and 2,4 dichlorophenoxyacetic acid as inhibitors of alkaline phosphatase and tyrosinase, respectively. As shown here, atrazine pesticide is measured with the following procedure: ① the distal pairs of pads are overlapped; ② the overlapped pads are folded on the sensor; ③ distilled water is added to run amperometric analysis of the enzymatic response (by the reaction of the tyrosinase enzyme with catechol substrate at correct pH and with buffer salts also loaded on paper) in the absence of inhibitor (blank signal); ④ pads are removed; ⑤ the strip is cut; ⑥ inhibitor (atrazine) is added on the red pad close to the sensor; ⑦ the pair of pads close to the sensor are overlapped on the sensor; and ⑧ distilled water is added to run amperometric analysis after enzymatic inhibition. Panel *c* adapted with permission from Reference 83; copyright 2019 Elsevier. (*d*) Origami paper-based electrochemical biosensing platform for quality control of agrifood waste. The origami is composed of two electrochemical sensors, printed on office paper, and modified with a dispersion of CB/PBNPs to detect H_2O_2 as a by-product of the reaction of GOx enzyme and glucose. Both sensors are overlapped with a pad; one contains only GOx enzyme for glucose detection and the other both GOx and Myr enzymes for sinigrin detection. After overlapping the pads on the sensors, glucose solution is dropped on the first pad while a sinigrin solution is dropped on the second pad, generating enzymatic reactions. Exemplary amperograms for the responses of both sensors are shown, with arrows indicating that the amperometric signals increase with increasing concentrations of glucose and sinigrin (from *black* to *blue curves*). Panel *d* adapted from Reference 93 (CC BY 4.0). Abbreviations: CB, carbon black; CE, counter electrode; GOx, glucose oxidase; Myr, myrosinase; NFC, near-field communication; PAD, paper analytical device; PB, Prussian blue; PBNP, PB nanoparticle; RE, reference electrode; SPE, screenprinted electrode; WE, working electrode.

A different paper function was exploited by Henry and coworkers (65) to quantify target complementary DNA by chemically modifying the paper cellulose network with a pyrrolidinyl PNA to detect the DNA COVID-19 sequence. The subsequent hybridization hindered the accessibility of the detection probe to the electrode, resulting in a decreased electrochemical response correlating to SARS-CoV-2 concentration.

2.1.4. Multiple biofluids. Several studies considered the use of fabricated paper-based electrochemical analytical devices to detect biomarkers in multiple biological matrices. Considering the different complexity of samples and depending on the biological fluid, most studies considered a pretreatment procedure (e.g., a dilution or filtering process) to effectively detect the biomarker.

Krishnakumar et al. (66) detected lidocaine in blood and serum samples by using the filter capacity of paper as a barrier to restrict the flow of complex protein in the fourfold diluted sample to efficiently detect the target analyte. A different approach was carried out by Amatatongchai et al. (67) to detect 3-nitrotyrosine and 4-nitroquinolin-*N*-oxide in serum, urine, and blood samples. A foldable configured platform was developed to detect the two carcinogen oxidative stress biomarkers in serum samples of cancer-affected patients in urine and blood. Although for serum and urine a previous filtration with nylon membrane and a dilution were performed, a Whatman LF1 filter paper was employed without pretreatment for blood analyses.

The fabrication of reagent-free analytical tools is always an appealing goal, but it is almost mandatory when wearable sensors must be developed. Parrilla et al. (68) utilized the loading

capability of Whatman grade 1 filter paper to effectively store an acid solution needed for analysis and to facilitate the sampling procedure for the detection of phenylalanine in saliva and serum samples using a wearable device.

Merging the reagent loading and electrode printing in the same substrate is a good strategy to deliver a reagent-free sensor device. For instance, Cinti et al. (69) developed a reagent-free sensor for chloride ion detection in serum and sweat, exploiting filter paper porosity to load HCl acid solution in the same printed electrochemical cell area. Bagheri et al. (70) utilized the porosity of filter paper to synthesize nanoparticles and load acid solution needed for copper detection in serum and urine samples.

As reported by Cao et al. (71), in addition to physical loading, chemical modification was selected to functionalize paper with biocomponents, i.e., enzymes, which actively modified the cellulose network with aldehydic function to chemically bind glucose oxidase for glucose detection in blood and sweat. Han et al. (72) used an unconventional lens-cleaning tissue paper substrate to chemically immobilize uricase with glutaraldehyde and bovine serum albumin cross-linkers for uric acid detection in saliva and serum samples.

2.2. Environmental Water

Environmental waters have been extensively analyzed by means of paper-based electrochemical (bio)sensors to monitor pollutants and water quality. In this field, paper can advantageously contribute to facilitating the analysis of real matrices with low detection limits by such means as filtration and preconcentration properties.

Indeed, preconcentration can enable trace element detection in water samples. A convenient strategy was conceived by the Dungchai group (73) for the simultaneous determination of heavy metals, e.g., Pb(II) and Cd(II), in bottled drinking water, tap water, pond water, and wastewater. They designed a heating preconcentration system by loading the water sample on a paper circular strip to be heated on a resistive heater. This procedure allowed the sample to evaporate, thus concentrating the metals and increasing the signal up to 29-fold. Another example was illustrated by Costa-Rama et al. (74), who applied drop-casting/evaporation cycles of the sample to preconcentrate and detect traces of anti-inflammatory drugs (e.g., diclofenac) in tap water.

Sensitivity can be an important issue when monitoring environmental waters because many substances must be detected at very low concentrations. An interesting approach to handle this relies on activating the capillarity of the paper using an alcohol pretreatment of the cellulose. This approach was presented by Shimizu et al. (75), who demonstrated the principle by applying a small volume (0.5 μL) of isopropanol on a pyrolyzed graphitic paper-based electrode, observing an improvement of the capillarity. This process allowed for hindrance-free redox reactions in the resulting active areas. These conditions resulted in fast penetration of the water samples, which were tested for the detection of a bulky analyte (phosphomolybdate complex for phosphate detection) in both lake and bottled drinking water, with a 99-fold improved detection limit over the nonalcohol-triggered device.

The preloading of chemical reagents in the cellulose network has also been used for water analysis, delivering ready-to-use (bio)sensors for the detection of phosphates, bisphenol A, and nerve agents (76–78). For instance, Bui et al. (79) preloaded metal nanoparticles to catalyze the reduction of nitrate and Hg(II), improving the sensitivity of the paper-based analytical tool. The paper's porosity is also suitable for covalent immobilization, e.g., of antibodies. With this approach, paper-based electrochemical immunoassays were developed to detect emerging pollutants such as ethinyl estradiol and microcystin-LR toxin (80, 81) and herbicides (e.g., atrazine and acetochlor) (82) in different water bodies.

The combination of reagent preloading and origami-like folding of the paper matrix has been explored as another way to design smart devices for surface water analysis based on the relevant features of the paper. Arduini et al. (83) developed a paper-based device composed of three foldable strips of wax-patterned filter paper, in which the enzymes and their corresponding substrates were preloaded separately to react only after controlled sequential steps for the detection of paraoxon, 2,4-dichlorophenoxyacetic acid and atrazine in river water (**Figure 2*c***). In this case, by folding, cutting, and adding the samples, the authors developed a lab-on-a-chip paper device to detect multiclass pesticides.

2.3. Extracts from Agrifood Samples

Food monitoring can be conducted through extracting substances that give information about food quality, the presence of toxins, or other relevant parameters. In this way, the extracted liquid samples can be analyzed by electrochemical sensing in the liquid phase. Also in this case, paper can be used to facilitate the sensor application owing to smart designs (e.g., pump-free sampling of the solution) or by preloading the needed reagents in the cellulose network. In particular, the reagent preloading can be used to adjust the conditions after the extraction process directly in the paper device (e.g., buffer solutions at the working pH or ionic strength), delivering ready-to-use samples.

Jiang et al. (84) designed a single-layer filter paper biosensor with a central area where the sample solution was dropped and driven by capillarity forces toward two opposite detection areas for dual simultaneous detection of the Ara h1 antigen allergen using two printed sensors functionalized with the selected aptamer. The paper microfluidics has also been combined with a three-dimensional (3D)-printed device by Silva et al. (85). They designed a 3D-printed mold in which a layer of filter paper provided the microfluidic channel to drive the sample from the inlet area through the electrochemical cell for detection of carbendazim pesticide in honey, wine, and commercial drinks diluted in Britton-Robinson buffer.

The capillary forces of paper can efficiently work as pumping forces that can be customized to improve the flow rate within the system. This concept was explored by the Henry group (86) in their paper-based flow injection analysis for caffeic acid analysis by using an inlet reservoir placed upstream of the system and filled with buffer as a carrier solution. This provided constant flow through the paper channels and was combined with a second reservoir constituted by a 270° fan-shaped paper layer with a surface area large enough to efficiently pump the carrier solution. In this configuration, 2 μL of the sample were dropped next to the inlet reservoir and carried by the flow through the electrochemical cell (composed of thermoplastic electrodes), where the detection of caffeic acid took place.

Among the versatile aspects of paper, capillarity can be valuable not only in lateral flow configurations but also in vertical flow, as reported by Prasertying et al. (87) for the electrochemical detection of SO_2 gas in situ generated in wine samples. Khamcharoen et al. (88) assessed L-cysteine in wheat flour, bread, and cake, whereas Sun et al. (89) quantified glycoprotein ovalbumin in diluted egg white samples.

As already discussed, a critical feature of paper is its suitability as a support for the immobilization/loading of biomolecules. Indeed, porous papers offer a large surface area that can increase the loading capacity. Several examples were reported in the agrifood sector, including aptamer immobilization with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide (EDC/NHS) for capturing gliadin protein in cereals (90) or the preloading of glucose oxidase together with $[Fe(CN)_6]^{3-/4-}$ electrochemical mediator for glucose detection in honey and drink samples upon dilution (91).

A further improvement in enzymatic biosensor was reported by Amor-Gutiérrez et al. (92). The authors used glass fiber strips to adsorb the sample and mix it with the buffer solution through capillarity, obtaining the desired dilution of the sample and the optimum pH/ionic strength conditions for the measurement of glucose in cola beverages and orange juice. Additionally, Colozza et al. (93) developed a bienzymatic sensing platform using glucose oxidase and myrosinase for the dual simultaneous analysis of glucosinolates and glucose in *Brassicaceae* plants as diagnostic substances for monitoring good nutritional value of agrifood waste (**Figure 2*d***).

3. GAS AND AEROSOL PHASES

Monitoring airborne substances is fundamental for tracing environmental pollution and threats to living beings. However, analysis in the gas or aerosol phases is one of many analytical challenges in the electrochemical sensor field. Among these challenges that require special attention are anthropogenic substances coming from industrial processes, urban emissions, the agrifood sector, and security conflicts. Continuous and real-time monitoring of the gas/aerosol phase is typically required in all types of applications, from air quality monitoring to biomarker detection in exhaled breath. One issue stems from the collection of representative gas/aerosol samples, which are highly dependent on the time of sampling, volume of the surrounding environment, presence of any perturbations (e.g., air streams), and application of passive or active sampling approaches. In this context, porous papers can provide outstanding advantages based on their adsorption capability of the sample. However, one critical issue involves the lack of ionic conductivity of air and, in general, of aerosol samples, which leads to biased electrochemical measurements. In addition, the cellulose itself does not present enough ionic conductivity. Fortunately, several researchers have found smart solutions to these issues, delivering highly efficient paper-based electrochemical (bio)sensors to monitor analytes transported by air or aerosol in different applications.

A good approach consists of utilizing the porosity of the paper to entrap an electrolyte solution in the cellulose matrix by drop-casting a few microliters and letting the paper dry. When the paper device is used for the desired detection, the electrolyte salts can be redissolved to create ionic conditions suitable for the electrochemical analysis. A breakthrough example of this approach was presented by the Dincer group (94). In their work, the porosity of a chromatography paper (Whatman grade 1) allowed them first to load the electrolyte (phosphate buffer) to ensure the ionic conductivity and then to adsorb the target sample, namely exhaled breath, to start measuring the vapor matrix (**Figure 3*a***). Considering the importance of real-time monitoring of respiratory diseases, the authors developed a wearable electrochemical device to monitor exhaled H_2O_2 in artificial breath based on electrochemical paper-based sensors.

Fiore et al. (95) also employed this strategy to monitor aerosolized H_2O_2 used as a disinfectant. Indeed, air disinfection has recently become a priority concern, especially after the COVID-19 pandemic. Therefore, Fiore et al. used filter paper to develop a sensor for continuous monitoring of H_2O_2 in the aerosol phase from the nebulizer to customize the disinfection process (**Figure 3*b***).

Park et al. (96) demonstrated another approach to provide electrolyte conditions to filter paper for electrochemical measurements. They used a gel electrolyte placed under a filter paper sensor to provide sufficient ionic mobility within the cellulose substrate during the measurement. In this case, the electrolyte solution was gradually adsorbed owing to the capillarity forces of porous hydrophilic paper. Specifically, they developed a paper-based peptide sensor for label-free detection of airborne *Bacillus subtilis* spores, namely a nonpathogenic *Bacillus anthracis* simulant.

Paper's porosity can also be utilized to entrap the reagents needed for analysis. Colozza et al. (97) provided several examples of how to design an enzyme inhibition assay within a paper device by combining the reagent preloading and filter paper adsorbent properties to perform aerosol

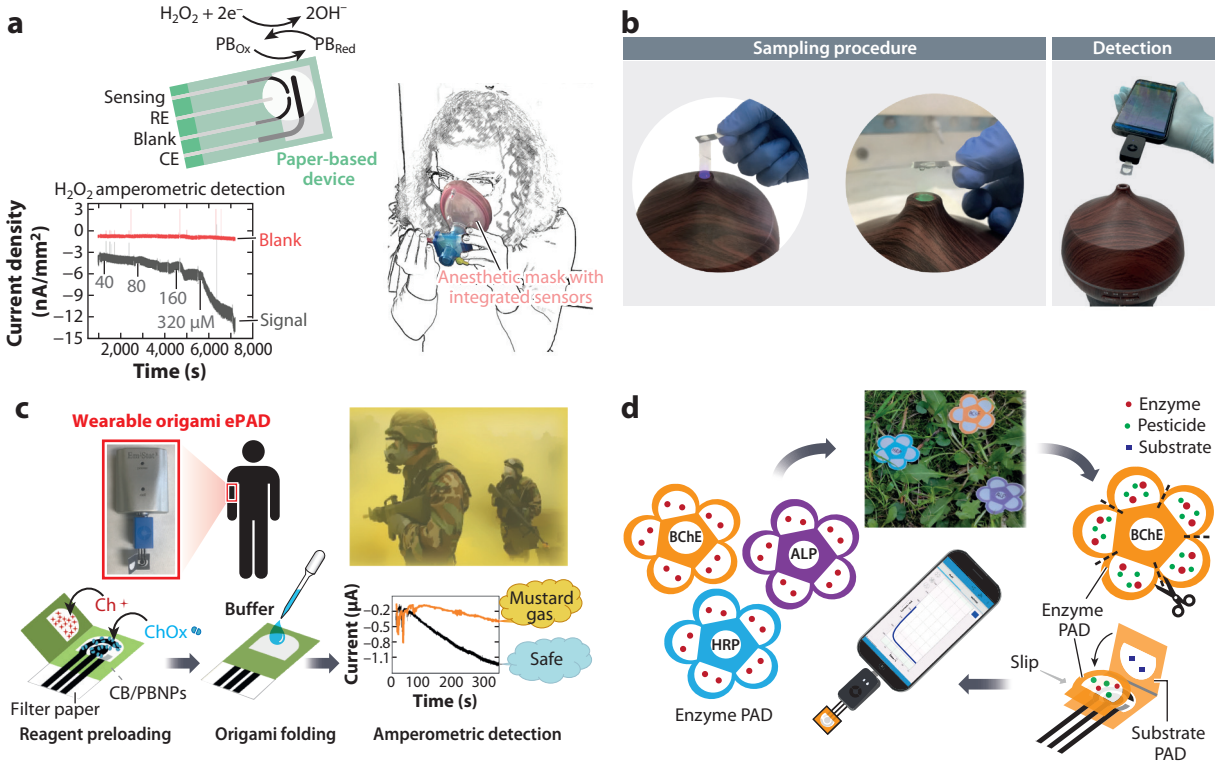


Figure 3

(*a*) A paper-based wearable sensor for real-time H_2O_2 measurement in a simulated breath. The paper-based sensor is placed inside a wearable respiratory mask. The exhaled H_2O_2 in artificial breath is monitored using a PB-mediated carbon electrode (signal) while a CB electrode records the background signal (blank). Panel *a* adapted from Reference 94 (CC BY 4.0). (*b*) A paper-based electrochemical sensor for H_2O_2 detection in aerosol phase. The sensor samples the aerosol containing H_2O_2 by direct exposure to the aerosol. The measurement is done using a miniaturized potentiostat connected to a smartphone. Panel *b* adapted with permission from Reference 95; copyright 2021 Elsevier. (*c*) A wearable origami-like ePAD for sulfur mustard detection. The origami is composed of an electrochemical sensor, printed on filter paper, and a pad that can be overlapped by folding. Ch substrate is preloaded on the pad while ChOx enzyme is preloaded on the hydrophilic area of the electrochemical cell. The working electrode is modified with a dispersion of CB/PBNPs to detect H_2O_2 enzymatic by-product. After overlapping, buffer solution is added, and the enzymatic reaction is monitored by amperometry. In the presence of mustard agents, the enzyme is inhibited and amperometric signal is decreased (*orange line*). Panel *c* adapted with permission from Reference 97; copyright 2019 Elsevier. (*d*) A paper-based flower-like origami biosensor for electrochemical detection of pesticides in the aerosol phase. Each flower-like paper device is preloaded with the corresponding enzyme and exposed to the ground where pesticides can be accumulated. The device is then cut, and one pad is placed on the electrochemical sensor printed on office paper; the pad containing the substrate is then folded onto the enzyme pad. Amperometric analysis is done inserting the sensor into a miniaturized potentiostat connected to a smartphone. Panel *d* adapted with permission from Reference 98; copyright 2022 Elsevier. Abbreviations: ALP, alkaline phosphatase; BChE, butyrylcholinesterase; CB, carbon black; Ch, choline; ChOx, choline oxidase; CE, counter electrode; ePAD, electrochemical paper-based analytical device; HRP, horseradish peroxidase; PB, Prussian blue; PBNP, PB nanoparticle; RE, reference electrode.

analysis. For instance, Colozza et al. presented a simple inhibition enzyme sensor based on a single-layer, foldable, paper-based device for the early detection of mustard gas, which is the most feared chemical weapon typically used as an aerosol spread into the air. This device was conceived as a miniaturized wearable analytical tool to be integrated, e.g., into military uniforms, to provide a prompt alarm of airborne mustard agents nearby (**Figure 3*c***). In this case, the porosity of filter paper was used for a dual purpose: (*a*) to separately preload reagents such as choline oxidase enzyme

and choline substrate into the cellulose matrix and (*b*) to adsorb the aerosolized mustard agents for real-time detection. After optimization in standard solutions, the resulting device could reveal sulfur mustard with a limit of detection of 19 mg/m^3 after 60 s of exposure.

Caratelli et al. (98) presented another example of a paper-based enzyme inhibition assay for monitoring pesticide residue after its nebulization over crop fields (**Figure 3*d***). A flower-like sampling device was designed with multiple petal-like pads, and each one was preloaded with an enzyme that was selectively inhibited by a specific pesticide. These devices can be left on the ground of crop fields to expose the enzyme to pesticides. Subsequently, one petal is cut and sandwiched between a filter pad, then preloaded with the enzymatic substrate and an office paper sensor to monitor the enzymatic reaction by chronoamperometry. This versatile approach was tested for the detection of paraoxon, 2,4-dichlorophenoxyacetic acid, and glyphosate pesticides at parts-per-billion levels using butyrylcholinesterase, alkaline phosphatase, and peroxidase enzyme.

The preloading of solutions within the cellulose matrix of porous papers has also been applied to preconditioning reagents (e.g., buffer solutions). In this case, the paper device can be designed to drive the sample through microfluidic channels in which it contacts preconditioning agents for a sensitive electrochemical measurement.

Taking advantage of this simple strategy, Mettakoonpitak et al. (99, 100) designed paper-based devices based on anodic/cathodic stripping voltammetry to achieve multiple detection of polluting species, including Cd(II), Pb(II), Cu(II), Fe(II), and Ni(II), from aerosolized particulate matter. The authors detected metals in particulate matter by first collecting aerosolized particulate matter on sampling filters followed by treatment through microwave-assisted acid digestion to liberate the metal ions from particulate matter (99). Those steps were followed by preconditioning the treated samples on paper driven by capillary forces through paper channels (100). Acid-resistant noncellulosic materials (Teflon and quartz fibers) were used for the sampling, followed by the microwave-assisted acid digestion treatment. Digested samples were then dropped on the filter paper, where the preconditioning agents (buffer solutions) established the correct pH conditions for detection of particulate matter.

4. SOLID PHASE

As previously discussed, the detection of target analytes in solid samples is typically approached through the extraction process or digestion of the solid sample. Direct analysis on a solid surface remains a challenge in analytical chemistry. Regarding electrochemical sensors, the main obstacle is the need for ionic conductivity in the electrochemical cell for a correct measurement. However, the ionic mobility in a solid material is generally not sufficient to enable electrochemical analysis.

Once again, paper offers solutions to these limitations. Indeed, paper cellulose can be used as a porous material capable of retaining an electrolyte solution and letting it permeate into a solid porous surface at the same time. Thus, ions are allowed to move between the cellulose material and solid substrate, establishing an electrochemical contact suitable for carrying out the analysis.

Colozza et al. (101–103) were the first to explore how to take advantage of paper porosity to conduct direct electrochemical measurements on a solid surface, namely reinforced concrete. Owing to its cost-effectiveness and resistance to mechanical strain, reinforced concrete is today the most common material used in the building sector but is also used to create artworks. However, reinforced concrete constructions are subjected to deterioration over time due to corrosion phenomena that are catalyzed mainly by water infiltration, pH decrease, and exposure to chloride-rich environments. Importantly, concrete is a porous solid material rich in ions that favor ionic conductivity. In light of this, our group developed paper-based potentiometric devices capable of exploiting the characteristics of concrete by means of porous filter paper.

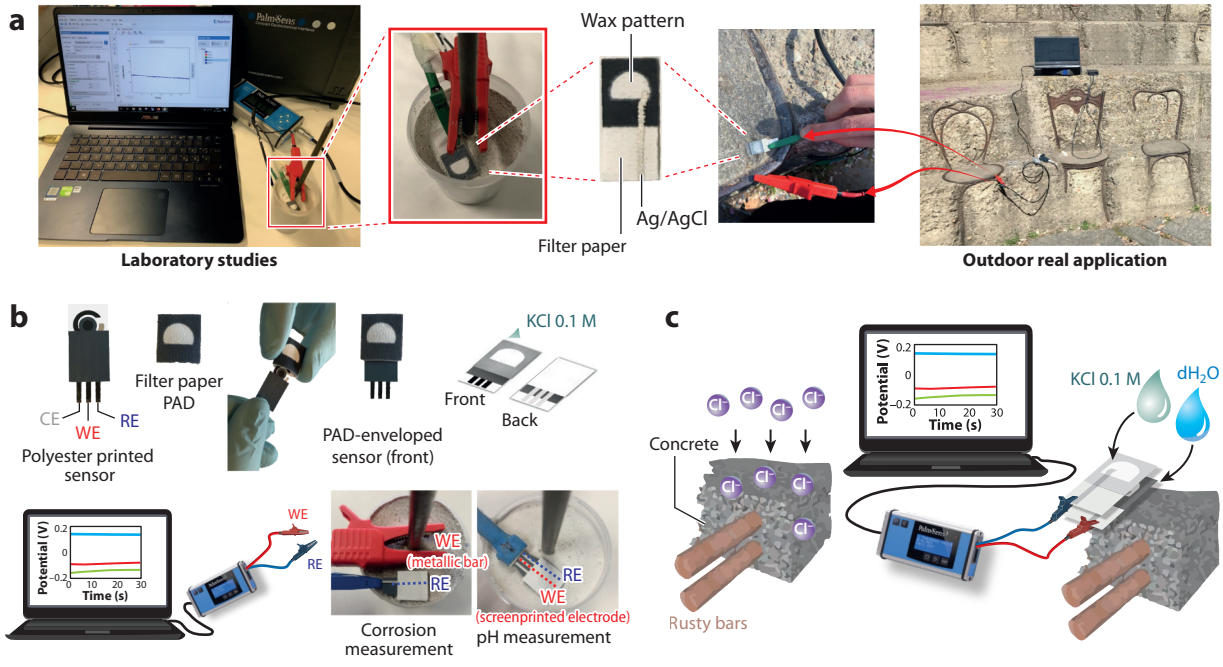


Figure 4

(*a*) A paper-based potentiometric sensor (*middle*) for corrosion valuation of reinforcements embedded in concrete structures. (*Left*) Experimental setup used for laboratory-prepared sample of reinforced concrete, where the paper-based sensor is placed on the concrete surface as an RE while the metallic bar of the sample is clamped to be used as a WE. (*Right*) Application of the experimental setup to a real concrete artwork. Corrosion is evaluated by measuring the potential difference between the WEs and REs. Panel *a* adapted with permission from Reference 101; copyright 2020 John Wiley & Sons. (*b*) Multiparametric analysis by paper-assisted potentiometric sensors for diagnostics and monitoring of reinforced concrete structures. Use of the PAD envelope allows for adsorbing the KCl solution and potentiometric measurement by directly placing the front side of the sensor onto the concrete surface. Corrosion evaluation is made using the sensor as an RE while the metallic bar of the sample is clamped to be used as a WE; the potential difference between them is then measured. pH measurement is done by measuring the potential difference between the RE and WE printed on the sensor, where the WE is modified with iridium oxide. Panel *b* adapted with permission from Reference 102; copyright 2021 Elsevier. (*c*) Vertical flow paper sensor for onsite evaluation of chloride contamination in concrete structures. The presence of chloride ions in concrete, which accelerates the corrosion of the metallic (rusty) bars, is monitored by a potentiometric paper-based device composed of three layers of paper. An Ag/AgCl electrode is preloaded with KCl solution and used as the RE. Another Ag/AgCl electrode is preloaded with distilled water and used as the WE. A paper pad is used to put the top and bottom layers in contact. The potential difference measured between the electrodes is correlated to the presence of chloride ions in the concrete matrix. Panel *c* adapted with permission from Reference 103; copyright 2021 American Chemical Society. Abbreviations: CE, counter electrode; PAD, paper analytical device; RE, reference electrode; WE, working electrode.

Colozza et al. (101) demonstrated the possibility of monitoring the probability of corrosion in reinforced concrete using a filter paper Ag/AgCl reference electrode to measure differences in the electrochemical potential between the metallic reinforcement (used as the working electrode) and the concrete itself. The paper sensor was placed on the concrete surface near the metallic reinforcement, and 70 μL of KCl electrolyte were drop-casted on the electrochemical cell (**Figure 4*a***). The adsorbent nature of the paper allows for retaining the electrolyte solution while it permeates into the concrete material, ensuring ionic conductivity. This method successfully distinguished among different levels of corrosion in laboratory-made concrete samples according to the expected correlation (i.e., the more negative potentials, the higher the probability of corrosion).

Later, Colozza et al. (102) combined the measurement of corrosion probability with the measurement of concrete pH as another diagnostic parameter for reinforced concrete degradation. Considering that fresh-prepared concrete has a pH of 12–13, a decrease of pH below 8 is associated with a high risk of corrosion. pH is commonly tested with a colorimetric assessment with phenolphthalein or by destructive methods. In this study, Colozza et al. used a pH-sensitive IrOx film electroplated on the working electrode to reveal pH variation through potentiometric analysis. The authors used paper as an envelope of a three-electrode, cell-based plastic that was screenprinted to ensure contact between the electrochemical system and concrete surface (**Figure 4*b***). This sensor enabled the dual monitoring of corrosion probability and of the concrete pH, yielding results consistent with the colorimetric assay.

In a final study, Colozza et al. (103) further improved the capability to monitor reinforced concrete degradation by designing a novel paper-based device for the measurement of excess chloride ions permeated in the concrete from the environment (e.g., near the sea). In this case, three layers of filter paper were overlapped in a vertical design composed of two layers with Ag/AgCl screenprinted electrodes and a junction filter paper pad placed in between (**Figure 4*c***). By loading different chloride concentrations on the two layers of Ag/AgCl-printed paper, their diffusion through the junction pad created a potential difference that could be easily measured. Thus, keeping constant the chloride concentration on the top, the bottom layer of this sandwich-like device was placed on the concrete surface to monitor its chloride concentration.

It is noteworthy that, to bridge the gap between the laboratory bench and the real world, Colozza et al. (101–103) tested the paper-based sensors characterized by a portable and easy-to-use analytical setup and fast response (i.e., 30 s). These were tested on reinforced concrete artworks (e.g., the Music Collection Session by Arman in Milan, Italy) and buildings (e.g., Giacomo Manzù Museum, Ardea, Italy), proving the practical suitability of paper-based devices for real applications.

Recently, Martins et al. (104) applied a similar approach to solid samples to monitor pesticides on the skin of fruits and vegetables. To further highlight the importance of paper porosity, the authors investigated two kinds of paper: kraft and parchment. They found that the higher porosity of kraft paper led to minor resistance to charge transfer and larger surface areas. The kraft paper was thus used as the substrate for screenprinted electrodes to be directly placed on the skin of apples and cabbages. In particular, Martins et al. demonstrated the nondestructive, onsite monitoring of carbendazim, which is a broad-spectrum fungicide of the carbamate group with toxic carcinogenic potential. The study revealed carbendazim concentrations at the micromolar level and close to 100% recoveries.

5. CONCLUSIONS

In this review, we described the configurations and applications of paper-based electrochemical devices for quantifying analytes in different types of samples. These include applications in the fields of biomedicine, the agrifood industry, the environment, and security and samples in different physical states such as liquids, aerosols, and solids. We have highlighted how the use of paper in the design of electrochemical devices confers additional features including (*a*) improved sensitivity by adding and drying of the sample, (*b*) reagent-free detection by loading reagent in the porous structure of the paper, and (*c*) the capability for multianalysis with origami designs, breaking down the limitation of commercially available plastic or alumina printed electrochemical sensors. Indeed, for the detection of target analytes in the gas phase or solid phase using sensors printed on conventional supports, sample treatment or an additional sampling system is required, whereas electrochemical paper-based devices can carry out analysis in the liquid, solid, and gas phases autonomously.

Furthermore, we highlighted how the versatility of paper presents several challenges in need of improved analytical tools. These would allow researchers to detect target analytes in the complex matrix without a laboratory setup treatment method, conduct multianalysis using the same device, and develop smart devices with the required analytical features.

Supplemental Material >

In this regard, in our **Supplemental Materials**, we report the analytical features of paper-based electrochemical devices for analyte measurement in biological fluids (**Supplemental Table 1**), environmental waters (**Supplemental Table 2**), agrifood samples (**Supplemental Table 3**), the aerosol phase (**Supplemental Table 4**), and the solid phase (**Supplemental Table 5**). These resources demonstrate the effectiveness and reliability of these devices when used for quantitative and accurate analyses.

Finally, the greenness of paper-based electrochemical devices is especially valuable because by incinerating each device after each measurement, we do not contribute to global waste. We conclude that paper-based electrochemical devices represent a superior solution for analytical chemists to detect the target analyte in a sustainable way.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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