International Journal of Pharmacy and Pharmaceutical Science

ISSN Print: 2664-7222
ISSN Online: 2664-7230
IJPPS 2025; 7(2): 553-564
www.pharmacyjournal.org
Received: 02-09-2025
Accepted: 05-10-2025

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A review on recent advancements in micro-fluidic paper based analytical devices

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DOI: https://www.doi.org/10.33545/26647222.2025.v7.i2g.250

Abstract
Microfluidic paper-based analytical devices (µPADs) are valued for their low cost, portability, and ease of use, making them ideal for point-of-need testing. Their flexibility in design enhances usability, allowing rapid detection and analysis using portable tools. µPADs meet critical needs in areas such as environmental monitoring, healthcare, and food safety by offering quick, reliable, and simple testing solutions.

Paper, an abundant and inexpensive cellulose-based material, is ideal for µPADs due to its micro-porous, capillary-like network that enables fluid flow without external pumps or power. These devices are fabricated by creating hydrophilic zones within hydrophobic barriers. The hydrophilic regions facilitate reactions and analysis, while the hydrophobic barriers control fluid movement and separate different test zones.

µPADs have proven particularly useful in clinical diagnostics, where they provide effective alternatives to time-consuming and expensive laboratory methods. Common detection methods include colorimetry, fluorescence, and electrochemistry. Recent advancements combine optical and electrochemical techniques to improve sensitivity and selectivity. Additionally, distance-based detection is emphasized for its instrument-free readouts, minimizing errors especially in low-resource settings.

This review summarizes the key components and principles in µPAD design and fabrication, as well as their broad applications across biological, pharmaceutical, and environmental fields. Their simplicity, speed, low cost, and minimal training requirements make µPADs a promising tool for widespread analytical use.

Keywords: Microfluidic paper-based analytical devices (µPADs), fabrication techniques wax printing, electrochemical detection, colorimetric detection, Point-of-care testing (POCT)

Introduction

Paper has been used as a substrate material in analytical testing for centuries, with scientific reports dating back to the early 1800s with litmus paper [1], and related porous hydrophilic materials offering unique advantages over traditional device materials, including power-free fluid transport via capillary action, a high surface area to volume ratio that improves detection limits for colorimetric methods, and the ability to store reagents in active form within the fiber network, enabling applications ranging from spot tests for metals [2] and paper chromatography [3] to lateral flow immunoassays [4]. Although largely ignored for microfluidic assays until 2007 when Martinez *et al* [5]. Reported the first microfluidic paper-based analytical device (µPAD) using hydrophobic photoresist to define hydrophilic flow channels for directing sample from an inlet to a defined location for subsequent analysis, this simple yet elegant development highlighted paper's potential for low-cost and portable applications where such features are critically important. [6] Microfluidic technology enables precise control and manipulation of microscale fluids on platforms like microfluidic chips, with traditional versions based on glass, silicon wafers, or polymers exploiting microscopic fluid properties, yet suffering from complex operation, poor flexibility, and high manufacturing requirements, whereas paper-based microfluidic chips, primarily made of paper fiber and controlling fluid via capillary force, overcome these disadvantages while

providing advantages like low cost, ease of manipulation, better biocompatibility, and good background contrast for colorimetric reactions [7]. µPADs, developed from paper-based microfluidic chips, trace their prototype to the 1940s with paraffin channels on filter paper for pigment diffusion and separation, and to immunochromatography in the 1980s, but are widely recognized as originating from Whitesides' group at Harvard in [8], which patterned paper for inexpensive bioassays, later combined with scanning devices for quantitative analysis, introduced three-dimensional (3D) stacking with double-sided tape, and proposed wax printing for simple low-cost fabrication alongside Lin's group, with further advances including electrochemical detection via printed electrodes by Dungchai *et al*., origami-based 3D assembly by Liu *et al*., and integration of nanomaterials, sealing devices, advanced sensors for fluorescence, electrochemical, and colorimetric sensing to address limitations and promote applications in biological and environmental analysis. *In vitro* diagnostics (IVD) involves analyzing human body samples to obtain biochemical and molecular parameters for diagnosis and treatment, traditionally requiring complex large instruments that hinder widespread use, thus shifting toward point-of-care testing (POCT) characterized by fast detection, low cost, and simple operation, where µPADs are rapidly growing due to meeting POCT demands; meanwhile, drug analysis [9, 10]. is fundamental to drug discovery, development, monitoring, and patient use in a global pharmaceutical market valued at U$930 billion in 2017 and projected to reach U$1.2 trillion by 2021[11], driven by rising chronic diseases, GDP growth, and lower regulatory barriers, with conventional techniques like HPLC, GC, CE, and MS being effective but expensive [12-13], sample-intensive, and lab-confined, prompting the rise of microfluidic devices including paper-based ones since 2007 for their low cost, rapid analysis, low reagent/sample consumption, high throughput, portability, automation [14, 15], wide availability, straightforward fabrication, self-pumping via capillary action, and easy disposal, applicable to diagnostics, environmental testing, food, pharmaceutical, and clinical samples, with emerging technologies focusing on fabrication, detection methods, and drug-specific analysis to overcome challenges in qualitative and quantitative determination across matrices.

Fabrication of paper microfluidic device

Selection of paper substrate

Various paper types with distinct properties are commonly employed in fabricating microfluidic paper-based analytical devices (mPADs) for drug analysis (Fig.1). Among these, Whatman filter papers stand out for their affordability and ready availability [16, 17], differing in key physical attributes such as thickness, porosity, and wicking rate [18] which directly influence sample transport speed within the device. Whatman Grades 16, 18, 19, and 20 are frequently used in paper-based sensors, including those for drug detection [1, 17, 20, 21], with selection often justified primarily by low cost and ease of patterning using simple printing methods [22, 23]. Pratiwi *et al*. found no notable performance difference when using Whatman Grades 1, 4, or 6 to make colorimetric dipsticks for allopurinol detection [20], and Evans *et al*. similarly observed comparable color development from glucose oxidase-peroxidase-KI reactions on Grades 1 and 4 [16]; however, Grade 3 yielded markedly weaker signals due to its roughly double thickness, as colorimetric readout depends heavily on surface-localized analytes. In contrast, Boehle *et al*. achieved better sensitivity with Grade 1 over Grade 4 in an enzyme-based mPAD, likely owing to Grade 1's slower wicking, which enhances reaction time, reagent rehydration, and release [24]. Whatman No. 1 chromatography paper shares thickness and retention traits with its filter counterpart but lacks whitening or strengthening additives, minimizing chemical interference [16], and has proven effective for on-device analyte separation, including electrophoretic resolution of dyes and proteins in 3D folded mPADs, with similar success on Whatman Grade 3 MM chromatography paper and glass fiber; specialized ion-exchange papers like Whatman P81 (cationic) and VWR 413 further enable pKa- or charge-based separations of compounds such as ascorbic acid and dopamine. Office copy paper offers an ultra-low-cost alternative cutting device expense by >90% versus Whatman No. 1 chromatography paper while remaining widely accessible, though additives like clay, calcium carbonate, rosin, and alum (added for brightness, printability, and water resistance) must be evaluated for potential assay interference. To guide fluid flow, hydrophobic barriers are created on hydrophilic paper using techniques such as wax printing [25], screen printing [19], inkjet printing [26], flexographic printing, wax dipping [23], and manual barrier drawing with wax pens, permanent markers, or correction fluid older methods like photolithography and plasma treatment, though effective, require costly equipment and are less practical. Most mPADs feature distinct sample inlet and detection zones linked by channels [20, 21, 24], suitable for multiplexed or multi-step assays, while some adopt simpler spot-test designs where sample addition and detection occur in the same area; beyond lateral flow in 2D devices, vertical flow is common in stacked multilayer formats. Wax patterning dominates drug-analysis mPADs [21, 24] due to its simplicity and compatibility with commercial wax printers or manual application for instance, Musile *et al*. wax-printed Whatman No. 1 chromatography paper, then laminated it at 160 °C to embed barriers for psychotropic drug detection, while hot-plate melting is routine, Narang *et al*. hand-drew wax barriers on Grade 1 paper and heated at 150 °C, De Oliveira *et al*. used craft-cut masks and brief heat-pressing at 70 °C after wax dipping, and Petroni *et al*. applied preheated metal stamps onto paraffin-coated copy paper. Despite wax's versatility, organic solvents can dissolve it, compromising barriers; to address this, Primpray *et al*. laser-cut Whatman SG81 into solvent-resistant free-standing mPADs for dexamethasone and prednisolone separation, and alternative solvent-compatible barriers include silicone resins and hydrophobic methylsilsesquioxane sol-gels [16].

Types of paper substrates used in µPADs for drug detection.

Paper substrate	Type of material	Thickness (µm)	Pore size (µm)	Flow rate	Analyte tested
Whatman 1 filter	Cellulose	180	11	NS*	Amoxicillin, ampicillin, allopurinol, ascorbic acid, ibuprofen, paracetamol, ketamine
Whatman 4 filter	Cellulose	210	25	NS*	Allopurinol, norepinephrine
Whatman 6 filter	Cellulose	180	3	NS*	Allopurinol
Whatman 1 chromatography	Cellulose	180	11	130 mm/30 min	Alprazolam, ascorbic acid, clenbuterol, clencyclohexerol, diclofenac, doxycycline, flucytosine, neomycin, norfloxacin, ofloxacin, oxytetracycline, ractopamine, salbutamol, terbutaline
Whatman P81	Cellulose phosphate	230	NS*	125 mm/30 min	Paracetamol
Whatman SG81	Cellulose, large pore silica	270	NS*	110 mm/30 min	Dexamethasone, prednisolone
VWR 413 filter	Acid-treated cellulose	NS*	5	NS*	Ascorbic acid, dopamine
Office copy paper	Cellulose	NS*	NS*	NS*	Caffeine, paracetamol, phenacetin
Vegetal paper	Acid-treated cellulose	NS*	NS*	NS*	Metamizole, midazolam, paracetamol
Pura-bind RP	Nitrocellulose	NS*	NS*	NS*	Toltrazuril
MF-Millipore membrane filter	Mixed cellulose esters	150	0.22	18 mL/min.cm^2	Acetaminophen
Glass fiber filter	Borosilicate	NS*	NS*	NS*	Tetracycline

Fig 1: Types of paper substrates used in µPADs for drug detection

Deposition of assay components:

Reagents can be added to mPADs using either straightforward manual techniques or more sophisticated printing methods. The simplest approach is to pipette or drop-cast the solutions directly onto designated areas of the device. This method requires no fancy equipment, but it frequently results in uneven reagent spread, which can compromise the reliability of results. A frequent drawback is the so-called coffee-ring effect, where drying droplets deposit solutes in a ring-like pattern around the edges. To promote more uniform distribution during drop-casting, additives such as polyethylene oxide, polyvinyl alcohol, polyvinylpyrrolidone, chitosan, or polyelectrolytes are incorporated into the reagent mixes [26]. In contrast, printing techniques offer much better consistency and precision. Though still uncommon in mPADs designed for drug analysis, ink-jet printing and related approaches greatly improve device-to-device reproducibility, allow for the creation of complex sensor layouts, and pave the way for large-scale manufacturing [27]. For instance, researchers have ink-jet printed silver nanoparticle ink onto Whatman No. 1 chromatography paper to produce a plasmonic platform for Raman-based drug detection [28]. In a similar vein, the technique has been used to deposit polyaniline onto screen-printed carbon electrodes in electrochemical mPADs for detecting ascorbic acid [29].

Fabrication Techniques

Based on recent advancements in the field of paper-based microfluidics, the fabrication techniques can be broadly categorized into two types: (i) chemical patterning for creating barriers by blocking the pores inside the paper and (ii) physical patterning or cutting to form a defined channel shape are represented in figure 2.

Fabrication Techniques	Equipment	Reagents	Advantages	Drawbacks
Photolithography	Lithography equipment, mask aligner, hot plate	Positive or negative photoresist	High resolution	Expensive equipment and reagents, complex steps
Wax Printing	Wax printer, hot plate	Solid wax	Simple and fast fabrication process	Low resolution, requires a heating step
Plasma Treatment	Vacuum plasma reactor, masks, hot plate, microplasma generator device	AKD, fluorocarbon	Reduces the cost of materials such as AKD or fluorocarbon	High cost, requires masks depending on different designs
Plotting	Plotter	Hydrophobic ink (PDMS, wax), permanent marker, pen	Low cost, a physically flexible device	Low resolution, unstable liquid barrier
Inkjet Printing	Customized inkjet printer	Hydrophobic chemical, AKD, UV curable acrylate ink	High resolution, requires only a printer to fabricate µPAD	Requires customized inkjet printers
Laser Printing	Laser printer	Commercial toner	High resolution, simple to print using commercial device	Mostly requires additional heating step, limitation of materials
Flexographic Printing	Customized printing equipment	Polystyrene, PDMS	Applicable to roll-to-roll process, no requirement for heating step	High cost, requires complex preparation and cleaning, printing quality depends on surface roughness of paper
Stamping	PDMS or metallic stamp	Commercial ink	Low cost, easy to fabricate, ink storage capability, suitability for rapid prototyping in lab environment	Inconsistent results, low resolution, requires a preparation step
Chemical Vapor-phase Deposition	Deposition equipment	Hydrophobic chemicals such as poly(o-nitrobenzyl methacrylate), PPX, chlorosilane	Hydrophilic channels in paper are not affected by solvents, simple steps	High-cost instrumentation
Wet Etching	Mask	TMOS, NaOH	Simple, quick	Low resolution, requires a mask depending on the design
Hand-held Corona Treatment	Corona generator, PMMA mask, nitrogen gun	OTS, hexane, water, nitrogen	Quick, cost effective, simple	Hard to mass produce, requires washing step
Screen-printing	Mask for patterning	Wax, UV curable ink, carbon, silver/silver chloride	Low cost, simple fabrication steps	Low resolution, unadaptable to mass production
3D Printing	3D printer	PDMS, 3D printer resin	Fast and accessible to mass production	Resolution depends on 3D printer, expensive 3D printing machine
Spraying	Acrylic mask, UV/Vis light	Commercial water repellent product, scholar glue	Easy-to-use, equipment-free method	Low resolution and uniformity
Knife Plotter	Computer, plotter	None	Sharp boundary, simple, reduces the fabrication time, can be scaled up	Wastage of remaining paper, requires additional barrier or cover

Fig 2: Fabrication Techniques and Their Conditions

Chemical Patterning

Photolithography: Whitesides and his team reported the first chip fabricated via photolithography in 2007, aiming to enable multiplexed assays on a single platform unlike traditional lateral flow paper strips. They selected photolithography for its accessibility, with plans to transition to alternative printing methods as outlined in later techniques. In the standard process, chromatography paper was immersed in SU-8 photoresist, then heated to evaporate cyclopentanone. UV exposure through a photomask was followed by a 95 °C post-bake to crosslink exposed regions. With photoresist embedded in the paper, cleanup involved propylene glycol monomethyl ether acetate, propanol, and oxygen plasma to restore hydrophilicity. To achieve a faster, simpler, and cheaper alternative, Whitesides' group developed fast lithographic activation of sheets (FLASH) [30], which substituted costly cleanroom photolithography with a basic UV lamp and hot plate. Another approach created hydrophobic surfaces by thermal deposition of TiO2 nanoparticles (NPs), then used a mask and UV irradiation to photocatalytically form hydrophilic channels [31]. Sones *et al*. explored laser-based patterning, directly writing on light-sensitive polymer-soaked paper with a UV laser to achieve 80 µm channel resolution in paper-based microfluidics. More recently, Rapp *et al*. introduced a photolithographic technique using a silane-photosensitizer mixture to define hydrophobic barriers [32], offering rapid processing and enhanced flexibility through localized wettability control.

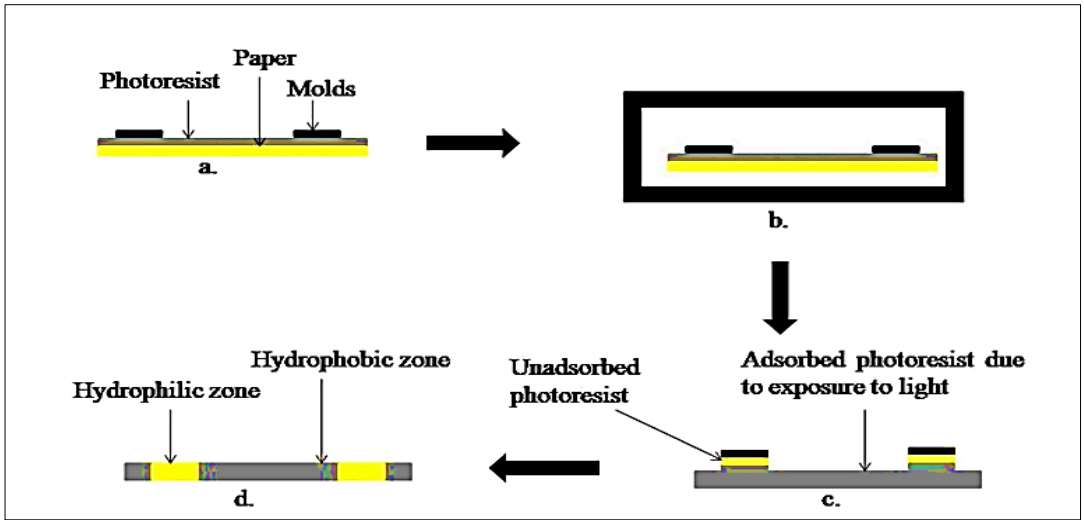

Fig 3: Photolithographic fabrication procedure

Photolithographic fabrication procedure: (a) paper, photoresist polymer, and moulds are held together, (b) exposure to radiation is done in contained chamber, (c) photoresist gets adsorbed in the areas exposed to radiation and unexposed region is left itself as such, and (d) completely fabricated PAD are showed in figure 3.

Wax Printing:

The prohibitive cost and susceptibility to hydrophilic channel contamination have rendered photolithography impractical for mass production. Nonetheless, its fundamental approach of establishing barriers within porous paper can be economically replicated using alternative materials. Wax was the first such material evaluated, patterned onto paper via a wax pen or printer [33], followed by heating on a hot plate or in an oven to enable wax penetration and formation of hydrophobic barriers. To mitigate contamination in open wax-printed channels, Kevin *et al*. subsequently devised an encapsulation strategy by toner-printing both sides of the wax-patterned paper, facilitating safe handling, preventing reagent evaporation, and sustaining solution concentration (Figure 2A) [34, 35]. Parallel techniques utilized metallic masks for molten wax pattern transfer [34]; notably, Songjaroen *et al*. employed laser cutting to fabricate an iron mold, sandwiched the paper between the mold and a glass slide, dipped the assembly in molten wax for one second, and, after cooling, separated the components to produce a finely patterned µPAD [34].

Plasma treatment

Plasma Treatment: To make the paper hydrophobic, it was first immersed into alkyl ketene dimer (AKD) with heptane solution, in a previous study [35]. It was allowed to dry and then heated at 100 ◦C for 45 min for curing the AKD, resulting in an entirely hydrophobic paper. The hydrophilic regions were then defined by plasma treatment with the help of metallic masks with the desired channel layout. Furthermore, plasma treatment was used to control the wettability of patterned paper, to create paper-based circuits [36]. In another method, fluorocarbon plasma polymerization was alternatively used for making the hydrophobic channel boundaries [37]. Positive and negative metal masks were used for better confinement of the liquid by the hydrophobic walls surrounding it from three sides instead of two. The advantages of this treatment are the simplicity of fabrication as well as the cost effectiveness of the reagents. However, the metal masks can be expensive; the plasma treatment also requires vacuum and expensive instrumentation. Circumventing these limitations, Kao *et al*. developed a portable, inexpensive, battery-operated microplasma generator device for fabricating µPADs [38].

3D Printing: Fu and his team used 3D printing to build a tiny paper-based device called a µPAD [39]. They started by printing a base with little channels in it, then brushed on some PDMS to close up the small gaps left by the printer. Next, they poured in a mix of cellulose powder and deionized water into those channels and let it dry in an oven. What's cool about this method is that it's super cheap,

quick, and easy to scale up just using a regular desktop 3D printer. Meanwhile, He and colleagues went with a desktop stereolithography 3D printer and a dynamic mask to make their µPADs [40]. They soaked the paper in UV resin first, shone UV light through the mask to pattern it, and then cured it to finish the job.

Physical Patterning

- **Knife Plotter**: Fenton and the team used a computer-guided knife plotter to neatly slice paper into the exact patterns they needed [41, 42]. To keep the paper from curling or ripping, they made three careful passes with the blade. It's a straightforward trick that speeds things up and lets them churn out tons of devices at once just cut a big sheet into dozens of tiny ones, perfect for cranking out loads in a factory.

- **Craft Cutting:** In a similar vein, Cassano and Fan used a digital craft cutter to whip up laminated paper-based analytical devices (LPADs) [43]. They kicked things off by gluing the paper to an adhesive sheet, then let the cutter carve out neat strips. The top and bottom covers got the same treatment cut, stacked, and laminated into a finished LPAD. When dealing with fragile stuff like nitrocellulose, they suggest slapping on a sacrificial layer on top to get nice, clean cuts without any tears [43]. It's a simple hack that saves material and shaves time off the process.On the other hand, Glavan and crew took omniphobic paper and a craft cutter to carve open channels straight into the surface [44]. They coated the cut zones with fluoroalkyltrichlorosilane to make them repel both water and oil, then sealed everything up with tape. The payoff? Super lightweight, flexible devices that are easy to carry around and cheap to throw away. The catch: unlike typical paper microfluidics that pull liquid along by themselves, these need an external pump to move things. And tweaking channel widths with just one blade? Not the easiest thing in the world.

- **Laser Cutting:** A quick zap from a laser turned hydrophobic paper into microfluidic patterns as tiny as 62 µm [45]. Using a CO2 laser cutter, the team carefully sliced and etched surfaces like parchment, wax, or palette paper. They fine-tuned the power and speed so the laser wouldn't punch all the way through just enough to shape the pattern. Then, they dusted the etched areas with silica microparticles to make them water-loving.
  In another clever setup, researchers used a backed nitrocellulose membrane sandwiched between two protective polymer sheets to prevent it from catching fire [46]. A CO2 laser then zapped away material to carve out the design. It's fast, works in both labs and factories, but scaling up for mass production is tough you'd need a bunch of laser systems running at once. To keep things even simpler, Nie and the team went with a one-step cut-and-engrave using a compact CO2 laser [47]. They sliced clean through the paper to create hollow, patterned structures that acted just like hydrophobic barriers. Bedin and colleagues used a 30W CO2 laser to craft the top and bottom layers one for fluid entry, the other for flow then stuck them together with adhesive film [48]. They even used laser, etching the glass-fiber bottom layer with the laser to control its thickness and shape precise reaction zones. The perks? Each device is done in about 20 seconds, the cuts are super consistent, and the laser machine won't break the bank.

Detection techniques

Detection techniques used in µPADs A number of detection techniques have been coupled with µPAD for various applications (Nery and Kubota, 2013). These techniques can be broadly categorized into optical and electrochemical detection techniques.

Optical detection

Optical detection has become the go-to method in labs because optical tools are everywhere [49]. It works by measuring things like light absorption or transmission, fluorescence, surface plasmon resonance, or chemiluminescence (CL). To pick up these signals, researchers often pair paper-based devices with microscopes, cameras, scanners, lasers, spectrophotometers, CCDs, or photomultiplier tubes. While many substances naturally interact with light, biosensing usually needs a labeled molecule that binds to the target and creates a visible optical signal [50a, b]. In many cases, simple color changes driven by chemical or biological reactions, like enzyme activity are used (this is colorimetry). The white, high-contrast paper background makes these color shifts easy to see, which is why colorimetric paper sensors are so popular in research today [51]. These assays can detect a wide variety of substances thanks to readily available reagents, good sensitivity, and fast results that even semi-trained users can interpret. You can often get a quick yes-or-no answer just by looking no equipment needed. But naked-eye readings can vary depending on who's looking and the lighting conditions. To fix that, a printed color reference chart or standard palette can be added right on the device, so anyone can compare the result objectively [50 a b]. Some reactions follow the Beer-Lambert Law, meaning color intensity scales directly with concentration, allowing real quantitative measurements on µPADs. For accuracy, devices like CCDs, CMOS sensors, scanners, or even smartphones capture the color, and software like ImageJ measures intensity; the value is then plugged into a calibration curve to calculate the exact analyte level [51]. One big advantage? No fancy gear is required your eyes are enough for qualitative or semi-quantitative checks. Many classic chemical or enzyme-based tests can be adapted for these sensors. The downside? Some reactions aren't very specific or sensitive they target chemical groups, not individual molecules and visible color changes can come from many sources. Overall, paper-based color detection lags behind lab instruments like UV-vis spectroscopy or HPLC in accuracy [52]. At very low concentrations, colorimetry often isn't sensitive enough (Giri, 2016), though enzymes or nanoparticles can boost performance in some cases (Ferreira *et al*., 2015). A few µPADs use fluorescence instead; for example, Yamada *et al*. (2013) deposited TbCl3 as a sensing and glowing agent, with NaHCO3 to enhance the signal. Studies show paper beats plastic for fluorescence work it's cheaper, thinner, and needs less sample [51]. Raman spectroscopy has been used too; wavelength-modulated Raman spectroscopy (WMRS) detected paracetamol and ibuprofen down to nanomolar levels. Surface-enhanced Raman scattering (SERS) substrates have been printed onto µPADs using nanoparticle

or nanorod inks [49], but it only works well with even coverage. Chemiluminescence (CL) offers great sensitivity and range; for instance, silver nanoparticles improved detection reliability and simplicity in a luminol-H2O2 system for measuring ofloxacin in eye drops [52]. (Liu *et al*., 2015a, 2015b).

Electrochemical sensing

Electrochemical detection paired with μPADs also known as electrochemical paper-based analytical devices or ePADs provides a budget-friendly way to achieve highly sensitive and selective measurements [50]. These clever devices blend the perks of paper-based systems, like being cheap, needing only tiny sample volumes, using capillary action to move fluids, and allowing multiple tests at once, with the power of electrochemical methods, including top-notch sensitivity, precise quantitative results, quick responses, and versatility across many analytes [54]. Typically, a three-electrode setup is printed right onto the paper, using techniques such as screen-printing, stencil printing, sputtering, sticking on metallic wires, or growing nanoparticles [50]. The main ways these devices generate signals are through voltammetry, amperometry, and potentiometry [50]. Recently, potentiometry has made its way into paper-based microfluidics: you simply press a solid-contact ion-selective electrode and a reference electrode against filter paper, which soaks up the sample and acts as both the intake point and holding area during measurement [55]. The paper's fibers naturally filter out big particles, making it ideal for ion-selective sensing. Paper-based electrochemiluminescence (ECL) has also gained a lot of attention lately because it combines the best of luminescence and electrochemistry think ultra-high sensitivity and a huge range for concentration measurements [53]. For instance, Wu *et al*. created a foldable, microfluidic paper-based ECL "origami" device with aptamer-coated 3D porous gold-paper electrodes to selectively grab cancer cells, while Zhang *et al*. built a 3D paper device that detects lead and mercury at the same time using ECL nanoprobes, all at point-of-care costs; you can even snap a photo of the glow with a smartphone after sealing it in clear plastic. These ECL paper platforms are now used to monitor environmental contaminants like 2-(dibutylamino)-ethanol, NADH, explosives, genotoxic compounds, Pb(II), and Hg(II)[50a, b]. Overall, electrochemical sensing stands out for compact analytical tools second only to colorimetry in paper-based applications thanks to its sensitivity, accuracy, low cost, easy fit into microfluidic designs, and minimal power use. That said, the electrodes can be finicky, reacting to changes in temperature, pH, or salt levels, which may shorten shelf life. Unlike colorimetric readouts, electrochemical ones cut through background noise in portable devices and aren't bothered by light, dust, or insoluble particles [51]. You can also switch from a still assay to continuous flow simply by attaching a strip of blotting paper to the channel exit, keeping fluid moving across the electrodes a setup that helps plate metals, clean electrodes, and works well under flow or stirring conditions. Paper devices even let you add electrolyte from behind the electrodes, creating a three-phase boundary between electrolyte, electrode, and air [56]. The natural roughness and porosity of paper boost the electrode surface area for better signals, though that texture can make secure electrode attachment tricky; and in cases where the electrode material gets used up during testing, limited loading can mean a shorter device lifespan [46a, b].

Camera-based readout devices

When a color signal appears on a colorimetric paper device, you simply take a picture of the assay area and analyze it to get quantitative results. Scanners are a popular choice for this because they deliver sharp, high-resolution images with perfect focus every time. Best of all, external lighting doesn't interfere with the image intensity [56, 50a b]; [55]. Smartphone cameras are another hugely popular option for capturing signals on paper devices (Erickson *et al*., 2014). Once the reaction finishes on the microfluidic device, the color change is easy to spot using a simple app on your phone. While smartphones win hands-down for portability and ease of use compared to flatbed scanners, they can be thrown off by shifting ambient light, leading to inconsistent image brightness [56 above 50 a b]. Luckily, you can fix this with intensity-correction software or by snapping the photo inside a light box that blocks outside light (Erickson *et al*., 2014). In many setups, the phone's built-in flash gives you steady, reliable lighting for accurate results. Today's smartphones come ready with both an LED flash and a high-quality camera, making them a fantastic all-in-one tool for image capture. People are now using camera phones to detect everything from phage and bacterial pathogens to pharmaceuticals, biomarkers, explosives, and toxic metals (Cate *et al*., 2015a, 2015b; Sicard *et al*., 2015). You can even use a regular digital camera as a detector with automatic white balance though it struggles to focus on anything closer than about 20 cm (Cate *et al*., 2015a, 2015b). Charge-coupled device (CCD) cameras have been paired with paper devices for optical readouts too. These are straightforward, affordable, and sensitive enough to scan large areas. In fact, their sensitivity matches that of ELISA plate readers. The catch? High-end CCD cameras drive up costs, making them less ideal for low-resource settings [54]. Webcams have also stepped up as versatile tools working as sensitive plate readers, microscopes, or even lens-less detectors for diagnostics. When you need extra sensitivity, smart image-processing software can boost the webcam's modest performance [55]. You'll find sensitive, multi-wavelength webcam-based fluorescence readers in labs everywhere, detecting biological and chemical assays through absorbance, fluorescence, or luminescence with a capable optical sensor [56].

Distance-based detection

Distance-based detection is refreshingly simple: no extra gear needed to read the signal on a paper device. That's why people call it a "chemometer" just like reading temperature on a thermometer. This approach is a game-changing, ultra-simplified way to get quantitative results from paper-based analytical devices (PADs) [57 above 50a 2b]. Here's how it works: colorimetric reagents are engineered to form a precipitate when they bind the target analyte. As the sample flows along the channel, the analyte gets used up bit by bit, leaving a colored band behind. The length of that band directly reflects the analyte concentration. You just measure it with a ruler. Reading length instead of color intensity cuts down on user error big time (Fu, 2014). Researchers have used this method to measure heavy metals, small biomolecules, reactive oxygen species, theophylline in blood and serum, cholesterol, streptavidin, and more [61].

Nanoparticle-based detection

Noble metal nanoparticles shine thanks to their incredible surface-to-volume ratio, opening doors to powerful biosensors, imaging agents, and drug delivery systems. They deliver detection that's not only robust but also far more sensitive and selective than traditional methods [55 above 46a, b]. Take silver nanoparticles (AgNPs), for example they've been used to detect ascorbic acid right on a paper device. Ascorbic acid is a go-to antioxidant in pharmaceuticals, cosmetics, and food. In one study, researchers prepared AgNPs and embedded them in paper sensors. When ascorbic acid was added, the color shifted from pale yellow to gray as the nanoparticles grew and clustered together [60].

Applications in different fields

Analysis of Biological Fluids

1.Detection of Creatinine in Urine

Creatinine is a critical biomarker for assessing kidney function, as it is an end product of creatine metabolism excreted at a constant rate through glomerular filtration. Abnormal creatinine levels in urine indicate potential kidney dysfunction, and its concentration also serves as an index for urine dilution. Traditional methods, such as Jaffe's reaction, lack specificity and require sophisticated lab setups. In 2015, Talalak *et al*. developed a low-cost, enzymatic paper-based analytical device (μPAD) for creatinine detection in urine. The method utilizes enzymes creatininase, creatinase, and sarcosine oxidase to generate hydrogen peroxide (H2O2) from creatinine, which then reacts with 4-aminophenazone and HTIB to produce a pink-red quinoneimine dye. This dye is detected colorimetrically using an inkjet scanner, offering a linearity range of 2.5-25 $mg \cdot dL^{-1}$ ($r^2 = 0.983$) and a limit of detection (LOD) of 2.0 $mg \cdot dL^{-1}$. Although 10-20 times less sensitive than conventional methods, this approach eliminates the need for complex instruments, trained personnel, or tedious sampling. Its portability, cost-effectiveness, and simplicity make it ideal for low-income and developing countries, enabling point-of-care kidney function monitoring with minimal resources. [62]

2. Detection of Thiocyanate in Saliva

Thiocyanate is an endogenous molecule found in physiological fluids, with elevated levels in saliva linked to tobacco smoke exposure due to cyanide detoxification in the liver. Conventional detection methods, such as UV-Vis spectrophotometry, fluorimetry, and atomic absorption spectroscopy, are time-consuming, solvent-intensive, and produce significant waste. In 2016, Pena-Pereira *et al*. introduced a credit card-sized paper-based analytical device with 40 test zones for thiocyanate detection in saliva. Fabricated on Whatman No. 1 filter paper, the device uses pre-loaded Fe(III) reagent to form a red iron(III)-thiocyanate complex, detected colorimetrically via an HP-4500 desktop scanner/printer. The blue channel was selected for intensity recording after comparing grayscale, red, green, and blue channels, achieving an LOD of 0.06 mM and an RSD of 3%. Recovery studies showed values between 96.1-103.6%, comparable to UV spectrophotometric methods, with no significant analytical differences. This method is time-efficient, uses minimal solvents, and provides reproducible results, making it a cost-effective and reliable alternative for monitoring smoking-related biomarkers in resource-limited settings. [63]

3. Detection of SARS-CoV-2 Humanised Antibody

The SARS-CoV-2 virus, responsible for COVID-19, necessitates rapid and accurate diagnostic methods to control its spread, particularly in underdeveloped regions. The WHO recommends reverse transcription polymerase chain reaction (RT-PCR) for detecting viral RNA, but it requires trained personnel, advanced instruments, and is costly. In 2020, Kasetsirikul *et al*. developed a paper-based ELISA for detecting SARS-CoV-2 humanized antibodies in serum, offering a low-cost, user-friendly alternative. The device, coated with recombinant SARS-CoV-2 nucleocapsid antigen and laminated for easy handling, uses a colorimetric reaction with 3, 3′, 5, 5′-tetramethylbenzidine (TMB) substrate and horseradish peroxidase (HRP)-conjugated antibodies. Digital image processing enables naked-eye inspection and quantifiable results via MATLAB R2018b. The assay achieves an LOD of 9 $ng \cdot L^{-1}$ (0.122 $IU \cdot mL^{-1}$) with an RSD below 10%, outperforming commercial ELISA kits (LOD: 5 $IU \cdot mL^{-1}$, RSD: 15-20%). This method's small sample volume, simplicity, and high sensitivity make it ideal for rapid COVID-19 diagnosis in low-resource settings, facilitating timely quarantine and treatment. [64]

Analysis of Foods and Food Products

1. Detection of Pesticides in Vegetables

The rising global population has increased food demand, leading to widespread use of organophosphate (OP) pesticides like methyl-paraoxon and chlorpyrifos-oxon in agriculture. These chemicals enhance crop yield but pose health risks when present as contaminants in food. Traditional detection methods, such as HPLC and LC-MS/MS, are accurate but require expensive equipment and skilled operators. In 2016, Nouanthavong *et al*. proposed a nanoceria-coated paper-based analytical device for detecting OPs in vegetables like cabbage and food like dried green mussels. The method relies on acetylcholinesterase and choline oxidase activity reduction, producing H2O2 that triggers a color change in ceria particles. Analyzed via colorimetric detection, it achieves LODs of 18 $ng \cdot mL^{-1}$ for methyl-paraoxon and 5.3 $ng \cdot mL^{-1}$ for chlorpyrifos-oxon. Recovery rates of approximately 95% align closely with LC-MS/MS results, confirming reliability. This approach is simple, cost-effective, and does not require sophisticated instruments, making it suitable for routine food safety testing in resource-constrained environments. [65]

2. Detection of Nitrite and Nitrate in Food Samples

Nitrite and nitrate salts are commonly used as preservatives and flavor enhancers in food, but excessive intake poses health risks, necessitating reliable detection methods. Traditional spectrophotometric methods are effective but require organic solvents and complex equipment. In 2020, Thonkam *et al*. developed a beeswax-based μPAD for simultaneous nitrite and nitrate detection in food using the Griess method. Beeswax emulsion, applied via screen printing on filter paper, forms hydrophobic barriers to define hydrophilic zones. Nitrite is detected by derivatization with sulphanilamide and N-(1-naphthyl) ethylenediamine, while nitrate is reduced to nitrite using vanadium (III) salt before detection. Color intensity is measured with ImageJ, yielding LODs of 0.1 $mg \cdot L^{-1}$ for nitrite and 0.4 $mg \cdot L^{-1}$ for nitrate,

with RSDs of 1.8-3.1%. This solvent-free, reproducible method closely matches spectrophotometric results and is ideal for routine food safety analysis due to its simplicity and low cost. [66]

3. Detection of Norfloxacin Residues in Food

The rise in large-scale animal agriculture has increased the use of antibiotics like norfloxacin, a fluoroquinolone, leading to risks of antibiotic resistance and bioaccumulation in humans. Traditional detection methods (e.g., spectrofluorometry, HPLC-MS) are labor-intensive and costly. In 2020, Trofimchuk *et al*. introduced a wax-printed µPAD utilizing the coffee ring effect to detect norfloxacin in meat and fish. The device, made on cellulose-based filter paper, uses iron (III) nitrate in 5 mM ammonia to induce a colorimetric shift upon reaction with norfloxacin. Images are analyzed with ImageJ, achieving LODs of 50 ppm (inner ring) and 5 ppm (outer ring). This method is faster, cheaper, and more user-friendly than conventional techniques, requiring minimal sample preparation. Its high sensitivity and portability make it suitable for routine monitoring of antibiotic residues in food supply chains, especially in resource-limited settings. [67]

Analysis of Water Samples

1.Determination of Reactive Phosphates in Water

Water pollution, particularly from phosphates, threatens potable water availability, critical for sustaining life. Traditional methods like HPLC/UPLC are reliable but require time, expertise, and costly equipment. In 2012, Jayawardane *et al*. developed a wax-printed µPAD for detecting reactive phosphate species in water via phospho-molybdenum blue formation. The device operates in a concentration range of 0.2-10 $mg \cdot L^{-1}$ of phosphorus, with an LOD of 0.05 $mg \cdot L^{-1}$ and an LOQ of 0.16 $mg \cdot L^{-1}$. Colorimetric detection ensures reproducible and reliable results compared to standard methods, with minimal solvent and equipment needs. This approach is highly portable and cost-effective, making it suitable for routine water quality testing in low-resource settings where access to advanced instrumentation is limited. [68]

2. Detection of Nitrate in Water

Nitrate, a natural component of the nitrogen cycle, contributes to water pollution when present in high concentrations from sources like fertilizers and wastewater. Excessive nitrate in drinking water is linked to health issues like colorectal cancer and thyroid disorders. Conventional detection methods are time-consuming and require specialized equipment. In 2020, Charbaji *et al*. developed a wax-printed µPAD with a folding architecture for nitrate detection in water. The folding design enhances detection zone uniformity, achieving an LOD of 0.53 ppm and an LOQ of 1.18 ppm. This simple, low-cost device requires minimal sample preparation and no trained personnel, making it ideal for routine water quality monitoring in areas with limited resources. [69]

3. Detection of Bisphenol A (BPA) in Water

Bisphenol A (BPA), an endocrine-disrupting compound used in plastics and coatings, poses health risks like diabetes and cancer when present in water. Traditional detection methods (e.g., HPLC, ELISA) are sensitive but time-consuming and expensive. In 2020, Jemmeli *et al*. proposed a wax-printed electrochemical µPAD with carbon black-modified ink for enzyme-free BPA detection. The sensor, integrated with a full electrochemical cell on filter paper, detects BPA at microgram levels in river and drinking water. Its low-cost materials and high sensitivity make it an effective tool for rapid pollutant monitoring. This method's simplicity and portability enable routine water safety assessments, particularly in regions lacking advanced analytical infrastructure. [70]

Recent Advancements

1. Paper-based Pump-Free Magnetophoresis

Magnetophoresis refers to the migration of particles under the influence of a magnetic field, categorized into positive magnetophoresis, where magnetic particles move in a diamagnetic medium, and negative magnetophoresis, where diamagnetic particles move in a magnetic medium. Traditional paper-based microfluidic devices (µPADs) are limited for magnetophoresis due to insufficient fluid velocities and particle entrapment within the porous structure of paper fibers, which hinders continuous separation processes. To address these challenges, Call *et al*. developed fast-flow paper-based analytical devices (ffPADs) by stacking two layers of wax-printed paper over a laser-cut double-sided adhesive, creating channels that facilitate rapid fluid flow. These channels prevent particles from becoming trapped, enabling efficient magnetophoresis. The ffPADs utilize a neodymium permanent magnet to drive the separation of magnetic particles, offering a pump-free solution that simplifies the setup compared to traditional microfluidics requiring external pumps. Call *et al*. also investigated critical performance parameters, such as flow rates and magnetic field strength, to optimize the separation process. This innovation expands the applicability of paper-based devices for analytical separations, providing a cost-effective and accessible platform for applications like diagnostics and environmental monitoring. The ability to perform magnetophoresis in a low-cost, portable format makes ffPADs particularly promising for point-of-care testing in resource-limited settings, where complex equipment is often unavailable. [71]

2. Development of 3D Fabrication

The fabrication of paper-based microfluidic devices (µPADs) involves creating hydrophobic barriers and fluidic channels for applications like lateral flow assays, analytical separations, and flow-based analysis. Traditional methods include photolithography, wax printing, inkjet printing, laser printing, flexographic printing, screen printing, and lacquer or glue spraying, all of which often require additional equipment like printers, ovens, or heat plates. Pen-on-paper (PoP) techniques have emerged as simpler alternatives, but many still necessitate post-processing steps like heating and face challenges with chemical compatibility and mechanical durability. Sousa *et al*. introduced an innovative 3D pen fabrication method, using acrylic resin to draw hydrophobic barriers directly on paper, which are then cured in under a minute using a portable flashlight, eliminating the need for extra tools or heating steps. These barriers demonstrate high chemical resilience against acids, alkalis, surfactants, and most organic solvents (except ethanol), enabling the creation of spot tests (2 mm diameter) and channels (3 mm width). Whitesides *et al*. developed 3D µPADs by stacking layers of patterned paper and double-sided adhesive tape, allowing

fluid wicking both horizontally and vertically without mixing streams, ideal for complex arrays of detection zones. Similarly, Liu *et al*. proposed an origami-based approach, constructing 3D devices from a single sheet of paper in one photolithographic step, assembled by manual folding. These advancements enhance the versatility of µPADs, enabling rapid, scalable production for applications like glucose and nitrite detection in saliva or environmental monitoring of iron, nitrite, and copper, particularly in low-resource settings. [72]

3. Development of Atom Stamp Printing and Paper-based Solid-Liquid Extraction Device

Guan and Sun introduced atom stamp printing (ASP), a novel fabrication technique for µPADs that creates precise hydrophobic barriers and hydrophilic channels using machine-etched or laser-engraved atom stamps (ASs) and polydimethylsiloxane (PDMS) as a hydrophobic solvent. ASP stands out for its low cost, simplicity, high production efficiency, and excellent resolution, achieving minimum hydrophobic barrier widths of 312 µm and hydrophilic channel widths of 328 µm. The use of PDMS, a clear liquid, prevents contamination of hydrophilic channels, unlike other methods that may leave residues. ASP-fabricated µPADs were successfully applied to detect Cu2+ concentrations as low as 1 $mg \cdot L^{-1}$ using a colorimetric method combined with distance-based detection, demonstrating high sensitivity for heavy metal analysis. Additionally, Guan and Sun developed a paper-based solid-liquid extraction device (PSED) with a "3+2" structure, leveraging paper filtration and capillary forces for efficient, recyclable extraction and filtering. This device simplifies traditional solid-liquid extraction by integrating multiple stages into a single platform, reducing processing time and complexity. The PSED's design makes it suitable for applications requiring high-throughput sample preparation, such as environmental testing or food safety analysis. Both ASP and PSED highlight the potential of paper-based platforms to offer scalable, cost-effective solutions for analytical chemistry, particularly in settings where advanced laboratory infrastructure is limited. [73]

4. Development of a Morphological Colour Image Processing Algorithm for PADs

Signal readout and detection mechanisms are critical for the functionality of paper-based analytical devices (µPADs), with color changes often assessed by the naked eye or digital image processing (DIP). Naked-eye evaluations are subjective and prone to variability, making DIP a preferred method for consistent and quantitative results. Hamedpour *et al*. developed a novel DIP algorithm based on mathematical morphology recognition to automate the selection of regions of interest (ROIs) in µPADs, minimizing errors associated with manual selection. This algorithm supports image transformation into color spaces like NTSC, YCbCr, and HSV, providing detailed quantitative descriptions of color changes. It enables automated recognition of circular and square forms, blank correction, and visual outlier detection, facilitating further chemometric analyses such as calculating limits of detection (LOD) and building regression models. Compared to traditional tools like ImageJ, the proposed algorithm significantly improves analysis speed, repeatability, and accuracy of color value measurements. This is the first study to design an image processing method specifically for µPADs, offering a robust framework for practical applications in diagnostics, environmental monitoring, and food safety. The algorithm's ability to process complex image data quickly and reliably enhances the scalability of µPADs, making them suitable for high-throughput testing in resource-constrained environments where precise, automated analysis is essential.[74]

Conclusion

Microfluidic paper-based analytical devices (µPADs) have emerged as low-cost, portable, and user-friendly platforms for rapid analysis in clinical diagnostics, food quality testing, pharmaceutical analysis, and environmental monitoring. Their ease of fabrication, capillary-driven flow, and compatibility with optical, electrochemical, and nanoparticle-based detection methods make them especially valuable in resource-limited settings. Recent advances in fabrication such as wax printing, laser cutting, 3D assembly, and atom stamp printing along with improved digital and distance-based readouts, have enhanced sensitivity, selectivity, and quantification. Overall, µPADs represent a highly adaptable and accessible technology with significant potential for widespread point-of-need analytical applications.

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