



Paper-based analytical devices for point-of-need applications

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Received: 10 October 2022 / Accepted: 28 March 2023 / Published online: 12 April 2023
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Abstract

Paper-based analytical devices (PADs) are powerful platforms for point-of-need testing since they are inexpensive devices fabricated in different shapes and miniaturized sizes, ensuring better portability. Additionally, the readout and detection systems can be accomplished with portable devices, allying with the features of both systems. These devices have been introduced as promising analytical platforms to meet critical demands involving rapid, reliable, and simple testing. They have been applied to monitor species related to environmental, health, and food issues. Herein, an outline of chronological events involving PADs is first reported. This work also introduces insights into fundamental parameters to engineer new analytical platforms, including the paper type and device operation. The discussions involve the main analytical techniques used as detection systems, such as colorimetry, fluorescence, and electrochemistry. It also showed recent advances involving PADs, especially combining optical and electrochemical detection into a single device. Dual/combined detection systems can overcome individual barriers of the analytical techniques, making possible simultaneous determinations, or enhancing the devices' sensitivity and/or selectivity. In addition, this review reports on distance-based detection, which is also considered a trend in analytical chemistry. Distance-based detection offers instrument-free analyses and avoids user interpretation errors, which are outstanding features for analyses at the point of need, especially for resource-limited regions. Finally, this review provides a critical overview of the practical specifications of the recent analytical platforms involving PADs, demonstrating their challenges. Therefore, this work can be a highly useful reference for new research and innovation.

Keywords Paper-based analytical devices · Colorimetry · Fluorescence · Electrochemistry · Distance-based detection · Dual/combined detectors

Introduction

The number of rapid tests for medical diagnosis has increased in the last years as a response to the global outbreak of coronavirus. The COVID-19 pandemic has intensified the development of millions of point-of-care tests. These tests have been globally used every day in hospitals, workplaces, airports, and people's homes. Despite the availability of tests for health systems and communities, the fabrication cost is always a deep concern for society, specifically for

underdeveloped countries. Additionally, their disposal can have unforeseen impacts on human health and the environment. Even though the devices are normally incinerated to reduce contamination risk, the commercial platforms are commonly fossil-derived unsustainable polymeric materials, which can generate toxic pollutants when improperly incinerated, causing detrimental effects on environmental and human health. As a result, Ongaro et al. [1] review ways to design a sustainable future for point-of-care diagnostics and single-use microfluidic devices. Different materials are reported for this purpose, including recycled plastics, such as polymethyl methacrylate (PMMA), bio-derived and biodegradable plastics such as shellac, zein, and polylactic acid (PLA), and natural fibrous materials (paper, cotton, and wood). The paper substrates have become an attractive substrate among natural fibrous.

A search on the "Web of Science" database using the keywords "paper-based device" shows a significant number of

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works exploring paper as an analytical platform from 2010 (Fig. 1). The publication's number achieved a maximum value after 2019, a landmark with the COVID-19 pandemic. Even though most previous works focused on techniques to fabricate the devices and looked for ways of coupling them with different detection systems, the new ones have extensively demonstrated the knowledge acquired over the years for analytical purposes.

PADs have received rapid development over the last decade (Fig. 1). They have been the focus of several reviews due to their advantages in comparison to conventional chips, normally made of glass and polymer platforms. The features include simpler fabrication, lower cost, and biological compatibility. Moreover, the paper has capillarity properties, dispensing the need for external pumps [2]. Xia et al. [3] published a review presenting different manufacturing techniques for microfluidic devices, employing two-dimensional (2D) and three-dimensional (3D) methods. The authors also highlighted the various types of applications for these devices, their advantages and disadvantages, and future trends for developments in this field. In 2020 Paixão et al. [4] published a review that discusses 10 years of development in paper-based devices focusing on electrochemical detection. The review presents that these devices have an enormous production variety, with different types of paper and ways of creating barriers. Moreover, these sensors can be applied to out-of-bench detection, performing analysis in-field detection. These devices have an appreciable form of detection, consisting of the main ones discussed in this review, colorimetric [5], fluorometric [6], electrochemical [7], distance-based [8], and dual readout [9]. They can also be integrated with other techniques, such as surface-enhanced Raman spectroscopy (SERS) [4], mass spectroscopy [10], and

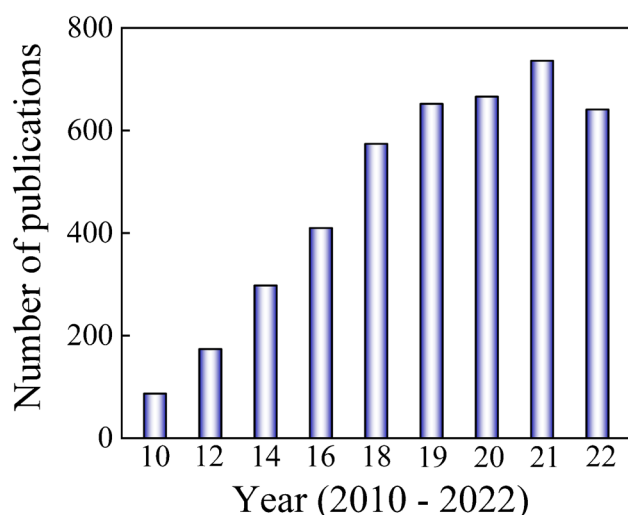


Fig. 1 Publications' number over the years obtained from the "Web of Science" database using the keywords "paper-based device"

Fourier-transform infrared spectroscopy (FTIR) [11]. For these devices, there are some examples in the market for the end user. However, they are often colorimetric detection.

Most recent advances in the fabrication methods of paper-based devices have received growing attention in the field of bioanalysis. To evaluate the state-of-the-art development of paper-based biosensors, Silva-Neto et al. [11] published a review that discusses important information about manufacturing methods, integrative sensing materials, the clinical relevance of biomarkers, and bioanalysis. Salentijn et al. [12] demonstrated how paper features could contribute to (bio)analysis in another review. Thus, while previous major reviews have focused on different manufacturing techniques and methods, this review aims to present the advances observed in the last 10 years for paper-based analytical devices, highlighting the relationship between the paper type and its analytical application. Furthermore, we carefully discuss the main detection systems, including the latest dual/combined and distance-based detection systems. In this way, we provide insights into PADs to serve as a reference for further research.

History of paper-based analytical devices

Most articles until 2022 show more laboratory solutions than point-of-need applications. However, since 2007, many applications using paper-based analytical devices have changed the focus of searching for new materials for sensing applications. They added a new look to an old material with a well-known chemical structure, the paper. Paper was used as an alternative material to transmit information from generation to generation, replacing animal skin, stone, wood, and papyrus. Some characteristics behind this change are due to some important properties of the paper, like lighting, porosity, price, and availability. The same features we look for in PADs [12, 13].

The analytical applicability of paper substrates is not new. However, it is not easy to precisely date the start. The first report came from the years 23 [14], where iron was detected in papyrus based on a colorimetric reaction. It opened the possibilities for pH detection using litmus paper in the seventeenth century [15] and free chlorine detection using iodide starch reaction in 1814 [16], followed by sugar dark-brown color detection based on tin(II) chloride reaction in 1850 [17]. In 1917, Feigl and Stern proposed a new color detection [18], culminating in the paper spot-test reaction. This article was the basis for many qualitative detections, leading to a landmark in analytical chemistry. After this period, Martin and Synge used paper as the substrate for chromatography separation, where the fibrous structure of the paper was essential to interact with the analytes resulting in a different time of elution [19]. This work opened the

possibility of using paper for electrophoresis separation by Durrum in 1950 [20], and for vital out-of-lab measurements using immunochromatographic side flow, which is currently used in the pregnancy test and other biosensing approaches for disease detection [21]. From 1982 to 2007, some authors attempted to highlight the potentiality of the paper. However, the way to look for the piece of paper only changed in 2007, making the paper old-new analytical platforms. This change was based on the Whitesides approach creating hydrophobic barriers on paper with photoresist [22]. In 2009, a clever and cheap approach [23] was proposed to fabricate the devices using wax-printing technology. Figure 2 summarizes the paper and its application in analytical chemistry.

Influence of paper type

The essential primary material for paper is cellulose, a natural polymer obtained mainly from wood and cotton. Based on the necessity of paper purity, the origin of the cellulose could vary, whereas cotton has purer cellulose than that came from wood. Instead of cellulose, the raw material has other compounds, such as hemicelluloses, lignin, and wood extracts. Cellulose is 40–50% of wood’s chemical composition, hemicelluloses represent 15–25%, lignin 10–30%, and extracts 0.5–5% [24]. During the paper manufacture, lignin is removed in the pulping process,

releasing the fibers and eliminating impurities related to discoloration and possible future paper breakdown. Depending on the application, paper substrates containing high lignin content, such as card boxes, have been used in the literature [7]. The polymeric structure and the attached groups are also essential for paper modification, especially biosensing applications. Consequently, the paper’s properties must match the specific applications, which will be discussed in this section.

Paper types typically differ in composition, pore size, surface area, porosity, and thickness. Thus, the paper provides a different feature in the PADs, since absorbing, flow controlling, filtering, reagent adsorption, and wicking [25]. PADs are often produced with chromatography and filter papers, considered traditional platforms [26, 27]. This fact is associated with the high percentage of alpha cellulose, the most stable form of cellulose, leading to a smooth surface and uniformity in this substrate. It is important to emphasize that the main difference between quantitative and qualitative papers is non-volatile substance content (called ash), which varies from less than 0.01% to 0.06% for quantitative and qualitative papers, respectively. The lower the ash content, the greater the purity of the paper. The chromatography paper is manufactured with pure cellulose obtained from the highest quality cotton without strengthening or whitening agents [28]. Table 1 summarizes the properties of common papers used to fabricate PADs.

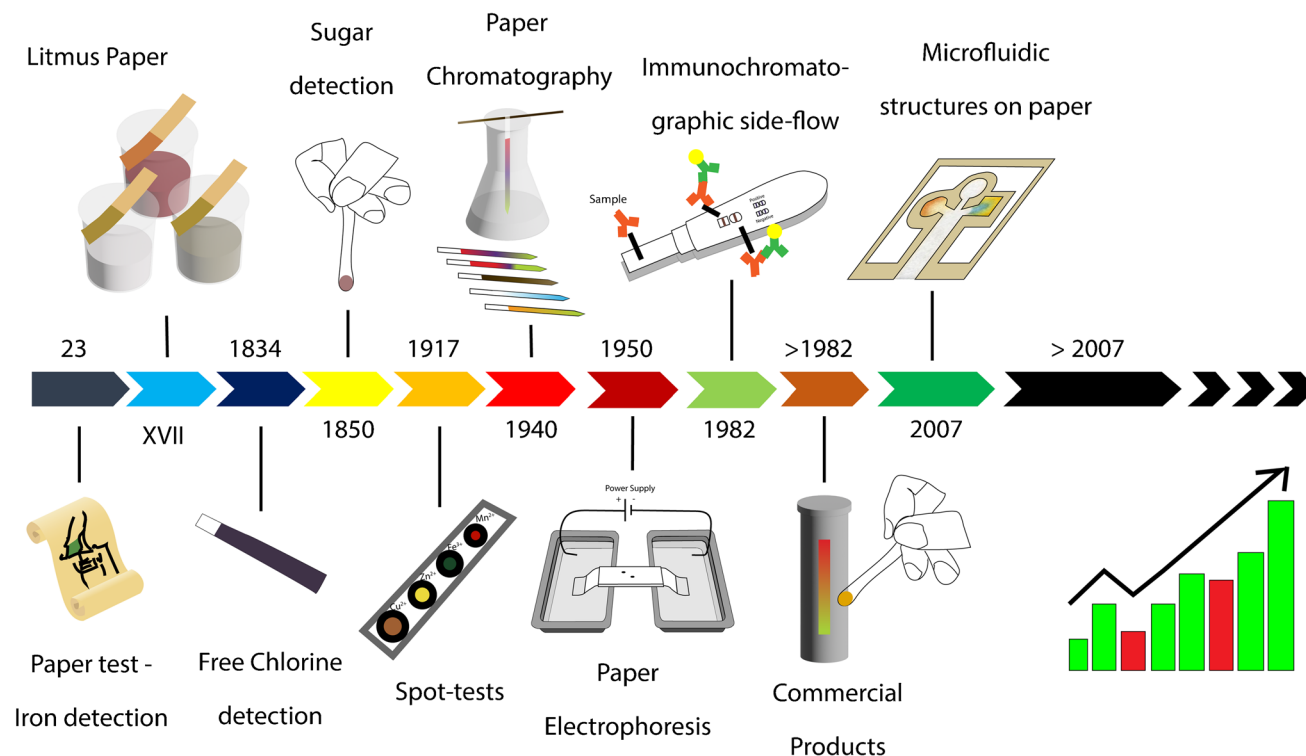


Fig. 2 Timeline involving paper substrates for analytical applications

Table 1 Properties of common papers applied for PADs fabrication [28]

Paper type	Thickness (μm)	Pore size (μm)	Nominal ash (%)
Quantitative filter paper Grade C42	200	1–2	0.07
Quantitative filter paper C40	200	4–7	0.07
Quantitative filter paper Grade 589/1	190	12–25	0.01
Quantitative filter paper Grade 41	215	20	0.07
Qualitative filter paper Grade 1	180	11	0.06
Qualitative filter paper Grade 3	390	6	0.06
Qualitative filter paper Grade 4	210	25	0.06
Qualitative filter paper Grade 5	200	2.5	0.06
Chromatography Cellulose Grade 3	360	NS*	NC**
Chromatography Cellulose Grade 1	180	NS*	NC**

NS not specified, NC no content

Paper characteristics have a significant influence on the analytical performance of electrochemical [7, 29–31], fluorescence [32], and colorimetric devices [33]. Pradela et al. [31] demonstrated the fabrication of paper-based electrodes with graphite ink. Filter paper and office paper were evaluated as substrates for the sensors. An improvement in electrical conductivity and, consequently, in the analytical response was observed for the filter paper. This behavior was attributed to the larger pore size of this substrate and its more uniform distribution, increasing the ink percentage immobilized onto the filter paper. Dias et al. [34] evaluated the influence of five paper types to fabricate electrodes, including filter paper, vegetal paper, office paper, photo paper, and chromatography paper. The vegetal paper provided the highest electrical conductivity and electrochemical performance for forensic applications. This fact was associated with the roughness of the vegetal paper, allowing higher graphite deposition. Ninwong et al. [32] proposed the first fluorescent distance-based device to determine trace mercury ions (Hg^{2+}) in water. Whatman N° 1 filter paper, anion exchange filter paper, cation exchange filter paper, and silica gel filter paper were evaluated to determine the best surface to deposit nitrogen-doped carbon dots (NCDs). The authors observed that the positive charges of diethylaminoethyl cellulose on the anion exchange filter paper could interact with the negative charge of the functional groups of the NCDs, facilitating their immobilization. Consequently, this paper was selected as a substrate, and the resulting device was applied for mercury quantification in water samples. Despite enhancing the analytical performance, the paper selection is not always studied or detailed during fabrication.

The paper choice is also an essential parameter for paper-based microfluidic devices once it can affect the flow rate, impacting the analytical performances of the devices [35]. A paper-based microfluidic device coupled with electrochemical detection showed higher analytical signals using Whatman filter paper N° 4 (25 μm) than the Whatman filter paper N° 1 (11 μm). This behavior was associated with an

increased flow rate by increasing the substrate's pore size, improving the analyte's mass transport to the electrode surface [36]. However, higher flow rates are not always advantageous depending on the application since the reagents previously deposited in the reaction zone can be easily washed out during the experiments, affecting the sensitivity and repeatability of the measurements [37]. Boehle et al. [38] showed that the Whatman filter paper N° 1, which has smaller pores relative to Whatman 4, improved enzyme-based colorimetric detection performance. Pradela et al. [39] demonstrated the possibility of using office paper as a substrate to fabricate paper-based microfluidic devices. However, care must be taken in choosing this substrate since additives may interfere with the measurements [40].

Although filter and chromatography paper are often used for PADs fabrication, other cellulose-based substrates can also be applied. Hunt et al. [41] reported a substantial improvement in the analytical signal by comparing cellulose-based paper and non-cellulose paper to produce colorimetric paper-based biosensors. The proposed biosensor was assessed for SARS-CoV-2 RNA determination in human saliva samples. In addition, combining the different papers has been applied to improve PAD performance by integrating multiple steps in a single device [42].

The paper modification has also been evaluated during device fabrication. The modification typically provides a variety of functional groups to the paper substrate, improving the uniformity of color development (e.g., colorimetric detection) and the stability of immobilized molecules [43]. Most approaches to modify paper substrates are based on electrostatic forces, hydrogen bonding, stacking, and/or van der Waals forces as well as some reversible non-covalent interactions [44]. Evans et al. [45] report the silica nanoparticles modified with 3-aminopropyltriethoxysilane incorporated on a Whatman grade 1 filter paper which facilitates the adsorption of selected enzymes and prevents the washing away effect that creates color gradients in the colorimetric measurements. Filter paper substrates chemically modified

by oxidation allow the covalent coupling of enzymes on the cellulose surface, providing an enhanced colorimetric measurement [46]. Gabriel et al. [47] propose a microfluidic paper-based device (μ PAD) modified with chitosan for colorimetric detection of glucose and uric acid (UA) in biological fluids. The modification incorporates chitosan on the paper surface, resulting in better solid support to adsorb enzymes, ensuring a more effective reactive area in the whole detection zone.

Considering the works discussed in this section, we propose a guide (Fig. 3) containing ways to select the paper type according to application. Figure 3 shows that papers with different characteristics have been used to produce analytical devices, demonstrating that a unique paper does not fit all applications. Therefore, evaluating the paper is significantly important to understand how this parameter can influence the analytical response of the device.

Detection and readout system for point-of-need applications

The need to obtain rapid results for disease diagnosis, real-time environmental and health monitoring creates the demand for point-of-need (PON) analytical devices that can be used remotely outside the hospital or laboratory environments. They are especially desired in locations with a lack of infrastructure and shortages of trained personnel. The development of point-of-care (POC) tests, in particular, must follow the ASSURED criteria set by the World Health Organization (WHO), that is, being affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users [48]. In this context, PADs can be an excellent alternative to these purposes. Paper is an

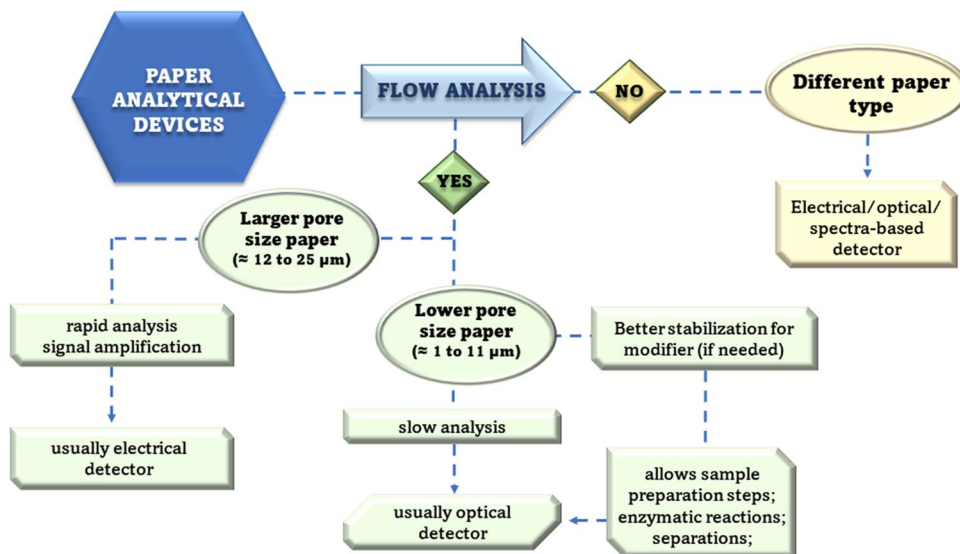
abundant, low-cost material whose porous structure allows reagents and biological sample storage and fluid flow control by capillary action without needing external equipment. PADs are also disposable, portable, and easy to use, which is very interesting for POC tests [49, 50].

Several detection methods can be associated with PADs, such as colorimetric [22, 51], electrochemical [52, 53], surface-enhanced Raman scattering [54], chemiluminescence [55], fluorescence [56], and electrochemiluminescence [57]. However, it is essential that these methods also attend the ASSURED criteria to be used as point-of-need or point-of-care devices. This section will critically discuss the most recent point-of-need applications found in the literature for paper-based analytical devices, which will be divided into the most common detection methods, also considering dual/combined readout devices [58] and distance-based detection [59].

Colorimetry

Colorimetry is the most common analytical technique used with PADs. A colorimetric sensor detects analytes through color changes that can be visually observed [60]. Paper is an excellent substrate for this type of detection due to its white background, contrasting with the color appearance [61]. Different strategies can be applied for color formation, including enzymatic reactions, redox indicators, nanoparticles formation, acid/base indicators, and complexometric reactions. The next step immediately after color formation is the readout process, which can be done by the naked eye, especially considering qualitative tests with YES or NO output originated by a color change due to the presence or absence of an analyte. It is also possible to perform quantitative data acquisition by constructing a calibration curve, where the color intensity is proportional to the concentration

Fig. 3 Scheme to select paper type according to the analysis's mode (static or flow analyses) for optical and electrochemical detection



of the analytes. In this case, office scanners or smartphones are required to digitize the images, which are processed in specialized software (e.g., Adobe Photo-Paint, ImageJ, PhotoMetrix) by decomposing the images into primary colors, known as RGB (red, green, blue), or secondary colors CMYK (cyan, magenta, yellow and black), among other systems [62].

Overall, paper-based colorimetric devices are a good fit for point-of-need tests regarding numerous applications, such as early pregnancy detection through hCG hormone in urine samples [5]; glucose, cholesterol, lactate [63], and creatinine [37] monitoring via noninvasive fluids; and detection of viruses like human immunodeficiency virus (HIV) for acquired immunodeficiency (AIDS) [64] and SARS-CoV-2 for COVID-19 diagnosis [65, 66], as well as bacterial diseases such as tuberculosis [67] and *E. coli* infection [68], and important cancer biomarkers [69, 70]. Table 2 summarizes recent findings in the literature related to PADs with colorimetric detection for point-of-need applications.

Currently, most of the available paper-based devices only identify single targets. However, it would be interesting to evaluate different parallel biomarkers or specific combinations, consequently improving the test's clinical value and saving on reagents, time, and cost. In this context, Pomili et al. [63] proposed a multiplexed colorimetric device for simultaneously detecting salivary biomarkers, i.e., glucose, cholesterol, and lactate. The multiplexed PAD, which was

manufactured in a single CO₂ laser cutting step, comprised a chromatographic paper with a small central area for saliva sampling, connected with three microfluidic channels. Each channel contains a detection zone spotted with gold nanoparticles (AuNPs) and a layer of the corresponding enzymes (glucose (GOx), cholesterol (ChOx), and lactate oxidase (LOx)) for the respective analytes. In addition, pre-treatment zones functionalized with halogens (NaI) were interposed between the sample and the test zones (Fig. 4). The colorimetric detection is based on the oxidation of the biomarker by its specific enzyme, generating hydrogen peroxide (H₂O₂) as a byproduct, which leads to a morphological change in the AuNPs, thus promoting a blue-to-pink color change. The colorimetric response is achieved within 10 min due to the presence of NaI, which boosts the oxidation process, promoting a rapid color change in case of a pathological concentration of the biomarkers in saliva that can be either read by the naked eye or using a smartphone camera. A prototype device for POC tests was also developed by adding a protective adhesive mask on the paper surface, designed with holes in the sample and detection zones. The prototype provides easy handling for home testing and could be adapted to detect several other biomarkers. Although this platform offers low-cost and rapid preliminary testing, the qualitative readout does not accurately assess health conditions, requiring conventional tests to provide more quantitative information.

Table 2 Summary of recent PADs found associated with colorimetric detection for point-of-need applications

Paper type	Analyte	LOD	Diagnostic	Sample	Reference
Whatman chromatography N° 1	Glucose, cholesterol, lactate	n/a (qualitative)	Diabetes	Saliva	[63]
Whatman chromatography N° 1	Glucose and lactate	0.312 and 0.297 mmol L ⁻¹	Diabetes	Human serum	[71]
Whatman filter N° 4	hCG hormone	10 ng mL ⁻¹	Pregnancy	Urine	[5]
Whatman filter N° 4	Creatinine	0.27 mmol L ⁻¹	Renal disorders	Artificial urine	[37]
Whatman chromatography N° 1 and 3	HIV-1 p24 antigen	0.03 ng mL ⁻¹	AIDS	Human blood plasma	[64]
Ahlstrom chromatography Grade 222	SARS-CoV-2	200 genomic copies μL ⁻¹	COVID-19	Saliva	[72]
Whatman chromatography N° 1	Dengue virus serotypes	5.23–38.17 nmol L ⁻¹	Dengue	Plasmids	[73]
Whatman chromatography N° 3	Tuberculosis DNA	0.0195 ng mL ⁻¹	Tuberculosis	Human disc tissue	[67]
Cellulose paper	<i>E. coli</i>	10 ⁴ CFU mL ⁻¹	Bacterial infection and drug resistance	Mice wound tissue	[68]
Whatman filter N° 1	PCA3 antigen	0.34 fg μL ⁻¹	Prostate cancer	RNA from cancer cells	[69]
Whatman filter N° 1	Citrate	0.4 mmol L ⁻¹	Prostate cancer	Urine	[74]
Nitrocellulose membrane	AFP and MUC16 proteins	1.054 and 0.413 ng mL ⁻¹	Liver and ovarian cancer	Synthetic serum	[70]
Whatman filter N° 1	Cytochrome <i>c</i>	0.5 mmol L ⁻¹	Hematologic cancer	Human serum	[75]

hCG human chorionic gonadotrophin, *HIV* human immunodeficiency virus, *AIDS* acquired immunodeficiency syndrome, *CFU* colony-forming unit, *PCA3* prostate cancer antigen 3, *AFP* using alpha-fetoprotein, *MUC16* mucin-16

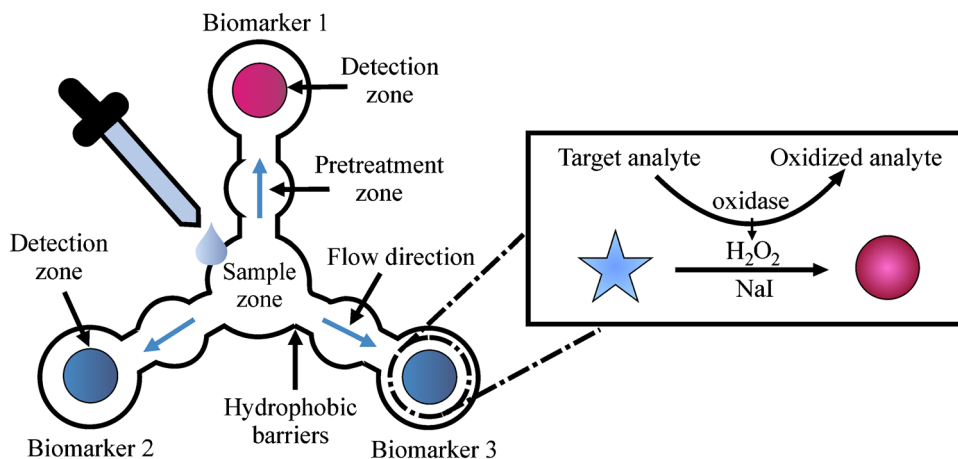


Fig. 4 Schematic illustration of the colorimetric paper-based device for the simultaneous detection of three salivary biomarkers, where a drop of saliva is deposited in the sample zone, flowing through the pretreatment zones, where it is mixed with the deposited NaI, after which it reaches the detection zones, functionalized with AuNPs and

oxidase enzymes. The insert shows the colorimetric reaction mediated by the H_2O_2 byproduct in the presence of NaI, which leads to a visible color change from blue to pink for nonphysiological levels of the target analyte. Copyright 2021, MDPI [63]

Regarding quantitative colorimetric analyses, Aydinoglu et al. [70] developed a paper-based spot test using AuNPs bioconjugates as a color provider for the quantitative detection of alpha-fetoprotein (AFP) and mucin-16 (MUC16), which are well-known protein biomarkers for liver and ovarian cancer. The AuNPs were first immobilized on a nitrocellulose membrane, which was pretreated with bovine serum albumin (BSA) solution to facilitate the formation of circular shapes as in a spot-like platform. Next, the *anti*-AFP or *anti*-MUC16 antibodies were immobilized on the spot's surface using glutaraldehyde as a crosslinker. Then, different concentrations of AFP or MUC16 antigen solutions were incubated for 30 min. Afterward, smartphone images via ImageJ software were used to evaluate color changes on the GNP surface, whose intensity depends on the biomarker concentration. The limit of detection (LOD) for AFP was 1.054 ng mL^{-1} which agrees with the serum cut-off value reported in the literature (20 ng mL^{-1}), demonstrating acceptable analytical performance for this species. On the other hand, the LOD for MUC16 (0.413 ng mL^{-1}) was not compared with reference values. The authors described that the cut-off value is reported in a different unit (35 U mL^{-1}), limiting the comparison. The sensing platform was only stable over 4 days, which is considered a drawback for commercial purposes. Nevertheless, it still represents a rapid and noninvasive alternative for preliminary cancer detection, which is an unmet need worldwide. It also holds great potential for the study of other molecules of interest.

Another approach presented by Wang et al. [69] used a reverse transcription loop-mediated isothermal amplification (RT-LAMP) chip with colorimetric detection for point-of-care testing of prostate cancer 3 (PCA3) biomarkers. The

chip was composed of an amplification zone, where the samples and reagents were loaded on a sponge-like polyvinyl chloride (PVC) pad, and a calcine-preloaded dry filter paper was positioned in a detection zone, both connected by a microfluidic channel. The paper discs were prepared by using a desktop paper cutter. After an incubation time using a battery-powered thermal module, the RT-LAMP products were pressed by a stick, flowing from the sponge to the paper. The color change was imaged using a smartphone, and the PCA3 gene could be detected from RNA samples at concentrations as low as 0.34 fg mL^{-1} .

Despite the ease of using smartphones for accurate quantification in colorimetric assays, Davidson et al. [72] presented a PAD for directly detecting SARS-CoV-2 in saliva using LAMP via a distinct colorimetric response that can be read using the naked eye. The device consists of a reading layer and two reaction paper strips. In addition, instead of using wax printing, they used polystyrene spacers to prevent cross-talk between samples, enabling large-scale production. Under positive results, the paper color changes from red to yellow. Although presenting an analytical sensitivity of 76%, specificity of 100%, and ~91% accuracy, the subjectivity in color perception of the control PAD can lead to false positive results, which do not occur with image processing. Additionally, the device exhibits the color change within 60 min and costs around \$10/test, which can be considered outdated compared to the COVID-19 lateral flow tests commercially available today.

It is also possible to perform semi-quantitative measurements by comparing the change in color with a color chart diagram without the need for external equipment, extending its application to resource-limited areas of the world. About this

topic, Chen et al. [64] developed an instrument-free μ PAD sensor based on the colorimetric enzyme-linked immunosorbent assay (ELISA) to detect the virological biomarker HIV-1 p24 antigen for early HIV diagnosis. Hydrophobic barriers were first patterned on chromatographic paper by wax printing to create sample/reagent wells. The paper was folded as origami and placed in a 3D-printed holder, enabling multi-step assays by sliding the μ PAD layers. The system comprises a test and a control detection well, both pre-modified with capture antibodies, forming an antigen–antibody complex in the test well by sample addition. It also includes a paper strip featuring two other wells containing dried horseradish peroxidase (HRP) conjugated detection antibodies that overlap with the detection wells when pulled down, and react with the complex by adding a washing buffer. Additionally, an electricity-free automated timer function was incorporated into the device, which works by delivering the washing buffer excess to indication wells at the appointed times, displaying a green color due to the presence of a food dye previously dried in the paper substrate. The correct delivery times were achieved by adjusting the viscosity of the buffer solution by treating the paper with dried sucrose, which is an efficient strategy for controlling the flow rate in μ PADs. The first indication well is triggered in approx. 4 min after the buffer is added. A green color indicates the right moment for 3,3',5,5'-tetramethylbenzidine (TMB) addition, which enables the development of a dark blue color in the test well due to the presence of the antigen, while the control well displays a light blue color. This step activates the second indication well, and the target analyte concentration can be determined (~5 min) by comparing the color on the test well with the color chart diagram. The latter shows the detection results based on different antigen concentrations, providing a semi-quantitative detection of the HIV-1 p24 with a satisfactory LOD of 0.03 ng mL⁻¹.

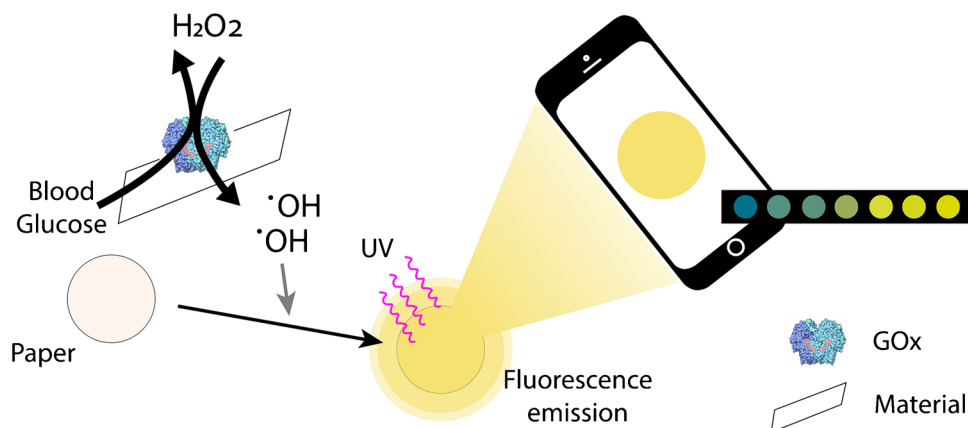
Fluorescence

Fluorescence is the electromagnetic radiation emitted by a molecule when electrons, previously excited by the absorption of light, return to their ground state or lower energy than the excited state [76]. Paper-based devices with fluorescence detection are usually fabricated by immobilizing fluorescent materials, such as organic dyes, metal nanoclusters, and different types of nanoparticles, on a piece of paper that produces spectral responses in the presence of target analytes. The fluorescence response can be obtained, for example, by using a smartphone camera, as previously described for colorimetric systems. Fluorescence detection has increasingly attracted the attention of researchers due to its high sensitivity, fast response time, easy implementation, and simplicity. In addition, there is a high commercial availability of fluorescence dyes that can be directly visualized by the naked eye under UV irradiation, increasing the possibility of applications [77–80].

Paper-based fluorescence devices have generally been applied in bioanalysis and medical diagnostics [6, 81–83]. The popularity is associated with the paper substrate's inherent advantages and the fluorescence response's efficiency, making this combination an attractive alternative for POC diagnostics [80]. Xu et al. [84] developed a paper-based platform for detecting microbial species (*Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium pan*) responsible for causing malaria in humans. The device comprises five paper panels with a hydrophobic barrier delimited by wax using wax printing. These panels are folded after adding the sample, generating an origami-shaped device. This design is an interesting approach since it allows sequential steps in a single device, including DNA extraction, loop-mediated isothermal amplification (LAMP), and fluorescence detection. After adding the sample to the device, the analyte solution is guided by capillarity to four different points containing the control and LAMP reagents for each analyte. The system is then closed to avoid evaporation of the reagents during the incubation, and subsequently, the amplification is carried out at 63 °C for 45 min. The results were observed by the naked eye using a portable UV lamp. Compared with gold-standard assay polymerase chain reaction (PCR), the proposed method provided high sensitivity and specificity to determine *Plasmodium* in blood samples. However, this test failed for some samples from PCR-positive patients who had already started antimalarial treatment. Consequently, this fact can be considered a limitation for POC testing, and the proposed method needs further studies for comparable analytical performance.

Al Lawati et al. [83] developed a new alternative for glucose monitoring (Fig. 5) using a paper-based fluorescence device with a built-in 2D cobalt-terephthalate framework (CoMOF). These well-organized three-dimensional structures contribute to the formation of highly porous and extraordinarily stable surfaces, which add to these devices a high surface area and the possibility of application in the catalysis of chemical reactions. Two-dimensional cobalt-terephthalate was synthesized using a solvothermal method and later incorporated with the enzyme glucose oxidase (GOx) into the paper through a simple drip-drying process. A yellow–brown color is observed when the sample (2 μ L) containing glucose is injected into the device. After 1 min, the device is exposed to UV irradiation (365 nm) in a dark box, generating fluorescent irradiation. The emitted fluorescence was captured by using a smartphone camera. The results showed that 2D CoMOF surprisingly increased both the performance and stability of GOx, remaining stable for at least 2 months and decreasing the time required for glucose oxidation. In addition, 2D CoMOF accelerated the reaction of H₂O₂ produced by the enzymatic oxidation of glucose with o-phenylenediamine (OPD). The proposed method provides a wide linear range (50 μ M–15 mM) and

Fig. 5 Schematic representation of the analytical application performed with a paper-based fluorescence device proposed by Lawati et al. [70]



great LOD (3.2 μM) for glucose quantification. The system's applicability was successfully evaluated to monitor glucose in blood samples. Despite all advantages, the time and cost required to prepare the CoMOF should also be considered parameters to compare the proposed device with commercial devices or other devices able to simultaneously determine glucose and other biomarkers [85, 86].

Considering the need for more sensitive and quantitative detection methods for the detection of biomarkers of non-communicable diseases, Natarajan et al. [79] have developed a fluorescent lateral flow paper-based microfluidic device for point-of-care detection of cardiac Troponin I. According to the American Heart Association and American College of Cardiology (AHA/ACC), this substance is considered the most specific biomarker for acute myocardial infarction. The proposed device is a sandwich-type, which uses a monoclonal antibody immobilized onto the nitrocellulose membrane using an Easy print to capture and an antibody conjugated to Alexa Fluor dye to detect the analyte. After 10 min of incubating the sample, cardiac Troponin was quantified by the immunoanalyzers. The fluorescent lateral flow immunoassay resulted in less than 10 min with a detection limit of 0.019 ng/mL.

Another device for biomarker detection was developed by Xu and colleagues [87]. A paper-based upconversion fluorescence resonance energy transfer biosensor was developed and applied to detect carcinoembryonic antigen (CEA). This compound is commonly associated with several types of cancer, including colorectal, pancreatic, and gastric carcinoma. The device was made on standard filter paper with a simple nano-printing method. The upconversion nanoparticles labeled with specific antibodies were printed on the test areas on the test paper, followed by the introduction of the test antigen. Fluorescence upconversion measurements were directly performed at the test sites after the antigen–antibody reactions. Besides good stability and reproducibility, the device showed a low detection limit (0.89 ng/mL). In addition, the authors highlight the device's ease of fabrication

and operation, making it a promising alternative for point-of-care clinical testing. Table 3 summarizes other recent works related to PADs with fluorescence detection for point-of-care applications.

Electrochemistry

Combining paper-based devices with electrochemical detection has distinctive features since this set can be produced in miniaturized size, aligning portability, low cost, and sensitivity [52]. In addition, potentiostat, the main instrument used in electrochemical measurements, can be easily miniaturized, allowing portability and field analysis [93]. Selectivity is a challenge faced in electrochemistry [94]. However, this parameter can be modulated by choosing the appropriate detection potential and/or the electrode material, significantly enhancing the device's analytical performance [4]. Commercial electrodes [95], pencil graphite [96], and metal wires [42] have been used as electrochemical detectors integrated into paper-based devices. Besides that, different approaches can be found to fabricate electrochemical sensors directly on the paper surface, including stencil printing, screen printing, inkjet printing, pencil drawing, and laser pyrolysis [97].

Screen printing and stencil printing use high-viscosity conductive ink for electrode fabrication [98]. The process consists of using an open mask, made of transparent film or adhesive tape, as a mold to design the electrode geometries. The ink is then applied onto the paper substrate using a squeegee [99, 100]. Before ink drying, this mask is rapidly removed from the paper substrate, generating the three-electrode electrochemical system. Unlike screen printing, stencil printing does not have sophisticated screen apparatus [100]), affording electrode fabrication even in resource-limited laboratories. In contrast, inkjet printing is a cutting-edge technology to deposit high-precision ink lines [97, 101]. This technique dispenses the use of masks and allows scalable electrode fabrication.

Table 3 Recent literature findings related to PADs with fluorescence detection for point-of-need applications

Paper type	Analyte	LOD	Diagnostic	Sample	Reference
Absorbent paper	Dipicolinic acid	0.8 nmol L ⁻¹	Anthrax	River water; Orange juice	[77]
Whatman chromatographic Grade 1	Hepatitis C virus DNA	5.0 pmol L ⁻¹	Hepatitis C	HCV cDNA	[78]
Nitrocellulose membrane	Cardiac Troponin I	0.019 ng mL ⁻¹	Acute myocardial infarction	Human serum	[79]
Whatman filter N° 4	Alkaline phosphatase and butyrylcholinesterase	3.6 U L ⁻¹ and 0.4 U mL ⁻¹	Liver diseases	Human serum	[6]
Whatman filter N° 1	Immunoglobulin E	0.13 IU mL ⁻¹	Allergic diseases	Human serum	[81]
Whatman filter	Glucose	3.2 μmol L ⁻¹	Diabetes	Blood	[83]
Filter paper	<i>Plasmodium falciparum</i> , <i>Plasmodium vivax</i> , and <i>Plasmodium pan</i>	n/a (qualitative)	Malaria	Blood	[84]
Whatman filter N° 3	Cooper ions	0.41 pmol L ⁻¹	Anemia, pancytopenia, Wilson's disease, and Alzheimer's disease	HepG-2 cells, mouse urine, human serum, and bovine serum	[88]
Filter paper	Albumin	16.4 nmol L ⁻¹	Hepatic impairment and chronic kidney disease	Human serum	[89]
Nitrocellulose membrane	Norovirus	3.3 ng mL ⁻¹	Acute gastroenteritis	Mussel	[90]
Whatman filter	Antibiotic resistance genes	n/a (qualitative)	Antibiotic resistance	Blood	[91]
Whatman filter N° 1	Sulfamethazine, oxytetracycline, and chloramphenicol	0.47, 0.48, and 0.34 ng mL ⁻¹	Antibiotic resistance	Shrimp	[92]
Filter paper	Carcinoembryonic antigen	0.89 ng mL ⁻¹	Colorectal, pancreatic, and gastric cancer	Human serum	[87]

HCV hepatitis C virus, cDNA complementary DNA, HepG-2 human liver cancer cell line

Unlikely ink-based techniques, pencil drawing consists of obtaining carbon conductive tracks from the mechanical friction between the paper substrate and graphite pencil [102]. Pyrolysis uses a laser source to induce the formation of graphite structures onto the paper substrates. This automated approach dispenses the need for reagents and controlled environmental conditions during electrode fabrication. Besides paper, other substrates have also been used for this purpose, including phenolic resin and polyimide [103]. However, each substrate can provide specific properties to the resulting device, including flexibility, stability, and hydrophobicity. Besides using these techniques for electrode fabrication, hydrophobic barriers can also be created onto the paper substrate (e.g., using wax printing technique), delimiting the volume of the electrochemical cell or creating microfluidic paths.

Once fabricated, paper-based electrochemical devices can be used in static or hydrodynamic conditions [104, 105]. In static mode [106], hydrophobic barriers are created in the paper to delimit the analysis zones (Fig. 6A). Then, micro volumes of the analyte solution, which contains supporting electrolytes, are dropped on the sensors to record the electrochemical measurements. Another approach [107, 108] involves transporting the analyte to the

electrochemical detection zone by the capillarity of the paper substrate. The electrochemical measurement is subsequently recorded when the solution stops flowing. This is an interesting strategy since multiple electrochemical detectors can be integrated into a single device, increasing the system's applicability. In hydrodynamic conditions, a constant flow of supporting electrolyte can be produced by using two reservoirs (Fig. 6B) [39, 103, 109, 110]. The inlet reservoir is constantly filled with supporting electrolyte, which flows by gravity and capillarity. A sorbent pad is used as an outlet reservoir and is responsible for wicking the carrier fluid. The electrochemical sensors are placed between the two reservoirs. The injections of the analyte solution are performed at a point located between the inlet reservoir and the electrochemical detector. Another approach involving hydrodynamic conditions involves coupling batch injection analysis with paper-based microfluidic devices (Fig. 6C) [96]. The electrochemical system is fabricated on a circular paper substrate. The analyte solution, prepared in the supporting electrolyte, is directly injected into the electrode surfaces, resulting in transient signals associated with the redox process. During the analysis, the analyte solution is gradually spread on the edges of the paper by capillarity, and

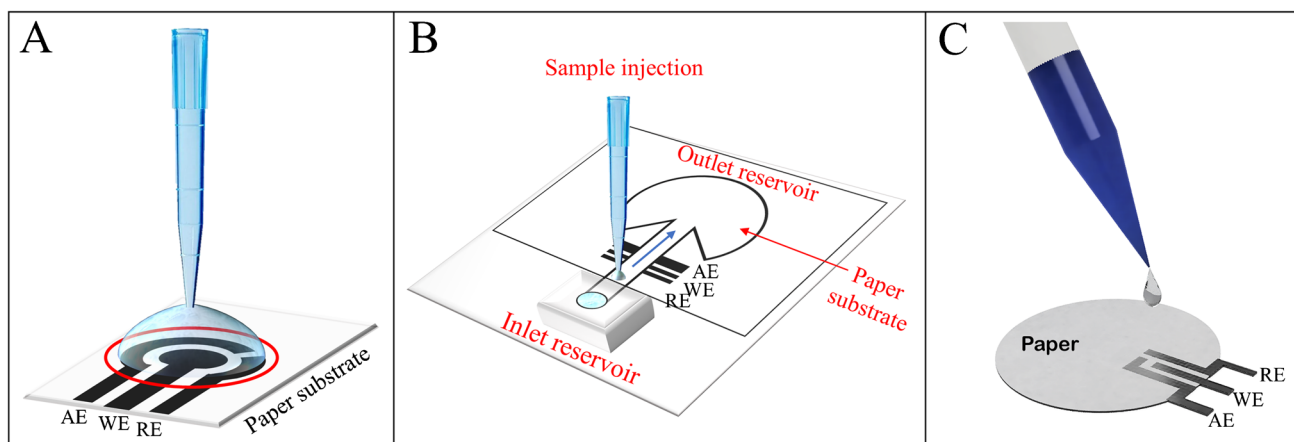


Fig. 6 **A** Paper-based electrochemical device used in static conditions. **B** Paper-based microfluidic device under a constant flow of supporting electrolyte produced by using two reservoirs [36]. **C**

Paper-based microfluidic device coupled with batch injection analysis. Reprinted with permission from Iana V.S. Arantes and Thiago R.L.C. Paixão [96]. Copyright 2022 Elsevier

new injections are sequentially performed in the device. In general, paper-based microfluidic devices bring remarkable advantages since the system can substantially improve the analysis time and reduce sample consumption.

Pagkali et al. [111] reported the development of an enzymatic biosensor capable of monitoring glucose in food samples. The electrochemical platform on the paper surface was obtained from the pencil-drawing technique, combining a commercial writing pencil to obtain the analysis zones and a hydrophobic marker pen to delimit the fluidic path of the sample zone. Subsequently, the enzyme glucose oxidase and the mediator ferricyanide were added to the analysis zones. Glucose quantification was performed by the electrochemical technique of amperometry, showing LOD of 0.08 mmol L^{-1} , a relative standard deviation of analytical response smaller than 12%, and recovery studies between 94 and 106%.

Dempsey and Rathod [112] described an electrochemical immunosensor, on commercial acetate paper, using screen-printed electrodes (SPE), by combining stencil printing process and ELISA approach for determining cardiac Troponin T (cTnT), exploring microfluidic properties of different papers substrate for analysis in lateral flow mode. The first stage was to manufacture the device. This was performed by the screen-printing technique using mixtures of graphite powder and epoxy resin. Then, the anti-cardiac troponin T antibody (Ab-cTnT) was fixed on the surface of the working electrode ($5 \times 2 \text{ mm}^2$) followed by the addition of bovine serum albumin. In the second stage, Whatman filter paper (membrane, $5 \times 50 \text{ mm}^2$) was fixed on the working surface of the device, followed by immobilization of anti-cardiac troponin T antibody-HRP (Ab-HRP) at a distance of 30 mm from the working electrode. Samples containing cTnT were added at a distance of 50 mm from the working electrode.

During the percolation process, a complex (Ab-HRP/cTnT) is formed, followed by a sandwich on the working surface (SPE/Ab/BSA/cTnT/Ab-HRP). The detection and quantification of cTnT are performed through a redox mediation after adding H_2O_2 on the working surface of the device, followed by enzymatic conversion of *o*-phenylenediamine into diamine. The electroanalytical method presented a linear range of $100\text{--}700 \text{ ng mL}^{-1}$ and a LOD of 0.15 ng mL^{-1} . The results were obtained relatively in a short analysis time compared to recent literature works (20 min). Therefore, the work reports a disposable device for the detection of a global relevance biomarker in preventing myocardial infarction.

Wei et al. [108] developed a microfluidic label-free paper-based aptasensor for detecting and quantifying prostate cancer using a prostate-specific antigen (PSA) as a biomarker. The device fabrication used wax printing to form hydrophobic barriers and screen-printing to obtain the reference, auxiliary, and working electrodes. The working surface was modified with AuNPs, reduced graphene oxide (rGO), and thionine (THI), followed by immobilization of the DNA aptamer. Biological recognition between DNA aptamer and PSA was mediated and translated by THI. The method presented lower LOD (10 pg mL^{-1}) than those reported in serum samples, demonstrating the possibility for prostate cancer diagnoses (4 ng mL^{-1}).

Torul et al. [113] developed a paper-based biosensor that microRNAs (miRNA-155 and miRNA-21) correlated to lung cancer. The fabrication method combined wax printing and pencil drawing. The working electrode surface was then modified with rGO or molybdenum disulfide nanosheets (MoS₂) modified with AuNPs (AuNPs/RGO, AuNPs/MoS₂). The analysis presented short times (35 min) and reduced sample volume ($5.0 \text{ }\mu\text{L}$) compared to recent works. The LODs obtained for miRNA-21 and miRNA-155 were

12.0 and 25.5 nmol⁻¹ for AuNPs/RGO against 51.6 and 59.6 nmol⁻¹ for AuNPs/MoS₂ biosensors.

Cincotto et al. [107] developed a paper-based microfluidic device with electrochemical detection to determine creatinine and uric acid biomarkers. The ePAD consisted of two spot sensors in the same working electrode. Each spot was modified differently for each biomarker. The first spot was modified with graphene quantum dots (GQDs) to monitor uric acid. The second spot was modified with quantum dots containing creatininase and the redox mediator ruthenium to determine creatinine. The modifications were performed by using the “drop-casting” method. The analytical signals of the proposed sensors were compared to the unmodified electrode, demonstrating the electrocatalytic properties attributed to the GQDs. Square wave voltammetry was used as an analytical technique for both biomarkers, showing a linear range of 0.01–3.0 μmol L⁻¹ and limits of detection (LOD) of 3.7 and 8.4 nmol L⁻¹ for creatinine and UA, respectively. The applicability of the proposed devices was evaluated to determine these species in real urine samples since the presence of both biomarkers can indicate kidney malfunction. The values obtained for the recovery tests ranged from 101.5 ± 0.7 to 98.9 ± 0.5%, demonstrating the potential application of the developed ePAD. Despite the scientific merit, a brief discussion about the effective parameters that could affect the reproducibility, performance, and large-scale production should be more detailed in this work. In addition, other parameters should be better discussed in this article, including paper types, electrode fabrication method, device design, and analytical signal amplification [114].

Considering the current outbreak of the COVID-19 pandemic caused by SARS-CoV-2 [115], the need for point-of-care testing has received considerable attention all over the world. As a result, ePADs have gained more space for biological analyses. Lomae et al. [116] developed a point-of-care device using filter paper and wax printing for reagent-free SARS-CoV-2 detection, assisted by a potentiostat integrated into the smartphone. The biosensor was modified with the biological recognition element pyrrolidiny peptide nucleic acid (acpcPNA). The PNA is responsible for capturing the target complementary DNA (cDNA) molecule. The synergistic interaction between PNA and cDNA results in the blocking of the [Fe(CN)₆]^{3-/4-} redox mediator signal. The method showed high selectivity and obtained concordant results for 10 nasopharyngeal swab samples compared to tests performed by RT-PCR.

Torres et al. [117] developed a point-of-care biosensor for the rapid determination of SARS-CoV-2. The electrochemical sensors were screen-printed using qualitative filter paper and phenolic paper circuit boards as substrates. The device consisted of a biosensor modified with the human angiotensin-converting enzyme-2 receptor immobilized by drop-casting on the surface of the glutaraldehyde polymer

previously sorbed on the phenolic paper. The device was stabilized with bovine albumin serum. Finally, Nafion was added to pre-concentrate the cationic species and protect the device from interfering with macromolecules. Electrochemical impedance spectroscopy was used as the analytical technique to determine SARS-CoV-2 in saliva samples. The estimated time for each analysis was only 4 min, which is considerably impressive. The proposed device demonstrated sensitivity and specificity comparable to nasopharyngeal/oropharyngeal tests. Torres et al. showed the possibility of performing multiple analyses in different media, temperatures, and virus activity. In addition, the authors showed a comparison with other analytical methods, evaluating the cost to afford large-scale production of the device, which is an essential factor in implementing the proposed device as a viable analytical platform for point-of-need applications. Considering the versatility of the systems, paper-based electrochemical devices (ePAD) have been used in several areas, including biological [118], pharmaceutical [118], forensics [119], and environmental applications [120]. Table 4 summarizes recent literature findings related to ePADs with electrochemical detection for point-of-care applications.

Dual/combined readout

The main detection systems for paper-based devices (PADs) are colorimetric [62], fluorescence [133], and electrochemical [134]. Despite the variety, each one has its intrinsic peculiarities and limitations. However, these systems can be integrated into a single device, generating the so-called combined sensor or dual devices [9]. These combinations are interesting for analytical applications since they can overcome individual barriers, making possible simultaneous determinations, and enhancing the sensitivity and/or selectivity of the proposed devices [135, 136]. Optical and electrochemical detection have been coupled to paper-based devices. The devices are generally designed for two main approaches. The detectors can work independently to generate answers for multiple analytes [137], or they can be combined for a single purpose, improving the analytical signal (selectivity and/or sensitivity) [135]. These approaches are promising alternatives to determine a variety of species, including biomarkers [138], glucose, total iron [136], bacteria [139], forensics [119, 140], cells [141], and plastic [142]. Table 5 summarizes the findings for dual detection. These devices can be made in miniaturized size, ensuring better portability to be used in the field. In addition, they can usually be fabricated by using low-cost materials, making the analyses cheaper than those from traditional methods. This feature is considerably helpful for non-privileged countries [40]. Electrochemical are commonly combined with colorimetric for POC. The other combinations are more usual for PON. Not all forms of dual detection are electrochemistry

Table 4 Recent PADs found associated with electrochemical detection for point-of-need applications

Paper type	Fabrication	Analyte	LOD	Diagnostic	Sample	Reference
WCP N° 1	SP	H ₂ O ₂	0.25 mg dL ⁻¹	Cholesterol	Serum	[121]
-	SP	3-NT and 8-OHdG	0.0138 and 0.0027 μmol L ⁻¹	Neurodegenerative disorders	Urine and serum	[118]
WCP N° 1	SP	CRP	6 ng mL ⁻¹	Cardiovascular diseases	Serum	[122]
WCP N° 1	SP and WP	CEA and NSE	2.0 and 10.0 pg mL ⁻¹	Lung cancer	Clinical	[123]
WCP N° 1	IP	Ferritin	19 ng mL ⁻¹	Deficiency anemia	Serum	[124]
WCP N° 1	WP	HBsAg and HCVcAg	18.2 and 1.19 pg mL ⁻¹	Hepatitis B and hepatitis C	Serum	[125]
WCP N° 1	WP	CRP, cTnI, PCT	0.38 ng mL ⁻¹ , 0.16 and 0.27 pg mL ⁻¹	Cardiovascular diseases	Serum	[126]
WCP N° 4	WP	Urinary albumin	0.072 mg dL ⁻¹	Chronic kidney disease	Urine	[127]
Fiberboard	PD	Catechol	0.01 mmol L ⁻¹	pollutant	Environmental	[128]
Office paper	PD	Furosemide	0.24 μmol L ⁻¹	Doping agent	Synthetic urine	[129]
WCP N° 1	PD	Glucose and Uric Acid	2.0 and 0.5 mmol ⁻¹	Diabetes	-	[130]
Kraft paper	LP	Nitrite	4.31 μmol L ⁻¹	pollutant	Tap water, soil, and saliva	[131]
Kraft paper	LP	Sulfites	1 mg L ⁻¹	pollutant	beverages	[132]

WCP Whatman chromatography paper, SP screen-printing, WP wax-printing, IP inkjet printing, PD pencil drawing, LP laser pyrolysis, 3-NT 3-nitrotyrosine, 8-OHdG 8-hydroxy-2'-deoxyguanosine, CEA carcinoembryonic antigen, NSE neuron-specific enolase, HBsAg hepatitis B surface antigen, HCVcAg hepatitis C core antigen, CRP C-reactive protein, cTnI troponin I, PCT procalcitonin

Table 5 Summary of recent PADs found for dual readout detection for point-of-need applications

Paper type	Analyte	Detection	LOD	Sample	Reference
Whatman chromatography N° 1	Bisphenol A	Electrochemical and fluorescence	0.002/0.002 μM	Plastic bottles and tin cans	[140]
Whatman filter N° 1	Thiocyanate ion	Electrochemical and colorimetric	0.006/0.2 mM	Saliva	[135]
Whatman filter N° 1	Glucose and iron	Electrochemical and colorimetric	0.15 mM/n.a	Human serum	[136]
Whatman filter N° 1	H1N1	Electrochemical and colorimetric	3.3/1.34 PFU mL ⁻¹	Saliva	[137]
Fiberglass filter paper	Chromium ion	Electrochemical and colorimetric	0.01/0.06 mg mL ⁻¹	Water sample	[141]
Whatman filter N° 1 or copy paper	<i>E. coli</i> and <i>E. faecalis</i> activity	Electrochemical and colorimetric	0.014/0.031 μM	Food and water	[139]
Whatman chromatography N° 2	MCF-7 cancer cells	Electrochemical and colorimetric	20/20 cells mL ⁻¹	Human serum	[143]
White office paper	Cocaine, levamisole, caffeine, acetaminophen, and lidocaine	Electrochemical and SERS	-	Seized cocaine	[140]
Photo paper	Bisphenol A	Electrochemical and LDI-MS	6.0 and 0.35 μM/10 nM	-	[10]
Qualitative filter paper	Cocaine	Colorimetric and electrochemical*	0.9 μM	Seized cocaine	[119]
Filter RS	KET	Electrochemical, fluorometric, and colorimetric	3.2/20 μM/10 mg mL ⁻¹	Soft beverages	[144]

H1N1 influenza virus type A,- PFU plaque forming unit, MCF-7 breast cancer cell line, SERS surface-enhanced Raman spectroscopy, LDI-MS laser desorption ionization mass spectrometry, KET ketamine hydrochloride

*Electrochemical technique was used as a pretreatment

combined with other techniques. Even though they are the most used, there are also examples of colorimetric [145] with SERS and colorimetric with fluorescence [146, 147].

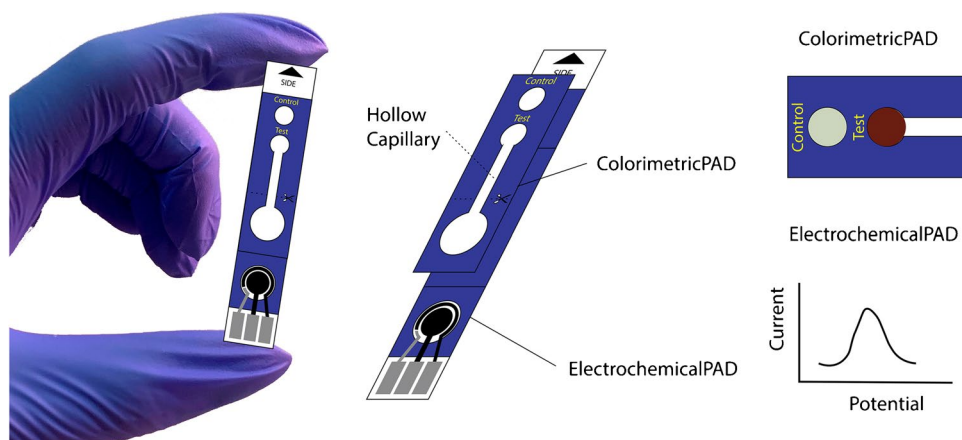
Wang et al. proposed a sensor to detect MCF-7 cancer cells [148]. The origami-like sensor detected the cells electrochemically and colorimetrically. This sensor has a simple design made with wax barriers and is modified with AuNPs and 3D-rGO. This Au@3D-rGO makes it possible to detect the cells electrochemically. This compound is synthesized in multiple steps, consisting of preparing 2D-rGO via “dropping and drying” into the paper matrix. This takes 2 h, and then put the paper in a vacuum freezer to dry for 8 h to form 3D-rGO. The AuNPs are formed by the chemical reduction of chloroauric acid (HAuCl_4) with ascorbic acid on the surface. Next, polyhedral-AuPd alloy nanoparticles (PH-AuPd NPs) is prepared to adhere to the surface H2 aptamer to ensure the interaction of the cell with the surface. Also, the cells need modifications with H1 aptamer, making it possible to produce hydrogen peroxide that the AuNP can sense. This peroxide is generated electrochemically for the colorimetric assay, reacts with the TMB in the colorimetric spot, and generates a deep blue color. This is a fully integrated sensor. A wide linear range was obtained from 50 to 107 cells mL^{-1} and a low LOD of 20 cells mL^{-1} . The proposed sensor could also work in human samples. The only downside is that electrode surface modification is time-consuming.

Pungjunun et al. presented a device to detect thiocyanate in saliva (Fig. 7) [135]. In the first instance, the saliva flow was investigated in the device. Saliva is a viscous non-Newtonian fluid, and its flow can be significantly decreased through the paper channel. As a result, different designs were evaluated for the proposed system. The best outcome was a sealed paper with a hollow capillary channel engraved with a laser machine. This set was selected to produce the device since this configuration showed the most traveled distance of the saliva samples. The analytical procedure initially

involves adding the saliva to the sample inlet zone. Then, it flows to the colorimetric region, generating a color change recorded with a phone. Subsequently, the colorimetric part is attached to the top of the electrodes to proceed with the electrochemical detection. This approach can evaluate a broader range of SCN—once the optical part detects higher concentrations and the electrochemical one quantifies the lower concentrations. However, colorimetric only works for those who smoke, which generally have higher concentrations than a non-smoker. Therefore, further studies could be performed to enhance the sensitivity of colorimetric sensors. In addition, other types of paper could be evaluated to help with saliva viscousness.

Mars et al. proposed a dual-sensing device with electrochemical and fluorometric detection [142]. This device is proposed to detect bisphenol A (BPA) in plastic bottles and cans. To achieve this, a curcumin@MIP is developed. MIP stands for molecularly imprinted polymer, which has specific sites for molecular recognition [149]. First, the curcumin nanoparticles are synthesized in boiling water, and then centrifugated and dried for almost 7 h. Then, the MIP is prepared with BPA, acrylamide (AA), ethylene glycol dimethacrylate (EGDMA), and benzoin ethyl ether (BEE) in a mixing, polymerizing, and cleaning step of 6 h. Then, the polymer is confined in the top region of the paper sensor and dried for 40 min before use. This curcumin@MIP is capable of sensing BPA in both electrochemical and fluorescence. The sensor consists of two parts, the bottom one is a three-cell electrode made of carbon paste mixed with Prussian blue [150], and both have a wax-patterned confining space with a part like a closing lead with a spot for the MIP, likewise the previous work, that can work for both detections. For fluorometric detection, the white spot paper allows the light to pass through, enabling the measure. This smart feature allows measurement without expensive materials such as fragile quartz cuvettes. This feature also works for electrochemical with a droplet of solution (20 μL), which is a good feature, using a small volume of solution. The reaction

Fig. 7 Schematic representation of the paper-based analytical device with dual detection system proposed by Pungjunun et al. [135]



occurs in the top part of the sensor, and the electrochemical measure is done in the bottom with the electrochemical cell.

For electrochemical detection, cyclic voltammetry is used to discover the oxidation and reduction peaks for sensing with differential pulse voltammetry (DPV). And for fluorescence, the sensor is trapped in a mounting bracket, and the particles are excited in 420 nm wavelength and an emission peak in 550 nm. In both analyses, the loss of signal/current is due to the binding of BPA with the MIP. The higher the concentration, the lower the signal. The pair exhibited a befitting detection limit (0.002 μM) with the previous in the literature and a better linear range (0.004–4.38 μM). This work could be a great alternative to commercial methods of BPA detection. However, it is important to mention that the whole sensor fabrication takes around 14 h.

Even though we did not dedicate a specific section for SERS, this technique is also considered a powerful tool for chemical and biological analysis since it provides a spectroscopic fingerprint for low-concentration analytes [151]. SERS is a sensing technique based upon the inelastic light scattering of molecules that is enhanced (by 108 or more) when molecules are absorbed onto a corrugated metal surface, normally composed of gold or silver nanoparticles [152]. Considering traditional instrumentation's inherent sensitivity and accuracy, this technique is a great candidate for point-of-care testing. However, traditional instrumentation is normally large, restricting applications at the point of need. Some portable instruments are commercially available to address this limitation, allowing the combination of SERS with paper-based devices. Despite the availability of portable devices, it is important to highlight that instrumentation's miniaturization typically suffers from decreased sensitivity [151].

Ameku et al. reported a combined readout sensor with AuNPs for electrochemistry and SERS for seized cocaine samples [151]. Because gold nanoparticles can not only detect cocaine but as well as its cutting agents (interferents) that drug dealers put into diluting the samples. These cutting agents work as fingerprints the police keep track of. This sensor is constituted of a working electrode made of AuNPs. These particles are made by a citrate-mediate method [153], a simple way to prepare AuNPs. Firstly, the paper is patterned with a wax printer pattern, which is molten on a hot plate. Then, the working electrode (WE) is done by adding in the WE spot 3 μL ($3 \times 1 \mu\text{L}$) of concentrated AuNP and drying under an IR-lamp, which allows the AuNP to dry in a confined way that leads to a homogeneous film. The counter, reference, and electrical contact were made of silver ink and dried under the IR lamp. The AuNP WE when compared to a commercial gold electrode presents better electrocatalytic performance. This occurs due to the packing structures with pores and uniform Au (111) fcc nanoparticles formed on the surface. This led to a sensitivity that a commercial

gold electrode does not have to detect acetaminophen and caffeine due to parallel reactions that occur on the surface. Cocaine and levamisole were detected with the SERS detection method, possibly due to the good interaction of the molecule with the gold surface. This work presents the benefit of having dual detection that allows detecting molecules that each method alone cannot, making a more complete sensor. Even though SERS has a portable sensor, it is still expensive and not accessible to everyone.

Distance-based detection

Even though electrochemical and optical systems are used as detectors in paper-based devices, the need for external equipment coupled to the analytical platforms can still be considered a limitation. To address this challenge, equipment-free readout systems have received considerable attention. A distance-based detection is a promising approach for point-of-need testing, especially in resource-limited regions. Distance-based detection measures the length of the color changing generated along a microfluidic channel as the analyte flows down and reacts with colorimetric or fluorescent reagents previously immobilized. When the sample is added to the device, the eluent fills the microfluidic channel over time, and the distance of the discolored/colored band is correlated with the analyte concentration. After the analyte reaction, fluorescence emission, in special, can be observed across the microfluidic channel by using a UV light source [8, 154]. Another approach involving distance-based detection measures the traveled distance of a colored solution [155]. A colored solution containing amphiphilic species (DNA, proteins, and surfactants) flows over a concentration-dependent distance. The amphiphilic analytes interact with the hydrophobic barriers of the microfluidic channel, reducing the final flow distance of the solution.

Consequently, higher analyte concentrations generate shorter colored bars. Distance-based detection is based on the visual response, and the color length can be measured with a ruler attached to the device. Compared with traditional optical detection, the devices are free of processing and recording equipment, which enhances the portability, reduces the cost of the analytical platforms, and circumvents the need for trained users. Additionally, distance-based detection is not vulnerable to ambient lighting influence, which is a great advantage since it does not measure colorimetric or fluorescence intensities [156]. Table 6 shows some analytical applications using paper devices coupled with distance-based detection.

Table 6 shows that different species have been quantified using paper devices combined with distance-based detection. Considering the number of applications, some works will be discussed below, providing information about device operation. Cate et al. [163] reported an analytical device

Table 6 Analytical applications using paper devices combined with distance-based detection

Paper type	Analyte	LOD	Sample	Reference
Whatman filter N° 1	Cocaine	1.8 $\mu\text{mol L}^{-1}$	urine	[59]
Filter paper	Chloride	1.7 mg L^{-1}	Tap water	[157]
Whatman filter N° 1	DNA	1 amol L^{-1}	Parasitic worm	[158]
Advantec No. 5C filter paper	Potassium ion	1 mmol L^{-1}	serum	[159]
Filter paper (80 g/m^2)	Glucose	0.1 mmol L^{-1}	tear	[160]
Whatman filter N° 1	Hydrogen peroxide	65.2 mmol L^{-1}	-	[161]
Whatman filter N° 1	Chloride	2 mg L^{-1}	mineral water	[161]
Whatman filter N° 4	Aluminum	100 $\mu\text{mol L}^{-1}$	water	[8]
Whatman filter N° 1	Bromide and bromate	10 $\mu\text{g L}^{-1}$ for Br^- and 0.5 $\mu\text{g L}^{-1}$ for BrO_3^-	Water, rice, cake flour, bread flour	[162]

with distance-based detection for glucose, nickel, and glutathione determination. Initially, wax barriers were printed on filter paper to create the microfluidic channel. Colorimetric reagents were then deposited across the channel. After drying at room temperature, the device was ready to use. The sampling solution was injected into the device and flowed downstream through the channel. Consequently, the analyte reacted with the reagents, generating the colored band. The results were achieved in less than 20 min, demonstrating encouraging findings. However, cysteine and homocysteine also generated color changing along the channel, acting as possible interfering species for glutathione determination. Considering the nickel quantification was based on the reaction with dimethylglyoxime (DMG), Co(II) could also react with DMG, causing interference with the signal [164]. Therefore, the system's applicability depends strongly on the selectivity of colorimetric or fluorescent reagents.

Wei et al. [165] developed a distance-based quantitative device produced by a wax printing method, and its applicability was evaluated to quantify cocaine in urine. A "sweet" hydrogel doped with glucoamylase (GA) was synthesized using an aptamer for cocaine as a cross-linker. Figure 8 shows a schematic representation of the proposed analytical method. The analytical procedure involved reactions performed in a centrifuge tube with the "sweet" hydrogel and the analyte. When cocaine is present in the sample, the "sweet" hydrogel releases glucoamylase into the solution, and this enzyme produces glucose by amylolysis. Then, the sampling solution can be added to the analytical device. Before the injection, a mixture of GOx and HRP solution is dropped on the sampling reservoir, and colorless 3,3'-diaminobenzidine (DAB) is dipped into the microfluidic channel to modify the detection zone. Finally, the sampling solution is injected into the microfluidic device. The glucose flows across the channel by capillary action, generating gluconic acid and hydrogen peroxide by GOx action. Then, the resulting H_2O_2 reacts with DAB in the presence of HRP, producing poly(DAB), which has brown coloration and is used for signal readout. The analytical device

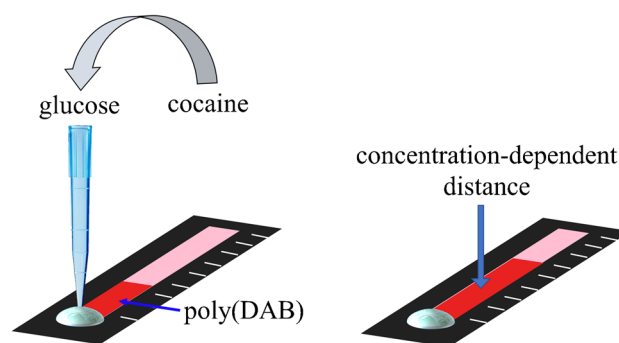


Fig. 8 Schematic representation of the analytical method used to determine cocaine proposed by Wei et al. [113]. Reprinted with permission from Wei et al. [165]

shows a great performance for cocaine determination in urine samples. The selectivity of the device was assessed for cocaine metabolites, such as ecgonine methyl ester and benzoylecgonine. A slight signal was only observed for higher concentrations of these species, demonstrating that the aptamer has favorable selectivity for cocaine. Besides these possible interfering species, the selectivity could also be evaluated for nitrite. The presence of some bacteria in urine produces nitrite [166], which might react with the hydrogen peroxide [167], causing interference in the analytical response. Despite that, the proposed device provides sensitivity and accuracy. In addition, the color changing is measured after 30 min, which can be considered rapid. Cocaine testing is of high interest for police agents once it could serve as an indispensable fast diagnostic for illegal drug seizures [34]. Hence, the device has demonstrated attractive advantages for cocaine determination.

Allameh et al. [160] produced a distance-based paper device to quantify glucose concentration in tear samples. In this case, the PAD was cut using a CO_2 laser, not requiring hydrophobic materials for channel fabrication. Glucose quantification helps to monitor diabetes. According to the WHO, ~422 million people worldwide have this disease.

Glucose is popularly monitored by blood analyses. However, sweat, interstitial fluid (ISF), saliva, tears, and urine can also monitor its concentration levels. Consequently, these samples can serve as minimally invasive alternatives, avoiding discomfort to patients. The proposed biosensing device was fabricated with GOx/HRP solution coupled with TMB. Only 10 μL of sample volume was required for the biosensor operation. The colorimetric assay was recorded with a smartphone camera. The video was processed with the software ImageJ and Tracker to get information about the color intensity and length, respectively. These data were important to evaluate the device's performance. The results were acquired within 5 min, dispensing the need for any additional equipment or trained personnel time.

Wang et al. [158] proposed a quantitative paper-based DNA reader for diagnostic soil-transmitted helminth (STH) infections. STH infections affect practically one-third of the global population. Most endemic areas are underprivileged regions, making distance-based detection a promising analytical tool for STH diagnosis. The signal readout was acquired by measuring the color distance in a wax-patterned device, eliminating the need for external readers. The analysis was based on the unique interfacial interactions of DNA intercalating dye, SYBR Green I, and unmodified chromatographic paper. The distance-based quantification took a few minutes (6 min). The analyses were conducted with real samples from children infected with *Trichuris trichiura*. The data were validated by comparing them with polyacrylamide gel electrophoresis, demonstrating consistent results.

Xia et al. [168] developed a paper-based lipase sensor. Pancreatic lipase, an exocrine enzyme from the pancreas, can be a biomarker for acute pancreatitis diagnosis. Pancreatitis is a common and severe gastrointestinal inflammatory disease. The proposed sensor consists of pH test paper strips supported on a PVC substrate. Phase separation is used to induce viscosity change. Firstly, lipase catalyzes triolein to produce oleic acid and glycerol. Calcium oleate is then produced by adding an excess of Ca^{2+} . The remaining Ca^{2+} binds with sodium alginate, promoting hydrogenation with an "egg-box" structure and resulting in phase separation. As a result, the quantification is performed via a phase separation-induced viscosity change. Hence, the lipase concentration is determined by measuring the solution flow distance on the paper. Thioflavin T was used as a fluorescent probe for the viscosity test. The sensor showed high sensitivity and specificity for lipase. The device was also applied for quantitative analysis in human serum samples, demonstrating the analytical potentiality for acute pancreatitis detection. Besides being portable, low-cost, and easy operation, the proposed device is considered an attractive platform for commercialization.

Conclusion

This work introduces a brief history of paper-based devices and critically discusses the practical and analytical specifications for POC applications. These devices have been used as promising analytical platforms to meet critical demands involving rapid and simple testing. They have been applied to quantify various chemical and biochemical species to evaluate food quality, environmental and health issues. The devices have been engineered in different layouts and detection systems to enhance analytical performance. In addition, the paper properties have shown a strong influence on the analytical performance of the devices.

Additionally, remarkable progress has been recently observed in analysis modes and applications. Optical detection can be performed using a simple smartphone, an indispensable tool. Electrochemical systems, in particular, have been used in static and hydrodynamic conditions, increasing the possibility of applications. Different strategies have also been used to integrate optical and electrochemical detectors with paper-based devices. Dual/combined detection has attractive advantages since it can overcome the individual barriers of the analytical techniques, making possible simultaneous determinations and enhancing sensitivity and/or selectivity. Distance-based detection is another exciting approach for PADs. These systems offer instrument-free analyses since the results are obtained by visual detection with a rule. Compared with traditional methods, the user can determine the analyte concentration by reading the length of the color changes across the microfluidic channel. This system precludes the need for external instruments and avoids user interpretation errors, which are outstanding features for point-of-need testing, especially for resource-limited regions. Although some paper-based devices are currently commercialized for point-of-need applications, the number of available devices is still considered low. This fact has been associated with some issues, including high LODs, poor reproducibility, low selectivity, and poor long-term storage stability. In general, the new proposed devices need to meet ASSURED criteria. However, the parameters must be carefully considered for the applications since some will be more significant than others. Therefore, further research can circumvent these challenges, extending the applicability of the PADs for point-of-need testing.

The final consideration is raised considering the following question "What to expect to be the new trend of these devices shortly?". To answer that, we first should consider that researchers have acquired knowledge about fabrication methods over the years. This has allowed them to develop complex architectures for chemical and biochemical analyses while being simple and easy to use. Second, the ability to perform advanced/

combined functions has significantly enhanced data quality, addressing some limitations involving analytical techniques. Finally, we can already expect the scalable manufacturing of low-cost devices considering the growing need for rapid medical diagnostics worldwide. Considering environmental analyses, we can also expect a next stage involving the collaboration of chemical scientists with engineers to provide new features for remote analysis. This includes using drone-board paper devices coupled with wireless data transmission. This will allow the monitoring of hard-to-reach locations. To achieve these goals, fundamental and applied research must keep in continuous progress with the aid of governments and private companies.

Acknowledgments We are thankful to FAPESP, CAPES, and CNPq for the financial support. The authors are also grateful to the University of São Paulo for the research laboratory.

Funding This research was financially supported by Sao Paulo Research Foundation (FAPESP) (Grant numbers: 2021/00205-8, 2022/11346-4, 2019/15065-7), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Grant Number: PROEX 88887.341030/2019-00, SACPAIS 88882.429169/2019-01), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Grant Number: 141854/2019-7, 140462/2021-0, 140463/2021-6).

Data availability The authors declare that the data supporting the findings of this study are available within the paper.

Declarations

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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