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# Paper-based sensors: affordable, versatile, and emerging analyte detection platforms

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Paper-based sensors, often referred to as paper-based analytical devices (PADs), stand as a transformative technology in the field of analytical chemistry. They offer an affordable, versatile, and accessible solution for diverse analyte detection. These sensors harness the unique properties of paper substrates to provide a cost-effective and adaptable platform for rapid analyte detection, spanning chemical species, biomolecules, and pathogens. This review highlights the key attributes that make paper-based sensors an attractive choice for analyte detection. PADs demonstrate their versatility by accommodating a wide range of analytes, from ions and gases to proteins, nucleic acids, and more, with customizable designs for specific applications. Their user-friendly operation and minimal infrastructure requirements suit point-of-care diagnostics, environmental monitoring, food safety, and more. This review also explores various fabrication methods such as inkjet printing, wax printing, screen printing, dip coating, and photolithography. Incorporating nanomaterials and biorecognition elements promises even more sophisticated and sensitive applications.

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## 1. Introduction

In the era of the internet and technological advancement, life is perpetually accelerating, and the demand for swift solutions to a wide array of challenges, problems, and health issues has never been more pressing.<sup>1</sup> In an increasingly interconnected world, individuals across different domains, from homemakers juggling household appliances to scientists conducting cutting-edge research, engineers overseeing complex construction sites, and healthcare professionals diagnosing and treating patients, all rely on a common but often unseen hero: sensors. Sensors, ranging from the commonplace to the highly specialized,<sup>2</sup> have become the linchpin of modern life, orchestrating machines and equipment with precision and enabling the success of various endeavours. They underpin a data-driven world, translating external inputs into meaningful outputs, thereby ensuring the smooth operation of an array of systems.

Broadly, a sensor can be defined as a device, module, or machine that interfaces with its environment, adeptly responding to external inputs, detecting these inputs, quantifying them, and, most crucially, transforming them into data or actions that can be stored, transmitted, displayed, or employed for diverse purposes.<sup>3</sup> The significance of accessible and easily operated sensors cannot be overstated in the context of today's world, where data acquisition is pivotal across a range of sectors, from healthcare to environmental monitoring, industrial automation, disaster management, and scientific research.

While there is a multitude of sensors, each with specific applications,<sup>4</sup> this article places a spotlight on a rapidly emerging and highly versatile category: paper-based sensors. These sensors have garnered immense attention due to their unique attributes and transformative potential. With roots dating back to earlier research endeavors,<sup>5</sup> paper-based sensors epitomize a novel frontier in sensing technology. They have charmed scientists and industries alike with their simplicity, cost-effectiveness, and easy portability, all of which are redefining the landscape of analytical chemistry and diagnostic applications. At the heart of paper-based sensors lies an elegantly straightforward principle.

### 1.1 Principle of operation

**1.1.1 Chemical or biochemical impregnation.** The paper substrate is treated or impregnated with specialized chemical or biochemical reagents. These reagents exhibit a remarkable ability to selectively react with specific target analytes present

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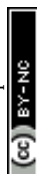
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in a sample. When such a reaction occurs, it triggers a visible change in color or intensity in the paper.

**1.1.2 Quantitative indication.** Crucially, the magnitude of the color change or intensity shift is directly proportional to the concentration of the analyte within the sample.

These attributes render paper-based sensors an ideal choice for a wide range of applications, particularly in settings where access to sophisticated laboratory equipment is limited or impractical. These sensors have found their place in a multitude of applications, from environmental monitoring to track pollution levels to healthcare, offering diagnostic solutions and point-of-care testing. Additionally, they are instrumental in ensuring food safety by providing rapid and accessible quality assessment. Their myriad advantages make them highly appealing:

**Cost-efficiency:** compared to conventional sensing technologies, paper-based sensors are more affordable and cost effective due to a number of reasons such as;

**Material cost:** the most common and affordable material used in paper-based sensors is cellulose-based paper. Paper is substantially less expensive to produce and get than other substrates used in typical sensors, such as silicon<sup>6</sup> or glass.<sup>7</sup>

**Printing techniques:** simple pen-on-paper techniques, screen printing, inkjet printing, and other printing techniques can all be used to create paper-based sensors. Due to their scalability and affordability, these methods enable the low cost per unit mass manufacture of sensors.

**Less complexity in manufacturing:** compared to their conventional counterparts, paper-based sensors frequently call for less complicated manufacturing procedures. Typically, the fabrication process entails printing or depositing sensing devices onto paper substrates, which are then simply encapsulated using techniques. The production costs are reduced as a result of this optimized manufacturing method.

Paper-based sensors are frequently made with the intention of being used only once or thrown away. The necessity for

expensive cleaning and maintenance processes connected with reusable sensors is eliminated by this disposability. In addition, paper sensors are reasonably priced for one-time usage in fields including environmental monitoring, food safety testing, and medical diagnostics.<sup>8</sup>

**Integration and miniaturization:** developments in micro-fabrication methods have made it possible to integrate and minimize sensing components on paper substrates. This integration keeps or even improves sensitivity and specificity while lowering the total cost by reducing the amount of sensing material needed for each sensor.

**Customization and versatility:** paper-based sensors are flexible in both their functional and design aspects. Customization is possible at a reasonable cost because researchers can modify the characteristics of paper substrates and sensor devices to fit certain applications. Because of its adaptability, sensors with reasonable prices can be developed for a variety of uses and sectors.

**Low power consumption:** a large number of paper-based sensors function without the need for complicated electronic components or external power sources. Rather, to identify analytes, they rely on passive methods like capillary action or colorimetric changes. Paper sensors are appropriate for environments with limited resources, such as those with expensive or restricted access to electricity, due to their low energy consumption, which also lowers operational expenses.

Paper-based sensors are generally less expensive because of their low-cost components, streamlined production methods, disposable nature, and adaptability in terms of design and integration. These qualities render paper-based sensors a desirable choice for a range of applications, especially those requiring scalable and reasonably priced sensing solutions.<sup>9,10</sup> In Table 1, a comprehensive comparison between paper and conventional substrates highlights the superiority of paper as an optimal choice for sensor development.

**Table 1** The comparison of traditional substrate with paper for making sensors

| S. no. | Property                    | Material         |                      |           |           |
|--------|-----------------------------|------------------|----------------------|-----------|-----------|
|        |                             | Paper            | PDMS                 | Glass     | Silicon   |
| 1      | Surface profile             | Moderate         | Very low             | Very low  | Very low  |
| 2      | Flexibility                 | Yes              | Yes                  | No        | No        |
| 3      | Structure                   | Fibrous          | Solid, gas-permeable | Solid     | Solid     |
| 4      | Surface-to-volume ratio     | High             | Low                  | Low       | Low       |
| 5      | Fluid flow                  | Capillary action | Forced               | Forced    | Forced    |
| 6      | Sensitivity to moisture     | Yes              | No                   | No        | No        |
| 7      | Biocompatibility            | Yes              | Yes                  | Yes       | Yes       |
| 8      | Disposability               | Yes              | No                   | No        | No        |
| 9      | Biodegradability            | Yes              | To some extent       | No        | No        |
| 10     | High-throughput fabrication | Yes              | No                   | Yes       | Yes       |
| 11     | Functionalization           | Easy             | Difficult            | Difficult | Moderate  |
| 12     | Spatial resolution          | Low to moderate  | High                 | High      | Very high |
| 13     | Homogeneity of the material | No               | Yes                  | Yes       | Yes       |
| 14     | Price                       | Low              | Moderate             | Moderate  | High      |
| 15     | Initial investment          | Low              | Moderate             | Moderate  | High      |



## 2. Selection of different substrates and nanoparticles for the synthesis of paper sensors

The selection of paper substrate and blending with different nanomaterials plays a pivotal role in the development of paper sensors, significantly influencing their performance, sensitivity, and overall reliability. The scientific community has explored a wide array of paper substrates and nanoparticles, nanotubes to design paper sensors that cater to diverse applications.

### 2.1 Cellulose paper

Regular cellulose paper, when modified with functional groups or coatings, becomes a versatile substrate for various paper sensors. This versatility allows scientists to tailor cellulose paper to meet specific application requirements. For instance, the addition of functional groups or coatings can enable cellulose paper to participate in colorimetric assays. In these assays, the paper interacts with analytes, resulting in detectable color changes that are proportional to analyte concentration. Moreover, cellulose paper can be employed in electrochemical sensors where chemical reactions at the paper's surface generate electrical signals, making it ideal for a wide range of electrochemical applications. Further the cellulose paper can be further designed to obtain filter paper and nitrocellulose paper as discussed below.

**2.1.1 Cellulose filter paper.** Filter paper's exceptional porosity and fluid-wicking capabilities make it an ideal choice for various paper-based sensors. The high porosity ensures efficient sample absorption and distribution, while its capacity to wick fluids allows for rapid and uniform interactions with analytes. Filter paper finds its application in diverse fields, including pH sensors and glucose sensors. In the case of pH sensors, filter paper can effectively detect pH changes by interacting with pH-indicating reagents. Similarly, in glucose sensors, filter paper can serve as a matrix for glucose-specific enzymes, facilitating the rapid quantification of glucose levels.

**2.1.2 Nitrocellulose paper.** Nitrocellulose paper has gained significant recognition for its role in lateral flow assays, particularly in widely-used diagnostic tests like pregnancy tests. Its standout feature is its exceptional capillary action, enabling swift liquid progression. This attribute is especially advantageous in diagnostic tests, where quick results are crucial. Nitrocellulose paper can efficiently immobilize biomolecules, enhancing its usability in diagnostic applications. When samples interact with nitrocellulose paper, they trigger specific biochemical reactions, leading to visible results, such as the appearance of lines or color changes.

**2.1.3 Chitosan coated cellulose paper.** Chitosan-coated paper is tailored for the detection of heavy metals such as lead and mercury. Chitosan, derived from chitin, possesses metal-binding properties, making it an effective material for capturing and quantifying metal ions in samples. The specific

interaction between chitosan and metal ions results in visible changes, such as color shifts or precipitation, indicating the presence and concentration of heavy metals.

**2.1.4 Graphene oxide-infused cellulose paper.** The infusion of paper with graphene oxide or other nanomaterials has ushered in a new era of enhanced sensor performance. Graphene oxide-infused paper is often utilized in the creation of sensors designed to detect heavy metals and organic compounds. The inclusion of nanomaterials, such as graphene oxide, amplifies the sensor's sensitivity. These sensors can efficiently recognize specific analytes in complex sample matrices due to their enhanced affinity, leading to more precise and reliable results.

**2.1.5 Carbon nanotube-infused cellulose paper.** Carbon nanotube-infused paper plays a unique role in the realm of paper-based sensors. It excels in creating conductive electrodes, which are indispensable in electrochemical and electronic sensors. The paper's interaction with analytes generates electrical responses, allowing for the quantification of analyte concentrations. In electronic sensors, the electrical properties of carbon nanotubes can be harnessed to enable electronic readouts and data transmission, expanding the scope of potential applications.

**2.1.6 Gold nanoparticle deposited cellulose paper.** Gold nanoparticles are often incorporated into paper substrates for creating colorimetric sensors. These sensors utilize the unique optical properties of gold nanoparticles to detect and quantify various analytes, including ions and biomolecules. The interactions between gold nanoparticles and analytes induce noticeable color changes, providing a straightforward and easily interpretable means of analysis. This makes them particularly valuable in applications where rapid and on-site testing is required.

**2.1.7 Silver nanoparticle deposited cellulose paper.** Silver nanoparticle-modified paper shares similarities with its gold counterpart and is also employed in colorimetric assays. These sensors can detect specific molecules by triggering color changes when the analyte interacts with the silver nanoparticles. This intuitive method of detection is valuable for various applications, including environmental monitoring and point-of-care diagnostics.

### 2.2 Polymer cellulose blended paper

The blending of various polymers with paper substrates enhances their properties, contributing to improved durability, flexibility, and stability. This blending is often used in microfluidic paper-based devices ( $\mu$ PADs), which require these characteristics. The  $\mu$ PADs find applications in various fields, from medical diagnostics to environmental monitoring, as their durability ensures they withstand complex sample matrices and rugged field conditions.

The selection of paper substrate plays a pivotal role in shaping the performance and applications of paper sensors. Researchers meticulously consider the nature of the target analyte, the sensitivity required, and the operational conditions when choosing the appropriate paper. In recent years, the





Table 2 Application of different types of paper sensors in detecting analytes in present

| S. no. | Type of metal ion detected | Paper substrate                                    | Sensing element                                             | Sample/matrix                                                        | Detection limit                      | Ref. |
|--------|----------------------------|----------------------------------------------------|-------------------------------------------------------------|----------------------------------------------------------------------|--------------------------------------|------|
| 1      | Hg(II)                     | Whatman paper no. 1                                | Rhodamine functionalized polymeric probe                    | Drinking water tap water                                             | 0.2 ppm                              | 11   |
| 2      | Hg(II)                     | Cellulose nanofiber                                | AuNPs                                                       | Water                                                                | 0.0002 ppm                           | 12   |
| 3      | Hg(II)                     | Filter paper                                       | CysA@AuNPs                                                  | Aqueous solution                                                     | 0.001 ppm                            | 13   |
| 4      | Hg(II)                     | Filter paper                                       | Terpyridine-quinolinium iodide                              | Aqueous solutions                                                    | 0.2 ppm                              | 14   |
| 5      | Hg(II)                     | Whatman paper no. 1                                | Oxalicalix[4]arene-AgNPs                                    | Waters                                                               | 0.014 ppm                            | 15   |
| 6      | Hg(II)                     | Cellulose filter paper                             | SiO <sub>2</sub> -AgNPs                                     | Aqueous media                                                        | 2.2 × 10 <sup>-4</sup> ppm           | 16   |
| 7      | Hg(II)                     | Filter paper                                       | CdSe/ZnS and TMB                                            | Tap water                                                            | 0.018 ppm                            | 17   |
| 8      | Cu(II)                     | Whatman filter paper                               | (8-Methoxy-1,3',3'-trimethylspiro [chromene-2,2'-indoline]) | Tap water                                                            | 38 ppm                               | 18   |
| 9      | Cu(II)                     | Whatman filter paper 2                             | Chrome azurol 5 pyrocatechol violet                         | Rain water and tap water                                             | 1.7 ppm                              | 19   |
| 10     | Cu(II)                     | Filter paper                                       | ZnSe nanostructures                                         | Environmental water                                                  | 1.9 ppm                              | 20   |
| 11     | Cu(II)                     | Chromatography paper                               | Rhodamine B with silica NPs                                 | Drinking water                                                       | 0.7 ppm                              | 21   |
| 12     | Cu(II)                     | Whatman cellulose filter paper                     | Tricyanofuranhydrazone                                      | —                                                                    | 50 ppm                               | 22   |
| 13     | Cu(II)                     | Whatman filter paper                               | Multi-dye system                                            | —                                                                    | 2.23 ppm                             | 23   |
| 14     | Cu(II)                     | Filter paper                                       | Rhodamine-fluorene                                          | Water                                                                | 0.072 ppm                            | 24   |
| 15     | Cr(VI)                     | Glass fiber filter paper                           | 1,5-Diphenylcarbazine                                       | Tap water, river water, surface water, and electroplating wastewater | 0.010 ppm                            | 25   |
| 16     | Cr(VI)                     | Nylon paper                                        | Chitosan with 1,5-diphenylcarbazine                         | Water                                                                | 0.06 ppm                             | 26   |
| 17     | Cr(III) + Cr(VI)           | Filter paper no. 1                                 | Diphenyl carbazine                                          | Drinking, mineral, and tap waters                                    | 0.003 and 0.002 ppm                  | 27   |
| 18     | Total Cr                   | Chromatography paper 1 CHR                         | Diphenyl carbazine                                          | Water and soil                                                       | 0.25 ppm                             | 28   |
| 19     | Cr(III) + Cr(VI)           | Whatman no. 1                                      | Diphenyl carbazine                                          | Water and wastewater samples                                         | 0.5 and 0.7 ppm                      | 29   |
| 20     | Pb(II)                     | Whatman no. 1                                      | Dithizone                                                   | Water                                                                | 10 ppm                               | 30   |
| 21     | Pb(II)                     | Quantitative filter paper                          | Rhodamine 6G hydrazide                                      | Tap water                                                            | 0.5 ppm                              | 31   |
| 22     | As(III)                    | Glass microfibre filter paper                      | Glucose-functionalized AuNPs                                | Environmental water samples                                          | 5.6 ppm                              | 32   |
| 23     | Pb(II)                     | Whatman paper no. 1                                | Dimethylglyoxime                                            | River samples                                                        | 7.7 ppm                              | 33   |
| 24     | Ni(II)                     | Whatman 1 filter paper                             | Dimethylglyoxime                                            | Industrial wastewater and river water                                | 2.9 ppm                              | 34   |
| 25     | Ni(II)                     | Whatman no. 1, 3, 4 and chromatography paper no. 1 | Boric acid-polyvinyl alcohol with dimethylglyoxime          | —                                                                    | 0.92 ppm                             | 35   |
| 26     | Tuberculosis DNA           | Whatman chromatography no. 3                       | AuNPs                                                       | Human disc tissue                                                    | 0.0195 ng mL <sup>-1</sup>           | 36   |
| 27     | <i>E. coli</i>             | Cellulose paper                                    | Zr-MOF                                                      | Mice wound tissue                                                    | 10 <sup>4</sup> CFU mL <sup>-1</sup> | 37   |
| 28     | HIV-1 p24 antigen          | Whatman chromatography no. 1 and 3                 | Anti-HIV                                                    | Human blood plasma                                                   | 0.03 ng mL <sup>-1</sup>             | 38   |
| 29     | Uric acid                  | Fiberglass and TLC papers                          | AuNPs                                                       | Urine                                                                | 1.0 μmol L <sup>-1</sup>             | 39   |
| 30     | Dopamine                   | Nitrocellulose membrane                            | AuNPs with 20T linkers                                      | Artificial urine                                                     | 10 ng mL <sup>-1</sup>               | 40   |
| 31     | Lactoferrin                | Whatman qualitative filter paper 541               | 2-(5-Bromo-2-pyridylazo)-5-diethylamino phenol              | —                                                                    | 110 μg mL <sup>-1</sup>              | 41   |

Table 2 (Contd.)

| S. no. | Type of metal ion detected                        | Paper substrate                                     | Sensing element                            | Sample/matrix | Detection limit                                   | Ref. |
|--------|---------------------------------------------------|-----------------------------------------------------|--------------------------------------------|---------------|---------------------------------------------------|------|
| 32     | Pesticide (methyl paraoxon and chlorpyrifos-oxon) | Whatman filter paper no. 4                          | Ceria nanoparticles                        | Vegetables    | 18 and 5.3 ng L <sup>-1</sup>                     | 42   |
| 33     | Chlorpyrifos                                      | Hyundai micro filter paper no. 51                   | Acetylcholinesterase and 3-indoxyl acetate | Food products | 8.6–12.46 ppm                                     | 43   |
| 34     | Organophosphate and carbamate pesticide           | Canson paper                                        | 5,5'-Dithiobis(2-nitrobenzoic)acid         | Food products | 0.27 mM and 6.16 mM                               | 44   |
| 35     | Nitrite and nitrate                               | Whatman qualitative filter paper 1 and 5            | Griess reagent and sulfanilic acid         | Food products | 0.1 mg L <sup>-1</sup> and 0.4 mg L <sup>-1</sup> | 45   |
| 36     | Norfloxacin                                       | Whatman qualitative filter paper 1, 2, 4, 5, and 42 | Iron(III) nitrate nonahydrate              | Meat and fish | 50 ppm per 5 ppm                                  | 46   |

utilization of different papers for colorimetric detection has been a growing focus for research groups. The choice of paper significantly influences the capabilities and versatility of paper-based sensors, further driving innovation in this field. Table 2 demonstrates a list of different types of paper used for designing paper sensors in the last two decades.

### 3. Different methods opted to fabricate paper sensors

In the field of analytical chemistry, paper sensors have emerged as a transformative technology due to their simplicity, cost-effectiveness, and versatility across a wide range of applications. Various methods have been applied to design and fabricate paper sensors, each offering unique advantages and opportunities for innovation. Paper sensors have emerged as a revolutionary technology in analytical chemistry, gaining widespread recognition due to their simplicity, cost-effectiveness, and remarkable versatility in diverse applications. Over the last two to three decades, various methods have been employed to fabricate paper sensors, each method bringing unique advantages and applications. The following section provides an extensive overview of some of these methods and highlights key recent advancements.

#### 3.1 Inkjet printing

Inkjet printing has ushered in a new era in paper sensor fabrication. This technique involves the precise ejection of tiny droplets of functional ink or sensing materials onto the paper surface using an inkjet printer. This method offers a level of control over deposition that is particularly suited for creating complex patterns and structures. Inkjet-printed paper sensors have found applications in diverse fields, including flexible electronics and wearable devices. In 2019, Ponram *et al.* introduced a different application of inkjet printing by depositing phosphorescent iridium(III) on paper surfaces. This innovation enabled the highly specific detection of Hg(II) ions.<sup>47</sup> This work demonstrated the method's effectiveness in addressing environmental and health-related challenges. In 2020, Lee *et al.* showcased the potential of inkjet printing by creating a colorimetric technique on photo paper. This technique enabled the detection of 2,4,6-trinitrotoluene (TNT) in contaminated soil with an impressive detection limit of 14 ppm.<sup>48</sup> This application exemplified the utility of inkjet-printed paper sensors in environmental monitoring and safety assessments. In 2021, Shrivastava *et al.* harnessed the power of inkjet printing to fabricate a colorimetric sensor using silver nanoparticles (AgNPs) on paper substrates. This sensor was specifically designed for on-site detection of mercuric ions (Hg<sup>2+</sup>) in water samples, further highlighting the adaptability and accessibility of inkjet-printed paper sensors.<sup>49</sup> In 2023, Lim *et al.* extended the capabilities of inkjet printing by fabricating paper-based sensors using graphene as a printing ink. These sensors were designed for breath monitoring, showcasing the potential applications of inkjet-printed paper sensors in the field of healthcare a schematic way as shown in Fig. 1.<sup>50</sup>



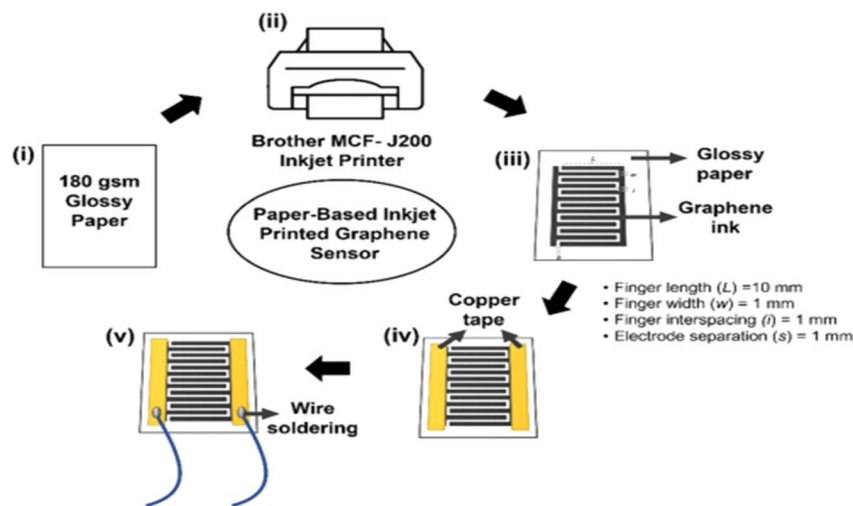


Fig. 1 Schematic representation of a paper-based inkjet-printed graphene sensor [reprinted with permission from ref. 50 Lim W. Y., Goh C. H., Yap K. Z. and Ramakrishnan N., One-step fabrication of paper-based inkjet-printed graphene for breath monitor sensors, *Biosensors*, 2023, **13**(2), 209, <https://doi.org/10.3390/bios13020209>. Copyright@MDPI].

Inkjet printing has paved the way for precise and customizable paper sensors, enabling applications that range from environmental monitoring to advanced healthcare solutions. These sensors, with their fine-tuned features, are at the forefront of addressing real-world challenges and improving quality of life. Table 3 gives the recent work done in paper sensors using inkjet printing.

### 3.1.1 Advantages and disadvantages of inkjet printing.

Paper sensor design benefits greatly from the use of inkjet printing technology, which is inexpensive, flexible, and capable of producing intricate patterns at high resolution. But there are a few drawbacks to employing inkjet printing for this kind of work.

**3.1.1.1 Limitations on resolution.** Inkjet printers can produce high resolutions,<sup>72</sup> but for some complex sensor designs—particularly those that call for extremely minute details or exact patterning—the resolution might not be enough.

**3.1.1.2 Limited ink compatibility.** While most inkjet printers can use a wide variety of inks, not all of them are compatible with sensor materials or appropriate for printing on paper substrates. This may limit the variety of materials that can be utilized in sensor fabrication.<sup>73</sup> Inkjet-printed inks have the potential to penetrate or spread unevenly on paper substrates, which could result in differences in the accuracy and performance of the sensors. This can be especially troublesome for sensors that need exact control over the layering of ink.<sup>74</sup>

**3.1.1.3 Limited compatibility with materials.** The varieties of sensor materials that can be utilized may be limited by the fact that inkjet printing is mostly appropriate for printing on porous or absorbent substrates like paper.<sup>75</sup> Certain sensor materials could not print well in an inkjet printer or stick to paper poorly.

**3.1.1.4 Durability issues.** Compared to sensors made using alternative methods or materials, inkjet-printed sensors on paper substrates could be less robust.<sup>76</sup> Environmental factors,

mechanical stress, and moisture can all have an impact on how long inkjet-printed paper sensors last and how reliable they are.

**3.1.1.5 Post-processing requirements.** To improve performance, stability, and longevity, inkjet-printed paper sensors may need to undergo extra post-processing procedures like drying, curing, or coating. These procedures may increase the fabrication process's complexity and expense.<sup>77</sup>

## 3.2 Wax printing: a versatile technique for microfluidic paper-based devices

In the realm of paper sensor fabrication, wax printing stands as a versatile and essential technique. This method revolves around the precise application of a hydrophobic wax to paper through various means, such as inkjet printing, screen printing, or using wax pens. The hydrophobic wax serves as a crucial barrier, dictating the flow of liquids within specific regions on the paper. The significance of wax printing lies in its role in creating microfluidic paper-based devices ( $\mu$ PADs) as shown in Fig. 2, a cornerstone in diagnostic applications.

In the year 2019, the wax printing technique experienced significant advancements, led by the work of Strong and colleagues. They made a remarkable leap in the precision and resolution of wax printing by fabricating microfluidic paths on paper.<sup>79</sup> The team achieved this feat by strategically altering the iodate concentration and initiating a series of chemical reactions. This breakthrough enhanced the technique's ability to achieve high-resolution designs, a crucial aspect for microfluidic paper devices. Wax printing boasts numerous advantages, which have contributed to its popularity. It is known for its cost-effectiveness, efficiency, rapid prototyping capabilities, and straightforwardness in usage. In fact, the prototyping of microfluidic devices can be completed in as little as five minutes, with subsequent adjustments being made even more swiftly.<sup>80</sup>

Wax printing remains at the forefront of paper sensor fabrication due to its precision, cost-effectiveness, and rapid





Table 3 The recent work done in paper sensor using inkjet printing method

| S. no. | Analyte                                                            | Detector                            | Printer               | Methods of detection      | Detection limit                                                                                                                                              | Ref.      |
|--------|--------------------------------------------------------------------|-------------------------------------|-----------------------|---------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 1      | pH, glucose, protein                                               | Scanner                             | Picojet-2000          | Colorimetry               | Glucose-2.8–28 mM<br>pH – 5–9                                                                                                                                | 51        |
| 2      | pH, human IgG, mouse IgG                                           | Scanner                             | Picojet-2000          | Colorimetry               | Human serum albumin-0.46–46 $\mu\text{M}$<br>Human IgG – at least 10 ng mL <sup>-1</sup>                                                                     | 52        |
| 3      | Paraoxon<br>Aflatoxin B1                                           | Digital camera, smartphone camera   | Dimatix DMP-2800      | Colorimetry               | Paraoxon – 100 nM<br>Aflatoxin B1 – 30 nM                                                                                                                    | 53 and 54 |
| 4      | Paraoxon<br>Bendiocarb<br>Carbaryl                                 | Digital camera                      | Dimatix DMP-2800      | Colorimetry               | Paraoxon – 1 nM<br>Bendiocarb – 1 nM<br>Carbaryl – 10 nM                                                                                                     | 55        |
| 5      | Malathion<br>Hg(II), Ag(I), Cu(II), Cd(II), Pb(II), Cr(IV), Ni(II) | Digital camera, scanner             | Dimatix DMP-2800      | Colorimetry               | Malathion – 10 nM<br>Hg(II)-0.001 ppm<br>Cu(II)-0.020 ppm<br>Ag(I)-0.002 ppm<br>Cd(II)-0.020 ppm<br>Pb(II)-0.140 ppm<br>Cr(IV)-0.150 ppm<br>Ni(II)-0.230 ppm | 56        |
| 6      | ATP                                                                | Human nose                          | Dimatix DMP-2800      | Odour                     | 1.16 $\mu\text{M}$ in buffer, 1.36 $\mu\text{M}$ in orange punch                                                                                             | 57        |
| 7      | pH, H <sub>2</sub> O <sub>2</sub>                                  | Scanner                             | Epson PX-101          | Colorimetry               | pH: 4–9<br>H <sub>2</sub> O <sub>2</sub> : 14.4 $\mu\text{M}$                                                                                                | 58        |
| 8      | Volatile primary amines                                            | Scanner                             | Dimatix DMP-2831      | Colorimetry               | Discrimination of <i>n</i> -C <sub>8</sub> H <sub>2n-1</sub> NH <sub>2</sub> ( <i>n</i> = 1–7)                                                               | 59        |
| 9      | Nitrite, ALP                                                       | Scanner                             | Canon PIXMA iP4500    | Colorimetry               | Nitrite (NO <sub>2</sub> <sup>-</sup> ): 0–5 mM                                                                                                              | 60        |
| 10     | DBAE, NADH                                                         | Smartphone camera                   | Canon PIXMA iP4500    | Electrochemiluminescence  | DBAE: 3 $\mu\text{M}$ –5 mM (0.9 $\mu\text{M}$ )<br>NADH: 0.2–10 mM (200 $\mu\text{M}$ )                                                                     | 61        |
| 11     | Nitrite, nitrate                                                   | Scanner                             | Canon P4700           | Colorimetry               | Nitrite (NO <sub>2</sub> <sup>-</sup> ): 10–150 $\mu\text{M}$<br>Nitrate (NO <sub>3</sub> <sup>-</sup> ): 50–1000 $\mu\text{M}$                              | 62        |
| 12     | Reactive phosphate                                                 | Scanner                             | Canon P4700           | Colorimetry               | 0.2–10 mg L <sup>-1</sup> P                                                                                                                                  | 63        |
| 13     | Metabolic volatiles from food products                             | Hg lamp, epifluorescence microscope | Hp Desk-jet 4280      | Fluorescence              | Monitoring of meats, dairy, fruit, grain, vegetables and fruit spoilage                                                                                      | 64        |
| 14     | Phenolic compounds                                                 | Scanner                             | Dimatix DMP-2800      | Colorimetry               | BPA, <i>p</i> -cresol: 1–200, dopamine, catechol: 1–300, phenol: 1–400, <i>m</i> -cresol: 1–500 $\mu\text{g L}^{-1}$                                         | 65        |
| 15     | hCG                                                                | Digital camera                      | SLT0505-HKF           | Colorimetry               | 1 ng mL <sup>-1</sup> in buffer solution, 4 ng mL <sup>-1</sup> in urine sample                                                                              | 66        |
| 16     | Volatile organic compounds                                         | UV lamp, digital camera, microscope | HP Desk-jet D2360     | Colorimetry, fluorescence | Discrimination of 10 organic solvent vapours                                                                                                                 | 67        |
| 17     | Morphine                                                           | Scanner                             | Dimatix DMP-2831      | Colorimetry               | 1 ng mL <sup>-1</sup>                                                                                                                                        | 68        |
| 18     | Cationic surfactants, anionic polymers                             | Digital camera                      | Details not available | Colorimetry               | Anionic polymers, cationic surfactants: at least 1 mM                                                                                                        | 69        |
| 19     | Glucose                                                            | Spectrophotometer                   | Epson ME1+            | Colorimetry               | 0–100 mM in urine                                                                                                                                            | 70        |
| 20     | <i>B. subtilis</i> , <i>E. coli</i>                                | Scanner                             | Epson Artisan 50      | Colorimetry               | <i>B. subtilis</i> , <i>E. coli</i> – 10 <sup>3</sup> CFU                                                                                                    | 71        |

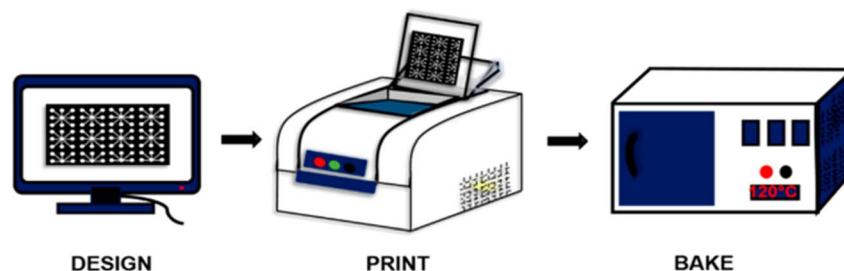


Fig. 2 Schematic representation of wax printing on paper microfluidic devices [reprinted with permission from ref. 78 Prasad A., Tran T. and Gartia M. R., Multiplexed paper microfluidics for titration and detection of ingredients in beverages, *Sensors*, 2019, 19(6), 1286, <https://doi.org/10.3390/s19061286>. Copyright©MDPI].

prototyping capabilities. While challenges related to the availability of wax printers persist, the innovative spirit of researchers ensures that alternative approaches will continue to be explored, guaranteeing the continued utility of this versatile technique in the development of paper-based microfluidic devices and sensors. The whole fabrication process and some parameters that can be used in wax printing are given in Table 4.

Recognizing the potential of wax printing, Chiang *et al.* introduced a 3D printing process in 2019 to fabricate paper-based

microfluidic devices using wax as a pivotal element.<sup>96</sup> In the same year, Wisang and colleagues harnessed the wax printing technique to construct microfluidic paper-based analytical devices designed for the colorimetric detection of lead(II).<sup>97</sup>

**3.2.1 Advantages and disadvantages of wax printing.** It is imperative to acknowledge the present challenge faced by the wax printing technique. The production of wax printers has seen a decline, which poses a hurdle to this otherwise advantageous method.<sup>98</sup> Along with this wax printing is often sensitive to temperature variations, which can affect the quality and

Table 4 Parameters for wax printing

| S. no. | Paper                                      | Printer                | Time   | Temperature (°C) | Design software                   | Heating equipment | Ref.      |
|--------|--------------------------------------------|------------------------|--------|------------------|-----------------------------------|-------------------|-----------|
| 1      | Filter paper 202 and 102                   | Xerox Phaser 8560DN    | 2 min  | 150              | —                                 | Oven              | 81        |
| 2      | Whatman chromatography paper no. 1         | Xerox Phaser 8560DN    | 2 min  | 150              | CleWin (Phoenix B.V.)             | Hot plate         | 82        |
| 3      | Whatman chromatography paper no. 1         | Xerox ColorQube 8570DN | 1 min  | 125              | CorelDRAW (Corel Corp.)           | Oven              | 83        |
| 4      | Whatman filter paper no. 114               | Xerox Phaser 8560      | 2 min  | 150              | Illustrator (Abode Systems Inc.)  | Oven              | 84        |
| 5      | Whatman filter paper no. 4                 | Xerox ColorQube 8750   | 30 s   | 150              | Illustrator (Abode Systems Inc.)  | Oven              | 85        |
| 6      | Whatman filter paper no. 4                 | Xerox ColorQube 8750N  | 5 min  | 120              | —                                 | Hot plate         | 86        |
| 7      | Whatman chromatography paper no. 1         | Xerox ColorQube 8570   | 45 s   | 120              | —                                 | Oven              | 87        |
| 8      | Whatman chromatography paper no. 1         | Xerox ColorQube 8750   | —      | 160              | Microsoft Paint (Microsoft corp.) | Laminator         | 88        |
| 9      | Whatman chromatography paper no. 1         | Xerox Phaser 8560DN    | 30 s   | 150              | Illustrator (Abode System Inc.)   | Oven              | 89        |
| 10     | Whatman chromatography paper no. 1         | Xerox ColorQube 8750   | 2 min  | 150              | Photoshop (Abode System Inc.)     | Hot plate         | 90        |
| 11     | Advantec no. 2 filter paper (Advantec MFS) | Xerox ColorQube 8570N  | 10 min | 100              | AutoCAD (Autodesk Inc.)           | Hot plate         | 91 and 92 |
| 12     | Whatman filter paper no. 114               | Xerox Phaser 8000DP    | 2 min  | 120              | —                                 | Oven              | 93        |
| 13     | Whatman chromatography paper no. 1         | Xerox ColorQube 8570   | 50 s   | 175              | Illustrator (Abode Systems Inc.)  | Hot plate         | 94 and 95 |



consistency of the printed patterns. This may pose challenges in environments where temperature control is difficult.

In response to this challenge, researchers are diligently exploring alternative approaches to fabricating microfluidic paper-based devices using wax as a hydrophobic substance. One innovative solution is a hybrid wax printing process that combines a mini CO<sub>2</sub> laser machine with a wax printer. This method allows for the simultaneous melting, heating, and piercing of wax for the design of  $\mu$ PADs. The commercial acceptance of  $\mu$ PADs continues to grow, encouraging manufacturers to consider the reproduction of wax printers, ultimately revitalizing this critical technique.<sup>99</sup>

### 3.3 Screen printing/stencil printing: precision crafting of paper-based sensors

In the realm of paper-based sensors, the art of screen printing or stencil printing is harnessed to create meticulously designed sensor patterns on paper. This method involves placing a stencil on the paper substrate and then meticulously depositing sensing materials through the openings in the stencil.<sup>100</sup> The technique offers unmatched precision in controlling the pattern's creation, enabling the formation of well-defined structures on the paper surface. The foundational principles of screen printing are elegantly illustrated in Fig. 3, providing a comprehensive visual representation of this process.<sup>101</sup>

In 2016, Cinti and colleagues showcased the capabilities of this technique by developing a paper-based electrochemical sensor that could detect phosphate levels with remarkable sensitivity.<sup>102</sup> Three years later, in 2019, Jarujamrus and team demonstrated how screen printing could be employed to craft a hydrophobic barrier on paper using a specially formulated rubber solution.<sup>103</sup> Within the same year, Caratelli *et al.* brought the advantages of screen printing to the construction of a three-electrode-based electrochemical sensor, catering to the

detection of cholinesterase inhibitors, an essential endeavor for healthcare and environmental monitoring.<sup>104</sup>

#### 3.3.1 Advantages and disadvantages of screen printing.

Screen printing is recognized for its holistic approach to sensor preparation. The necessary equipment and materials are readily available, contributing to its popularity. Additionally, the technique is known for its cost-effectiveness, making it an ideal choice for producing small batches of modified paper-based devices.<sup>105</sup> Notably, screen printing's suitability extends to bulk production, further underscoring its versatility and applicability in crafting paper-based sensors for various applications.

Compared to other printing methods like inkjet printing or photolithography, screen printing might not be able to produce patterns with a high level of resolution. This may restrict the intricacy and accuracy of patterns that may be printed, which may have an impact on the sensitivity and functionality of the sensor.<sup>106</sup> In addition to this, while using screen printing, it might be difficult to obtain exact control over the thickness of printed layers. For paper-based sensors to remain homogeneous and sensitive, layer thickness management is essential.<sup>107</sup> Changes in layer thickness could have an impact on the sensors' repeatability and performance. Multilayer registration: to construct functional elements like electrodes, sensing layers, and insulating layers, printing many layers of distinct materials is a common process in the fabrication of paper sensors. It can be challenging to precisely align these layers during the screen printing process,<sup>108</sup> which can result in misalignment problems that could impair sensor performance.

### 3.4 Dip-coating: precision crafting of functional paper-based sensors

In the realm of crafting advanced paper-based sensors, the dip-coating process emerges as a powerful technique. This method

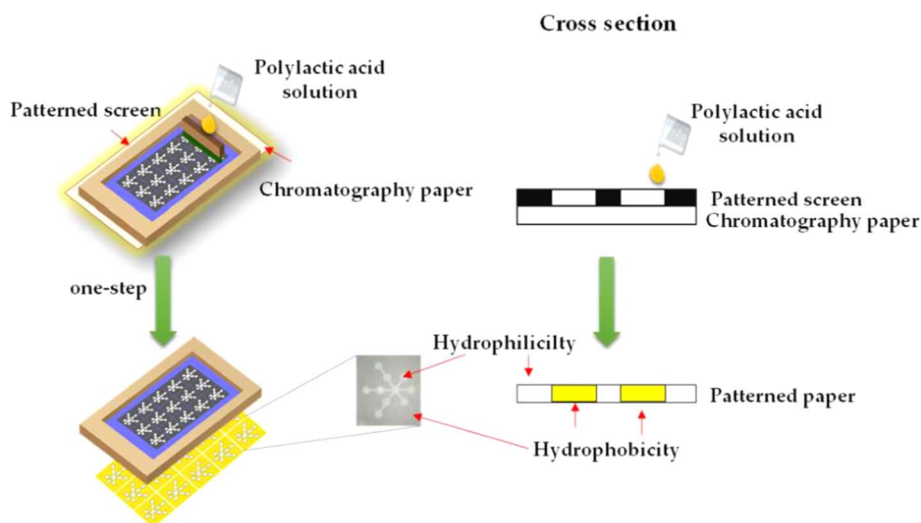


Fig. 3 The fundamental processing model of screen printing [reprinted with permission from ref. 101 Teepoo S., Arsawiset S. and Chanayota P., One-step poly(lactic acid) screen-printing microfluidic paper-based analytical device: application for simultaneous detection of nitrite and nitrate in food samples, *Chemosensors*, 2019, 7(3), 44, <https://doi.org/10.3390/chemosensors7030044>. Copyright©MDPI].



involves immersing a substrate, such as paper, into a solution or suspension, typically containing functional materials like nanoparticles, polymers, or chemical reagents. The moving unfolds as the substrate is gently withdrawn from the solution, orchestrated by the delicate dance of capillary and wetting forces.<sup>109</sup> This process creates a delicate and controlled deposition of a thin film of the functional material onto the paper's surface. The control over the film's thickness is achieved by manipulating parameters like withdrawal speed, solution concentration, and the number of coating cycles, granting precision and fine-tuning capabilities to this method.

The utilization of dip-coating in creating paper-based sensors has garnered significant attention in recent years, largely owing to a multitude of advantages it offers. These sensors stand out for being lightweight, flexible, and disposable, attributes that render them exceptionally suitable for an array of applications, ranging from environmental monitoring to medical diagnostics and food safety.<sup>110</sup>

One of the defining strengths of this technique lies in its adaptability. By skilfully tailoring the composition of the coating material, paper-based sensors can be meticulously designed to detect a wide spectrum of analytes. This versatility equips them to respond to diverse analytical needs, from identifying chemical pollutants and biomolecules to detecting ions, thereby serving as dynamic analytical tools.<sup>110</sup> Beyond its fundamental advantages, dip-coating bestows upon the sensor designer the ability to orchestrate intricate and multifaceted sensor designs. This enables the creation of paper-based sensors comprising multiple layers, each meticulously engineered to serve a distinct function. These layers can collectively capture target analytes, transduce signals, or facilitate the readout of critical data, showcasing the vast creative possibilities within this methodology.<sup>111</sup>

**3.4.1 Advantages and disadvantages of dip coating.** As the world of paper-based sensors continues to advance, the dip-coating process stands as a cornerstone in achieving sophisticated and multifunctional paper-based sensor designs. Its lightweight, flexible, and adaptable nature, coupled with the ease of manipulation,<sup>112</sup> makes it an indispensable tool for the development of sensors catering to multifaceted applications.

Taking about its disadvantages, certain materials used in dip coating formulae might not work well with paper substrates or might change the paper's characteristics in an unfavourable way, including reduction its flexibility or porosity.<sup>113</sup> Further, dip coating procedures can take a while, particularly if complicated sensor designs or several coating layers are needed. This may affect the production efficiency and scalability of paper-based sensors.<sup>114</sup> In addition to this, dip coating frequently calls for the use of chemicals and solvents, which could lead to issues with waste disposal, worker safety, and solvent emissions.<sup>115</sup>

### 3.5 Photolithography: crafting precision patterns for advanced paper sensors

In the world of sensor fabrication, photolithography, often referred to as “photolitho” or “photopatterning,” shines as a versatile and precise manufacturing technique that has,

historically, been the stronghold of the semiconductor industry. However, its capabilities have transcended boundaries, finding resonance in a multitude of applications, including the development of paper sensors.<sup>116</sup> Photolithography takes center stage in the creation of these sensors by empowering the precise patterning of materials onto the paper substrate.

The core of photolithography lies in the manipulation of light, usually in the form of ultraviolet (UV) rays, to transfer a meticulously designed pattern onto a photosensitive material. This photosensitive material, often a photoresist or light-sensitive polymer, is evenly coated onto the paper substrate, forming a blank canvas awaiting intricate details. The pivotal component of the process is a photomask, a meticulously crafted guide that maps out the pattern to be transferred onto the paper. These photomasks are born of computer-aided design (CAD) software, each possessing transparent and opaque regions corresponding to the desired sensor features.

The alchemy of photolithography unfolds as the coated paper is exposed to the brilliance of UV light, a moment of transformation. The areas of the paper exposed to this luminous energy undergo a chemical metamorphosis, typically in the form of cross-linking or dissolution processes, depending on the specifics of the design.<sup>117</sup> The shaded areas, however, remain unchanged, forming the negative of the intended pattern. After the illumination, a developer solution enters the stage, removing the unexposed or uncross-linked regions. This development reveals the precise and intricate sensor features, etched onto the paper like an artistic masterpiece.

The beauty of photolithography extends beyond pattern creation; it encapsulates a world of possibilities. Post-patterning, the paper becomes a canvas for functionalization with specific chemicals or materials to facilitate the sensor's primary function – the detection of target analytes. This could involve the introduction of enzymes, nanoparticles, or conductive materials that breathe life into the paper sensors, allowing them to interact with the chosen analytes.<sup>117</sup>

The symphony of photolithography unfolds with precision and exactitude, granting paper sensors well-defined patterns that underpin their ability to deliver accurate and reliable sensing.<sup>118</sup>

**3.5.1 Advantages and disadvantages of photolithography.** This technique is not only precise but also scalable, making it suitable for the mass production of sensors. Furthermore, it offers excellent repeatability, a critical aspect in ensuring consistent sensor performance, thereby marking it as a stalwart in the world of advanced paper sensors.<sup>119</sup>

But if we discuss its drawbacks, the acquisition and upkeep of photolithography equipment can be costly, which limits its availability for small-scale or low-budget projects like those using paper sensors.<sup>120</sup> Further, Photolithography operations necessitate a regulated setting with specific tools, materials, and skilled workers. Incorporating this intricacy into the production of paper-based sensors could not be practical or economical. Photoresists and etchants used in photolithography are frequently incompatible with the materials used in paper sensors.<sup>121</sup> These compounds' chemical interactions with the paper substrate may result in unintended reactions or a decline in sensor function. In addition to this, if



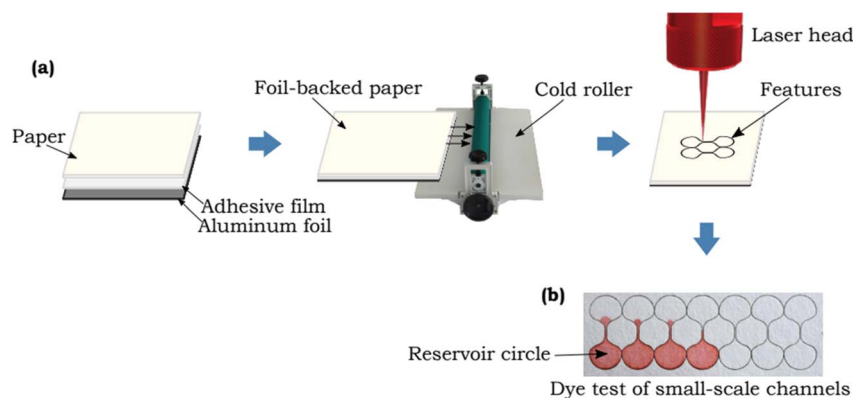


Fig. 4 Schematic representation of small scale channels generated by using laser cutting method [reprinted with permission from ref. 123 Mahmud M. A., Blondeel E. J., Kaddoura M. and MacDonald B. D., Features in microfluidic paper-based devices made by laser cutting: how small can they be?, *Micromachines*, 2018, 9(5), 220, <https://doi.org/10.3390/mi9050220>. Copyright@MDPI].

photolithography is not done correctly, the usage of different chemicals and processes could have an adverse effect on the environment.<sup>122</sup> The use of these methods to the production of paper sensors may give rise to questions regarding sustainability and the management of chemical waste.

### 3.6 Laser cutting: crafting precision and innovation in paper-based devices

Laser cutting has emerged as a formidable tool for creating meticulously designed patterns and intricate microfluidic channels on paper, giving rise to a new era of precise and rapid fabrication in the world of paper-based sensors. In 2018, Mahmud *et al.* introduced an innovative concept where they harnessed the precision of laser technology to fabricate microfluidic paper-based analytical devices ( $\mu$ PADs) capable of measuring the smallest feature sizes essential for the unhindered flow of fluids.<sup>123</sup> As depicted in Fig. 4, this achievement has opened up exciting possibilities in the field of paper sensors, promising to revolutionize their design and capabilities.

One of the remarkable techniques emerging from the world of laser-based fabrication involves the controlled heating of paper, pre-coated with hydrophobic materials. This inventive approach allows for the creation of aquaphobic barriers at high temperatures, effectively rendering the paper water-resistant. In 2019, Zhang *et al.* demonstrated their pioneering work by uniformly distributing a layer of wax on paper and utilizing laser-induced heating to engineer intricate microfluidic paths and water-resistant barriers.<sup>124</sup> This innovative method not only showcases the adaptability of laser technology but also enhances the functionality and durability of paper-based sensors.

Furthermore, laser cutting plays a pivotal role in the creation of aquaphobic paths by removing hydrophobic materials from the paper. In 2020, Hiep *et al.* utilized this methodology for the quantification of nitrite, marking a significant milestone in the evolution of paper-based sensor fabrication.<sup>125</sup>

**3.6.1 Advantages and disadvantages of laser cutting.** The dynamic nature of laser cutting and its ability to adapt to

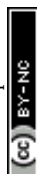
various sensor requirements and applications are pushing the boundaries of sensor technology.<sup>126</sup> The integration of laser technology into sensor fabrication is ushering in a new era of precision and innovation, promising a brighter future for paper-based sensors.<sup>127</sup>

Further, it has some limitation such as material compatibility. The variety of materials can be laser-cut, however not all papers are best suited for this technique. Certain papers might have coatings or additives that, when cut with a laser, release harmful vapours that could harm the machinery or cause health problems.<sup>128</sup> In addition to this, when laser cutting technique is used for fragile materials like paper, burnt edges may result. This may have an impact on the sensor's appearance as well as its ability to function, especially if the burnt edges weaken the paper's structural integrity or change its characteristics.<sup>129</sup>

## 4. Design and development of 3D paper-based sensors

The study on sensors made of paper has recently extended from sensors based on paper substrate with two dimensions (2D) to those with three dimensions (3D) coupled with different preparation techniques. In comparison to 2D sensors, more difficult multichannel analyses can be performed by 3D paper sensors.

Additionally, compared to its transverse flow, the longitudinal fluid flow device requires fewer samples and shorter flow times, allowing quicker and more effective detection and analysis. Currently, the manufacturing procedure of 3D paper-based devices mostly consists of stacking discrete 2D devices using techniques like folding and adhesion.<sup>130</sup> In year 2008, Martinez *et al.* demonstrated a technique to create 3D microfluidic devices utilising dual-sided tape and decorative paper. The fluids were spread vertically and horizontally by the 3D paper-based equipment, so they could straddle one another without blending, and the samples were then dispersed to the detection area, permitting concurrent and effective detection.<sup>131</sup> In year 2014, Wang *et al.* created a 3D paper-based sensor using wax printing and dual sided tape to detect several heavy metal ions concurrently.<sup>132</sup> In year 2020, Guan *et al.* created a three-



dimensional paper based sensor using glue adhesion, setting distinct functions on each layer to create a platform that could perform many operations including detection of blood fibrinogen and plasma separation.<sup>133</sup> However, employing double-sided adhesive tape to construct 3D paper-based devices has some disadvantages *viz.* difficult alignment, bonding, and punching procedures, which have negative impact on fluid flow. Additionally, a few scientists created 3D paper sensor using origami to attain numerous tests and simultaneous shunting.<sup>134</sup> In year 2016, Ding *et al.* constructed a 3D equipment using folding technique. The equipment could be utilised for a variety of bioassays by unfold and fold the structure of paper.<sup>135</sup> In year 2022, Lazzarini *et al.* created a 3D paper sensor using origami and wax printed ink for the detection of allergens in food sample.<sup>136</sup> It describes a sensor made of origami paper that uses magnetic microbeads to functionalize the paper substrate and conducts a competitive chemiluminescence immunoassay for ovalbumin in food samples. The schematic representation of the sensor as shown below in Fig. 5.

The construction of 3D paper-based sensors using the origami technique is easy, quick, and enables various 3D shapes to execute various tasks.<sup>137</sup> The preparation of 3D paper-based devices *via* stacking necessitates accurate alignment of several layers individually and appropriate interaction between hydrophilic channels in each layer.<sup>138</sup>

Researchers have developed 3D paper sensors on single paper by employing different preparation techniques. On two sides of the same piece of paper, different hydrophobic patterns are printed in order to implement the preparation process. In year 2016, He *et al.* used a laser-based direct writing technology

to construct and made a three-dimensional (3D) structure on paper substrate, utilising the 3D structure to enable multi-step analysis.<sup>130</sup> This approach regulates the hydrophobic barrier depth formed in the substrate by adjusting laser writing parameters. It is possible to build three-dimensional flow routes after thorough design and integration. In year 2021, Jeong *et al.* suggested 3D- $\mu$ PAD formation on one piece of paper by managing the paper absorption of several wax inks.<sup>139</sup> In year 2014, Li *et al.* suggested a novel approach for creating 3D paper sensors, instead of using sticky tape or origami. Wax printing was used to create patterns on both sides of a piece paper. By altering the printed wax's density and heating time, multi-layer patterned routes in the substrate were produced with varying wax penetration depths.<sup>140</sup> The method reduces the amount of paper layers required to create 3D sensors, facilitating the device quality control process simpler. It is suitable for 3D paper sensors and regular production processes. In year 2018, Punpattanakul *et al.* effectively generated a three-dimensional network of fluid channels with many fluids channel crossing each other using inkjet printer. Inkjet printer as a shield is used to create hydrophilic design on both sides of the paper, and then submerged in a non-polar solution that contains a water-resistant material to create a barrier that repels water. The mask design assisted in separating the printed area into hydrophobic and hydrophilic regions by preventing the non-polar solution from adhering to it. The mask design assisted to avoid the non-polar solution adhering to the printed section,<sup>141</sup> therefore make sections of the paper that are hydrophobic and hydrophilic. In conclusion, the 3D modeling method enables effective integration of additional fluid flow

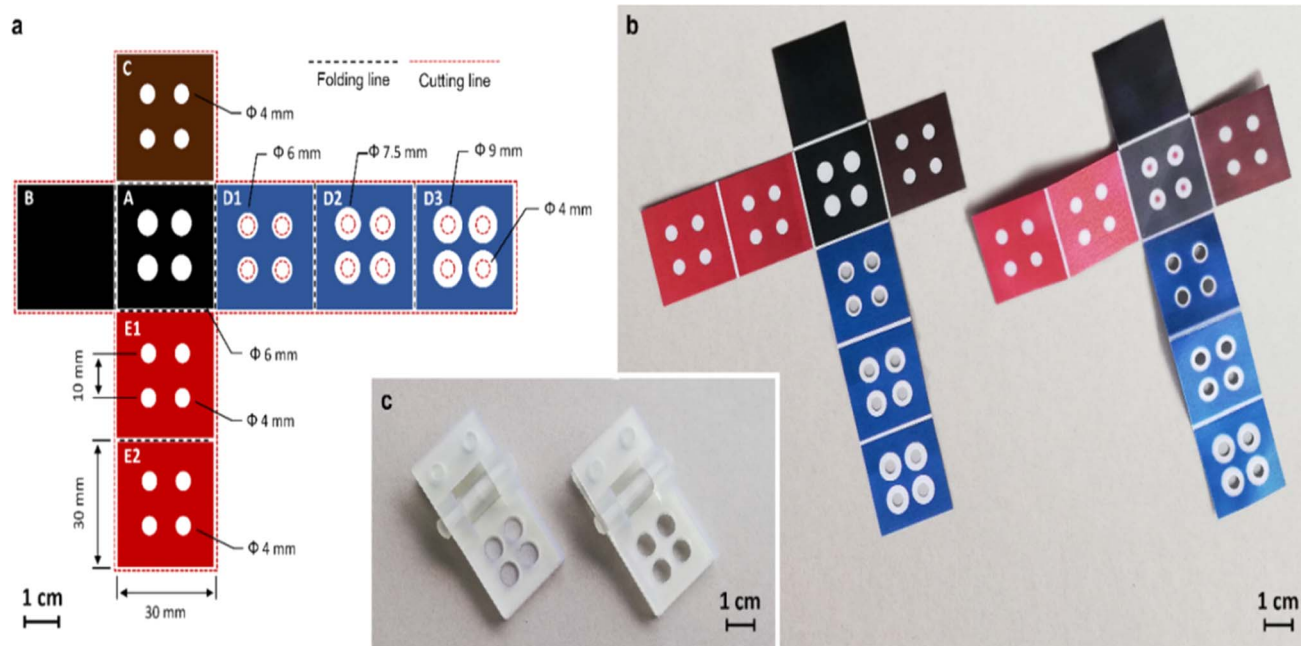
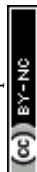


Fig. 5 The schematic representation of 3D paper based sensor using origami and wax printed ink method [reprinted with permission from ref. 136 Lazzarini E., Pace A., Trozzi I., Zangheri M., Guardigli M., Calabria D. and Mirasoli M., An Origami Paper-Based Biosensor for Allergen Detection by Chemiluminescence Immunoassay on Magnetic Microbeads, *Biosensors*, 2022, 12(10), 825, <https://doi.org/10.3390/bios12100825>. Copyright@MDPI].



channels, enabling more sophisticated samples analysis on a single device.<sup>134</sup> Currently, 3D paper-based sensors are being used for food safety, illness diagnosis and environmental monitoring research and detection.

## 5. Colorimetry detection method

Colorimetric detection methods are extensively used in paper designed sensors virtue of their cost-effectiveness, simplicity and ease of interpretation.<sup>142</sup> These methods rely on changes in color to indicate the presence of a specific analyte or target molecule. Here are some common colorimetric detection techniques used in paper sensors.

### 5.1 Indicator dyes

Indicator dyes are molecules that undergo a color change in response to a specific analyte. These dyes can be immobilized on the paper substrate or within the detection zone. When the target analyte reacts with the indicator dye, it triggers a chemical or physical change that results in a visible color shift. Examples of indicator dyes include pH indicators (*e.g.*, litmus paper), metal ion indicators (*e.g.*, metallochromic dyes), and enzyme substrates. In year 2017, Gunda *et al.* detect the *E. coli* in water sample using a litmus paper test (dip test) in which the enzymatic reaction is performed on the surface of a porous paper substrate. The detection limit of *E. coli* was<sup>143</sup>  $2 \times 10^5$  to  $4 \times 10^4$  CFU mL<sup>-1</sup>. In year 2018, Idros *et al.* developed a multi-dimensional colorimetric paper based sensor for the detection heavy metal ions *i.e.*, mercury, lead, iron, copper, nickel and chromium ions using triple indicator. The triple indicator changes color upon addition of metal ions and seen by naked eye. The detection limit for Cu<sup>2+</sup>, Hg<sup>2+</sup>, Pb<sup>2+</sup>, Ni<sup>2+</sup>, Cr<sup>2+</sup> and Fe<sup>+</sup> were 15, 0.1, 0.3, 0.5, 0.8 and 3.58  $\mu$ M respectively.<sup>144</sup>

### 5.2 Nanoparticles and nanomaterials

Nanoparticles such as gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), and quantum dots can be used in paper sensors. These nanoparticles exhibit unique optical properties that can be tuned to change color in the presence of specific analytes.<sup>145</sup> Functionalized nanoparticles can interact with the analyte and cause aggregation, leading to a color shift that is visible to the naked eye. In year 2021, Borthakur *et al.* synthesized copper sulfide porous reduced graphene oxide nanocomposite to detect the Hg(II) ions. The paper strips dip in Hg(II) ion solution it initiated the TMB oxidation and shows the faded bluish-green color with the detection limit of 0.010 ppm.<sup>146</sup> In the same year, Sahu *et al.* synthesized glucose functionalized AuNPs and deposited on paper substrate for the visual identification of arsenic (As(III)) and lead (Pb(II)) ions. The detection limits for As(III) and Pb(II) were 5.6 ppm and 7.7 ppm.<sup>34</sup>

### 5.3 Enzyme substrates

Enzyme-based colorimetric assays involve using enzymes that catalyse reactions causing color shifts.<sup>147</sup> For example, in the presence of a target analyte, an enzyme-substrate complex can produce a colored product. This approach is commonly used in

bioassays, where the analyte concentration determines how strongly the colour changes. In year 2023, Guan *et al.* developed a colorimetric paper based sensor for the real time detection of ascorbic acid based on AuNPs nanozyme activity. Its detection limit was 0.406  $\mu$ M.<sup>148</sup> In year 2023, Gao *et al.* fabricated a DNzyme colorimetric paper based sensor for the highly sensitive detection of antibiotic-resistant genes in different microorganisms' pathogens. The antibiotic resistance genes can be detected in femtomolar level.<sup>149</sup>

### 5.4 Lateral flow assays (LFAs)

LFAs, also known as lateral flow immunoassays, are a type of paper-based sensors, which work on the basis of capillary action and are commonly used for rapid diagnostic tests.<sup>150</sup> LFAs typically include four zones *viz.*; a sample application zone, a conjugate zone (where labeled antibodies or other recognition elements are immobilized), a reaction zone (where target analytes bind to the labeled antibodies), and a detection zone (where a color change occurs). In year 2017, Roh *et al.* developed an immunochromatographic strip using polydiacetylene on nitrocellulose membrane for the detection of Hepatitis B surface Antibody (HBsAb). A red line can be seen by naked eye on nitrocellulose membrane for up to 1 ng mL<sup>-1</sup> of HBsAb.<sup>151</sup> In year 2018, Loynachan *et al.* developed a lateral flow immunoassay (LFIs) for the sensitive detection of p24 which is the important biomarker for HIV. The detection level of p24 is in the femtomolar range.<sup>152</sup>

Colorimetric detection methods offer several advantages, including simplicity, rapid response, and visual interpretation. They are particularly suitable for point-of-care testing, environmental monitoring, and resource-limited settings. The design of a colorimetric paper sensor depends on the specific analyte of interest and the desired sensitivity and specificity of the assay.

## 6. Instrument-free readouts

Instrument-free in paper sensors refer to the ability of a paper-based sensor to provide a measurable response or signal without the need for specialized or complex instrumentation. These sensors are designed to be affordable, transportable and simple, making them particularly useful for point-of-care diagnostics, environmental monitoring, and resource-limited settings. Numerous approaches have been used for this kind of analysis such as radius-based, counting, and detection based on distance.<sup>153</sup> In any case, no external equipment is used for semiquantitative determination. In the year, 2018 Hofstetter *et al.*, used radius-based detection technique for the detection of Cu<sup>2+</sup>, Fe<sup>2+</sup>, Fe<sup>3+</sup>, and Zn<sup>2+</sup> metal ions using metal complexing reagents. The key benefit of radius/diameter-based detection technique was that it is quite time saving and takes only two to three minutes. Detection based on counting depends on specific components of a device to create colour. Number of coloured materials overall or wetted spots shows how much sample is there. This detection type has been utilised to measure albumin, glucose, antibodies, and hydrogen peroxide.<sup>155,156</sup> In year 2012 Lewis *et al.* used the counting-based technique by developing certain compound to detect H<sub>2</sub>O<sub>2</sub>.<sup>154</sup>



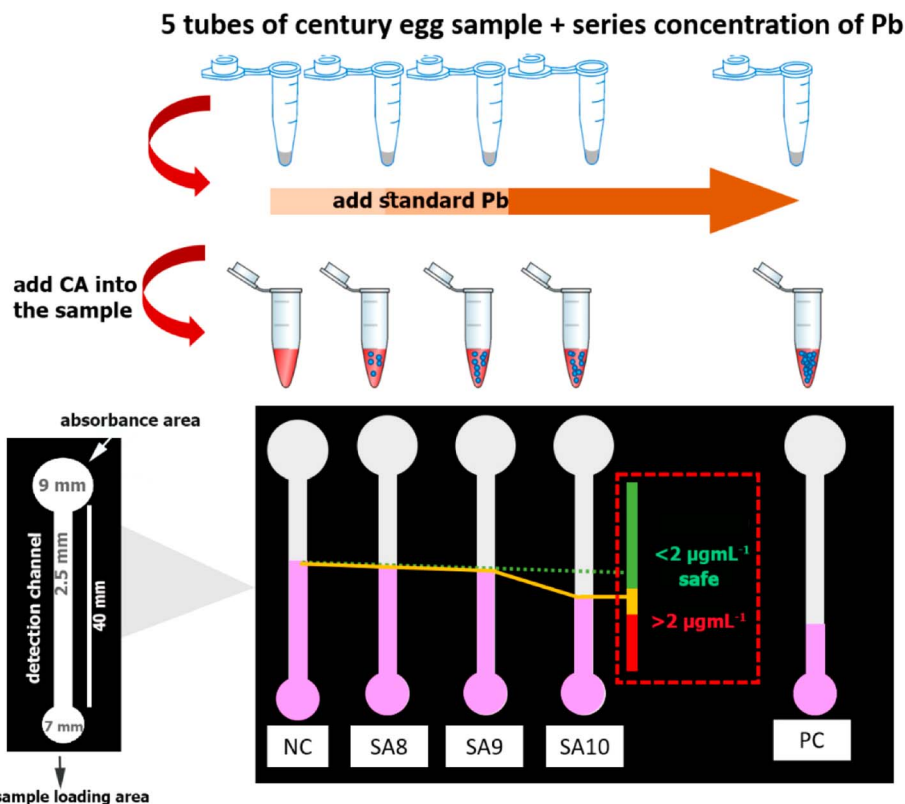


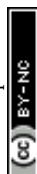
Fig. 6 The schematic representation of distance based paper sensor to identify lead in food samples [reprinted with permission from ref. 165 Katelakha K., Nopponpunth V., Boonlue W. and Laiwattanapaisa W., A simple distance paper-based analytical device for the screening of lead in food matrices, *Biosensors*, 2021, 11(3), 90, <https://doi.org/10.3390/bios11030090>. Copyright@MDPI].

Heavy metals, proteins, DNA, haematocrit levels, small molecules such as glucose, microbes have all been detected using distance-based technique.<sup>157–164</sup> Most of the time, distance-based detection depends on an interaction between the target and chemicals that have been dried in the paper channel. Upon complete consumption of the target, colouring will not continue to develop, and the distance at which it stops

can specify the targets concentration. In year 2021, Katelakha *et al.* fabricated a distance paper-based analytical device for the detection of lead in food products. Hydrophilic and hydrophobic barriers were produced by printing the specified pattern onto filter paper using the wax printing process. The schematic representation of distance based paper sensor for lead as shown in Fig. 6.<sup>165</sup>

Table 5 The various analytes to be detected using paper sensor with distance based detection

| S. no. | Analyte                       | Sample                                | Linearity range                                                                                                                   | Detection limit                                                                                        | Ref. |
|--------|-------------------------------|---------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|------|
| 1      | Cocaine                       | Urine                                 | —                                                                                                                                 | 1.8 µM                                                                                                 | 167  |
| 2      | Chloride                      | Tap water                             | —                                                                                                                                 | 1.7 mg L <sup>-1</sup>                                                                                 | 168  |
| 3      | DNA                           | Parasitic worm                        | 1 aM to 1 pM                                                                                                                      | 1 aM                                                                                                   | 169  |
| 4      | Potassium ion                 | Serum                                 | 1–6 mM                                                                                                                            | 1 mM                                                                                                   | 170  |
| 5      | Glucose                       | Tear                                  | 0.1–1.2 mM                                                                                                                        | 0.1 mM                                                                                                 | 171  |
| 6      | H <sub>2</sub> O <sub>2</sub> | —                                     | 117.5–587.5 mM                                                                                                                    | 65.2 mM                                                                                                | 172  |
| 7      | Chloride                      | Mineral water                         | —                                                                                                                                 | 2 mg L <sup>-1</sup>                                                                                   | 172  |
| 8      | Aluminium                     | Water                                 | 100 µM–1 mM                                                                                                                       | 100 µM                                                                                                 | 173  |
| 9      | Bromide and bromate           | Water, cake, rice, flour, bread flour | 25 µg L <sup>-1</sup> to 2 mg L <sup>-1</sup> for Br <sup>-</sup> and 0.5–50 µg L <sup>-1</sup> for BrO <sub>3</sub> <sup>-</sup> | 10 µg L <sup>-1</sup> for Br <sup>-</sup> and 0.5 µg L <sup>-1</sup> for BrO <sub>3</sub> <sup>-</sup> | 174  |
| 10     | KIO <sub>3</sub>              | Milk and salt                         | 0.05–0.5 mM                                                                                                                       | 0.05 mM                                                                                                | 175  |
| 11     | Tartaric acid                 | Wine                                  | 2.5–150 mm                                                                                                                        | 2.5 mm                                                                                                 | 176  |
| 12     | Lead ion                      | Gunshot residue                       | 50–500 mg L <sup>-1</sup>                                                                                                         | 50 mg L <sup>-1</sup>                                                                                  | 177  |
| 13     | RBCs                          | Whole blood                           | 28–57%                                                                                                                            | —                                                                                                      | 178  |
| 14     | Lactoferrin                   | Tear                                  | 0.1–4 mg mL <sup>-1</sup>                                                                                                         | 0.1 mg mL <sup>-1</sup>                                                                                | 179  |
| 15     | Nickel                        | Combustion ash                        | 0.7–92 µg m <sup>-3</sup>                                                                                                         | —                                                                                                      | 180  |



In year 2023, Giménez-Gómez *et al.* created a distance paper based colorimetric sensor for the real time monitoring of dissolved inorganic carbon (DIC) concentration in freshwater. The measurement of DIC concentration between<sup>166</sup> 50 to 1000 mg L<sup>-1</sup>. Table 5 shows the various analytes to be detected using paper sensor with distance based detection.

Instrument-free readouts in paper-based sensors are advantageous for their ease of use, low cost, and portability. They enable rapid and accessible testing in diverse scenarios, from medical diagnostics to environmental monitoring and food safety.

## 7. Applications of paper-based sensors

Paper-based sensors have gained significant attention in recent years due to their numerous advantages, including low cost, portability, biodegradability, and ease of fabrication. These sensors have found applications in various fields, and some of the key applications include.

### 7.1 Medical diagnostics

Paper sensors have gained significant attention in recent years for their potential applications in various fields, including medical diagnostics. These sensors are typically inexpensive, easy to fabricate, portable, and environmentally friendly, making them attractive for point-of-care testing and resource-limited settings.<sup>181</sup> Here are some medical diagnostic applications of paper sensors.

**7.1.1 Glucose monitoring.** Sensors based on paper substrate can be developed to measure the glucose levels in blood, similar to traditional glucose test strips. The paper-based

sensors may contain reagents that changes color in glucose presence, allowing individuals with diabetes to monitor their blood sugar levels easily. In year 2017, Gabriel created a colorimetric paper-based sensor using the wax printing method and 3,3',5,5'-tetramethylbenzidine (TMB) reagent for the detection of glucose in human tear sample as shown in Fig. 7.<sup>182</sup> The detection limit of glucose was 84 AU mM<sup>-1</sup> and 50 μM and the linear concentration range of glucose was between 0.1 and 1.0 mM.

In year 2019, Park *et al.* fabricated a colorimetric 3D paper based microfluidic sensor with plasma separation membranes for the detection of glucose in whole blood. The detection limit of glucose in whole blood was 0.3 mM.<sup>183</sup> In year 2019, Erenas *et al.* fabricated a combined microfluidic sensor of thread and paper for the colorimetric determination of glucose in blood sample. The 3 μL of whole blood sample is only used for the detection of glucose.<sup>184</sup> In year 2023, Khachornsakkul *et al.* created an enzymatic free colorimetric paper based sensor using nanoparticles for the monitoring of glucose and diagnosis of diabetes in humans. The gold nanoparticles are deposited on one end of paper substrate for oxidation of glucose and silver nanoparticles on other end for colorimetric detection. The limit of detection was 340 μM.<sup>185</sup>

**7.1.2 Urinalysis.** Paper sensors can be used to perform basic urinalysis tests, such as detecting the presence of proteins, glucose, ketones, and other substances in urine. This can aid in the diagnosis of various conditions, including kidney disorders and diabetes. In year 2019, Abarghoei *et al.* developed a colorimetric paper based sensor based on gold nanoparticles for the detection of citrate, which are employed in identifying the phases of prostate tumours and diagnosing prostate cancer. The detection limit of citrate found in urine sample was<sup>186</sup> 0.4 mmol L<sup>-1</sup>. In year 2021, Lewinska *et al.* created

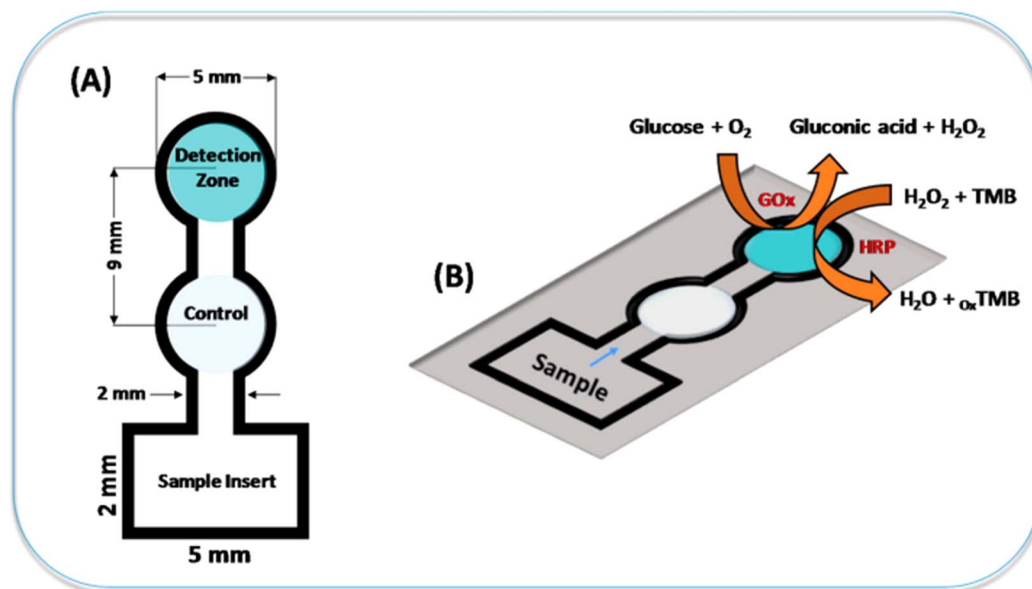


Fig. 7 The representation of colorimetric sensor to detect glucose using a paper substrate [reprinted with permission from ref. 182 Gabriel E. F. M., Garcia P. T., Lopes F. M. and Coltro W. K. T., Based colorimetric biosensor for tear glucose measurements, *Micromachines*, 2017, 8(4), 104. Copyright@MDPI].



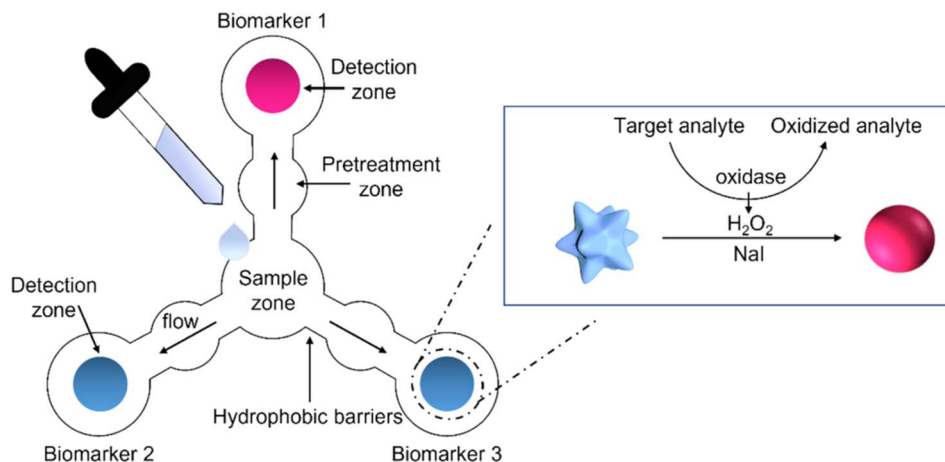


Fig. 8 The schematic representation of paper based sensor for the simultaneously detection of three salivary biomarkers [reprinted with permission from ref. 190 Pomili T., Donati P., Pompa P. P., Paper-based multiplexed colorimetric device for the simultaneous detection of salivary biomarkers, *Biosensors*, 2021, 11, <https://doi.org/10.3390/bios1110443>. Copyright@MDPI].

a colorimetric sensor using paper substrate for the analysis of creatinine in artificial urine. Its detection limit was<sup>187</sup> 0.27 mmol L<sup>-1</sup>. In year 2021, Mukhopadhyay *et al.* fabricated a colorimetric sensor on paper substrate for the same time detection of ketone and glucose in urine samples, which are important biomarkers for the identification of diabetic ketoacidosis (DKA). The detection limit for ketone and glucose was 1 mM.<sup>188</sup> In year 2021, Rahbar *et al.* created a colorimetric immunosensor as a new generation of point-of-care platforms that use paper substrate to detect the human chorionic gonadotrophin (hCG) hormone in urine samples and then showing whether a pregnancy is positive or negative. The detection limit of hCG hormone was<sup>189</sup> 10 ng mL<sup>-1</sup>.

**7.1.3 Saliva testing.** Paper sensors can be designed to analyze components in saliva, such as biomarkers or pathogens. This could be valuable for diagnosing oral health conditions, infectious diseases, and monitoring certain hormonal levels. In year 2021, Pomili *et al.* created a multiplexed

colorimetric paper based sensor for the simultaneously detection of three salivary biomarkers *i.e.*, lactate, glucose and cholesterol. The schematic representation of sensor as depicted in Fig. 8 in which the detection zones were loaded with gold nanoparticles and their respective enzymes and shows the blue to pink color change.<sup>190</sup>

In year 2021, Davidson *et al.* created a colorimetric paper based sensor that uses loop-mediated isothermal amplification (LAMP) to identify pathogen nucleic acids in complicated samples. This paper based sensor is used to detect the SARS-CoV-2 in human saliva without preprocessing. The detection limit was<sup>191</sup> 200 genomic copies  $\mu\text{L}^{-1}$ . In year 2021, Pungjunun *et al.* created a microcapillary grooved colorimetric paper sensor for analysis of thiocyanate present in saliva. The detection limit of thiocyanate was 0.2 mM.<sup>192</sup> Table 6 shows various analytes detection in saliva using paper based colorimetric sensor.

Table 6 Various analytes detection in saliva using paper based colorimetric sensor

| S. no. | Materials & structures  | Fabrication methods               | Analyte             | Detection limit               | Ref. |
|--------|-------------------------|-----------------------------------|---------------------|-------------------------------|------|
| 1      | Filter paper, 2D        | Wax printing                      | Glucose             | 0.37 dg mL <sup>-1</sup>      | 193  |
| 2      | Filter paper, 3D        | Wax printing + laser cutting      | Glucose             | 0.09 mM                       | 194  |
| 3      | Filter paper, 2D        | Wax printing                      | Glucose             | 0.3 mM                        | 195  |
|        |                         |                                   | Nitrite             | 14.8 $\mu\text{M}$            |      |
| 4      | Filter paper, 2D        | PMMA masking                      | Nitrite             | 7.8 $\mu\text{M}$             | 196  |
| 5      | Filter paper, 2D and 3D | Inkjet printing + screen printing | Thiocyanate nitrite | 1 mM                          | 197  |
|        |                         |                                   |                     | 0.2 mM                        |      |
| 6      | Filter paper, 3D        | Cutting + gel bonding             | Nitrite             | 0.05 $\mu\text{M}$            | 198  |
|        |                         |                                   | Nitrate             | 80 $\mu\text{M}$              |      |
| 7      | Filter paper, 3D        | Wax printing                      | H1N1                | 1.34 PFU mL <sup>-1</sup>     | 199  |
| 8      | Filter paper, 2D        | Cutting                           | Hepatitis B         | 104 copies mL <sup>-1</sup>   | 200  |
| 9      | Filter paper, 3D        | Laser cutting                     | Tuberculosis        | 100 bacteria mL <sup>-1</sup> | 201  |
| 10     | Filter paper, 2D        | Cutting                           | Cyanide             | 0.053 mg L <sup>-1</sup>      | 202  |
| 11     | Filter paper, 2D        | Cutting                           | Cu <sup>2+</sup>    | 1.2 ppm                       | 203  |
| 12     | Filter paper, 3D        | Laser jet printing                | Urea                | 0.18 mg dL <sup>-1</sup>      | 204  |
|        |                         |                                   | Ammonia             | 0.03 mg dL <sup>-1</sup>      |      |
|        |                         |                                   | CO <sub>2</sub>     | 0.06 mg dL <sup>-1</sup>      |      |



## 7.2 Food monitoring and food safety

When it comes to food monitoring and food safety, paper sensors offer several promising applications.

**7.2.1 Food contaminant detection.** Paper sensors can be designed to detect contaminants such as pathogens (e.g., bacteria, viruses),<sup>205</sup> chemical residues (e.g., pesticides, antibiotics), and adulterants (e.g., melamine in milk). The sensor's components are chosen to specifically interact with the target contaminants, resulting in color changes or other visual signals that can be easily interpreted by users. In year 2019, Govindarajalu *et al.* fabricated a portable colorimetric sensor on cellulose paper for the detection of starch in milk.<sup>206</sup> In year 2021, Dena *et al.* used bromothymol blue (BTB) and bromocresol purple (BCP) to construct lab-on-paper colorimetric sensor for bacterial meat deterioration.<sup>207</sup> Table 7 highlights some colorimetric paper sensors for detecting adulterants in different useful products.

**7.2.2 Freshness and shelf-life assessment.** Enzymes and indicators can be integrated into paper sensors to assess the freshness and spoilage of food products.<sup>222</sup> For example, enzymatic reactions that produce color changes as food deteriorates can be incorporated onto the paper, allowing consumers to determine if a product is still safe to consume. In year 2017, Chen *et al.* demonstrate a low-cost approach that uses the barcode on the meal as a colorimetric sensor array to keep track of the food condition. This colorimetric sensor is used for monitoring chicken aging and eventual spoiling at various temperatures.<sup>223</sup> In year 2018, Chen *et al.* developed a newly colorimetric food package label to check the freshness of lean pork. These indicator packaging label consisting the three

different pH-sensitive dyes groups, including a mixture of bromothymol blue and methyl red, bromocresol purple and bromothymol blue.<sup>224</sup> In the same year, the same group constructed a colorimetric indicator label for checking the freshness of packed fresh-cut green bell pepper based on pH responsive dyes. The indicator label made of methyl red and bromothymol blue mixture solution was used to check the pepper decay and it shows the color change from yellow-green to orange.<sup>225</sup>

**7.2.3 Foodborne pathogen monitoring.** Paper sensors can be designed to detect specific pathogens responsible for foodborne illnesses, such as *Salmonella*, *E. coli*, and *Listeria*. The sensors can utilize antibodies or DNA probes that bind to target pathogen biomarkers, leading to a visible signal upon binding. In year 2019, Kim *et al.* developed a paper-based diagnostic device for point-of-need testing, used duplex coloration to detect fecal-indicating *Escherichia coli* (*E. coli*) and highly pathogenic *E. coli*. The target bacteria 10 cfu mL<sup>-1</sup> could be detected in milk sample.<sup>226</sup> In year 2020, Asif *et al.* fabricating an analytical device on paper to detect the *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and their antibiotic resistant in milk samples using chromogenic substrate. Detection limits were found<sup>227</sup> to be 10<sup>6</sup> cfu mL<sup>-1</sup>.

**7.2.4 Point-of-care testing (POCT).** For food handlers and regulatory bodies, paper sensors can serve as efficient point-of-care testing tools to rapidly screen food samples for potential safety hazards, leading to quicker decision-making and improved food safety management as detailed in Table 8.

It is important to note that while paper sensors offer numerous advantages, they may not always replace traditional

Table 7 The paper-based colorimetric sensors used for detection of adulterants in different useful products

| S. no. | Source of analysis      | Adulterants           | Paper substrate                                      | Detection limit                           | Ref. |
|--------|-------------------------|-----------------------|------------------------------------------------------|-------------------------------------------|------|
| 1      | Milk samples            | 17 $\beta$ -Estradiol | Whatman AE99                                         | 0.25 $\mu$ g L <sup>-1</sup>              | 208  |
| 2      | Alcoholic beverages     | Ketamine              | Whatman filter paper no. 1                           | 0.001 M                                   | 209  |
| 3      | Milk samples            | Clenbuterol           | Whatman cellulose chromatography paper               | 0.2 ppb                                   | 210  |
| 4      | Milk samples            | Melamine              | Common filter paper                                  | 5.1 nM                                    | 211  |
| 5      | Milk samples            | Urea                  | Filter paper                                         | Urea-5 mg                                 | 212  |
|        |                         | Starch                |                                                      | Starch-17 mg                              |      |
|        |                         | Salt                  |                                                      | Salt-29 mg                                |      |
|        |                         | Detergent             |                                                      | Detergent- 20 mg in 10 mL of milk         |      |
| 6      | Milk samples            | Milk adulterants      | Whatman filter paper no. 1                           | NA                                        | 213  |
| 7      | Commercial food samples | Benzoic acid          | Whatman qualitative filter paper no. 3               | Multiple values for the different samples | 214  |
| 8      | Wheat flour             | Iron fortificant      | Whatman 1PS paper                                    | 3.691 $\mu$ g mL <sup>-1</sup>            | 215  |
| 9      | Milk samples            | Starch                | Whatman qualitative 1                                | NA                                        | 206  |
| 10     | Milk samples            | Melamine              | Chromatographic paper                                | 0.1 ppm                                   | 216  |
| 11     | Food samples            | Sulphite              | Cellulose paper                                      | 0.04 $\mu$ g L <sup>-1</sup>              | 217  |
| 12     | Fish (salmon)           | Mercury               | —                                                    | 15 nM                                     | 218  |
| 13     | Food products           | Nitrite and nitrate   | Whatman qualitative filter paper grade 1 and grade 5 | 0.1 and 0.4 mg L <sup>-1</sup>            | 219  |
| 14     | Food samples            | Borax                 | Whatman filter paper no. 1                           | 10 mg L <sup>-1</sup>                     | 220  |
|        |                         | Salicylic acid        |                                                      | 35 mg L <sup>-1</sup>                     |      |
|        |                         | Nitrite and nitrate   |                                                      | 0.4 mg L <sup>-1</sup>                    |      |
| 15     | Meat samples            | Nitrite               | Whatman qualitative filter paper 1, 2, 4, 5 and 42   | 1.1 mg kg <sup>-1</sup> of meat           | 221  |

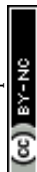


Table 8 Emerging point-of care (POC) colorimetric sensor for food safety testing

| POC sensors        | Sample type                                            | Analytes                                         | Assay time | Limit of detection                                                                    | Ref. |
|--------------------|--------------------------------------------------------|--------------------------------------------------|------------|---------------------------------------------------------------------------------------|------|
| Paper-based sensor | Chinese cabbage                                        | <i>E. coli</i> O157: H7                          | 1 h        | 10 000 CFU mL <sup>-1</sup>                                                           | 228  |
|                    | Milk                                                   | Clenbuterol                                      | 1 h        | 0.2 ppb                                                                               | 229  |
|                    | Water                                                  | <i>Cronobacter</i> spp.                          | 1 h        | 10 cfu cm <sup>-2</sup>                                                               | 230  |
|                    | Water                                                  | NO <sub>2</sub> <sup>-</sup>                     | 5 min      | 0.5 nM                                                                                | 231  |
|                    | Water                                                  | Benzoic acid                                     | 1 h        | 500 ppm                                                                               | 232  |
|                    | Water, tomato juices                                   | Copper ions                                      | 2 min      | 0.3 ng mL <sup>-1</sup>                                                               | 233  |
|                    | Phosphate buffered saline, milk, water and apple juice | <i>E. coli</i> O157:H7                           | 35 min     | 10 CFU mL <sup>-1</sup>                                                               | 234  |
| Paper-based sensor | Milk                                                   | Alkaline phosphate                               | 10 min     | 0.1 U L <sup>-1</sup>                                                                 | 235  |
|                    | Water                                                  | Nitrite                                          | 15 min     | 73 ng mL <sup>-1</sup>                                                                | 236  |
|                    | Water                                                  | <i>Pseudomonas aeruginosa</i>                    | 50 min     | 200 CFU mL <sup>-1</sup>                                                              | 237  |
|                    | Milk, water, spinach                                   | <i>E. coli</i>                                   | 1 h        | 10–1000 CFU mL <sup>-1</sup>                                                          | 238  |
|                    | Milk, juice                                            | <i>Salmonella typhimurium</i>                    | 1 h        | 100–1000 CFU mL <sup>-1</sup>                                                         | 239  |
|                    | Phosphate buffered saline                              | (i) <i>E. coli</i><br>(ii) <i>S. typhimurium</i> | 10 min     | (i) 10 <sup>5</sup> cfu mL <sup>-1</sup><br>(ii) 10 <sup>6</sup> cfu mL <sup>-1</sup> | 240  |
| Chip-based sensor  | Corn                                                   | Aflatoxin B1                                     | 1 h        | 3 ppb                                                                                 | 241  |
|                    | (i) Wheat                                              | (i) Gluten                                       | 15–20 min  | (i) 4.7 ng mL <sup>-1</sup>                                                           | 242  |
|                    | (ii) Peanut                                            | (ii) Ara h                                       |            | (ii) 15.2 ng mL <sup>-1</sup>                                                         |      |
|                    | Water                                                  | (i) Pb(II) ion<br>(ii) Al(III) ion               | 8–10 min   | (i) 30 ppb<br>(ii) 89 ppb                                                             | 243  |
|                    | Apple                                                  | Malathion                                        | 20 min     | 100 ppb                                                                               | 244  |
|                    | Water                                                  | Tetrabromodiphenyl ether                         | 12 min     | 0.01 µg L <sup>-1</sup>                                                               | 245  |

laboratory methods for highly sensitive or complex analyses. However, they can complement existing techniques, especially in resource-limited settings or for rapid preliminary screening.

### 7.3 Environmental monitoring and control

**7.3.1 Water quality monitoring.** Paper sensors can be designed to detect and quantify pollutants in water sources, such as heavy metals ions (*e.g.*, lead, mercury, cadmium), pH levels, nitrate, and microbial contaminants.<sup>246</sup> These sensors can provide real-time monitoring of water quality in both natural bodies of water and water treatment facilities. In year 2014, Wang *et al.* designed a paper colorimetric sensor using dual-sided tape layers of wax-patterned paper to identify metals ions in water samples with high selectivity.<sup>132</sup> In year 2018, Idros *et al.* prepared an affordable, portable, paper-based microfluidic analytical device, for the detection of Pb(II), Cr(VI), Hg(II), Cu(II), Fe(II) and Ni(II) ions.<sup>144</sup> Colorimetric indicators resulted observable colour change when metal ions were applied. Red, green, and blue (RGB) RGB's colour properties in three dimensions were quantitatively analysed by digital imaging and colour calibration techniques.<sup>246</sup> In year 2019, Firdaus *et al.* developed a low cost, straightforward, and portable analytical technique paired with a smartphone for quantifying mercury ions. Silver nanoparticles (AgNPs) in small quantities were used as a colorimetric agent, when mercury ions were added on the AgNPs the yellowish-brown color changed to colourless. It was detected over a lower limit of 0.86 ppb. The schematic representation for the fabrication procedure and acquisition of digital images for determination of mercury ions are shown in Fig. 9.<sup>247</sup>

In year 2020, Moniz *et al.* used 3-hydroxy-4-pyridinone chelators produced from ether as chromogenic reagents,

constructed a paper-based microfluidic device to detect Fe(III) ions in water samples. Utilising this new colour reagent increased the device detection limit, the detecting signal remained constant for four hours and the device can be stored for a minimum of one month.<sup>248</sup>

**7.3.2 Air pollution detection.** Paper-based sensors can be used to monitor air pollutants, including gases like nitrogen dioxide (NO<sub>2</sub>), sulphur dioxide (SO<sub>2</sub>), carbon monoxide (CO), and volatile organic compounds (VOCs). These sensors can be deployed in urban areas to assess air quality and pollution levels. In year 2012, Wang *et al.* developed a colorimetric sensor that combine with microfluidic to detect the total NO<sub>x</sub> (NO + NO<sub>2</sub>). The created sensor could continually track the quality of the air for up to 18 hours with a detection limit of 50 parts per billion per volume (ppbV).<sup>249</sup> In year 2016, Tang and Sun *et al.* have created a colorimetric sensor made of paper with a high sensitivity for airborne methyl isothiocyanate (MITC) detection. The detection limit was 100 ppb.<sup>250</sup> In year 2018, Guo *et al.* created a microfluidic colorimetric sensor to analyse the gaseous formaldehyde using 4-aminohydrazine-5-mercapto-1,2,4-triazole (AHMT) technique. The detection limit was 0.01 ppm. The schematic representation of the fabricated microfluidic sensor with their calibration is shown in Fig. 10.<sup>251</sup>

In year 2019, Nguyen *et al.* created a disposable paper-based sensor for chemically detecting gaseous with high selective and accuracy. Seven rings were used in the probe designed, which was wax-printed and coloured with various pH indicators. The probes colour was evaluated and RGB values were retrieved from pictures obtained with a handmade smart-phone applications.<sup>252</sup>

These applications highlight the versatility and potential impact of paper-based sensors in environmental monitoring and control. Table 9 highlights the paper based colorimetric



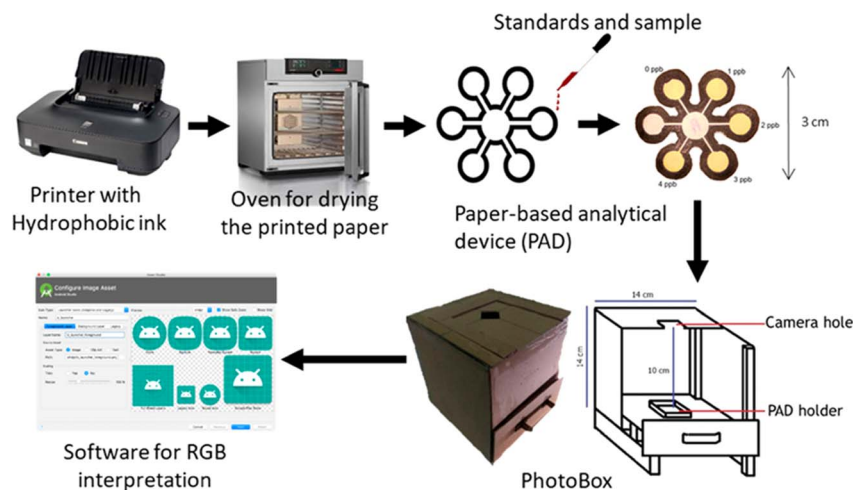


Fig. 9 The schematic representation for the fabrication procedure and acquisition of digital images for determination of mercury ions [reprinted with permission from ref. 247 Firdaus M. L., Aprian A., Meileza N., Hitsmi M., Elvia R., Rahmidar L. and Khaydarov R., Smartphone coupled with a paper-based colorimetric device for sensitive and portable mercury ion sensing, *Chemosensors*, 2019, 7(2), 25, <https://doi.org/10.3390/chemosensors7020025>. Copyright@MDPI].

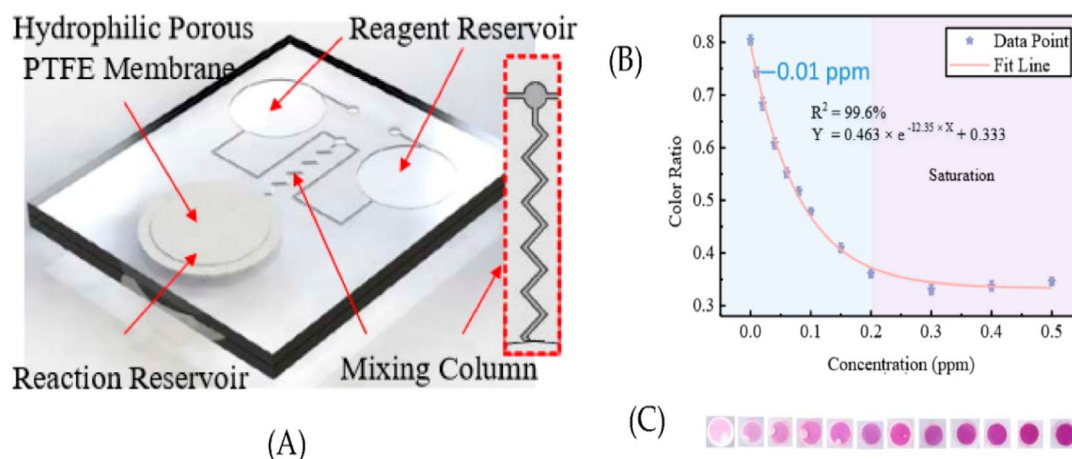


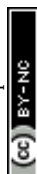
Fig. 10 The schematic representation of the fabricated microfluidic sensor with their calibration [reprinted with permission from ref. 251 Guo X. L., Chen Y., Jiang H. L., Qiu X. B. and Yu D. L., Smartphone-based microfluidic colorimetric sensor for gaseous formaldehyde determination with high sensitivity and selectivity, *Sensors*, 2018, 18(9), 3141, <https://doi.org/10.3390/s18093141>. Copyright@MDPI].

sensor for the detection of pollutants found in environment monitoring. However, it's important to note that while paper-based sensors offer many advantages, they also have limitations, such as sensitivity and durability. Therefore, the choice of sensor technology should be carefully considered based on the specific monitoring requirements and environmental conditions.

## 8. The REASSURED criteria for new POCTs and their updates

In 2002, the World Health Organization (WHO) and the Special Programme for Research and Training in Tropical Diseases (TDR) recognized the urgent need for innovative, useful, and cost-effective diagnostic methods for sexually transmitted infections in humans, especially in developing countries.

ASSURED was a set of criteria that described the desired features for new POCTs. Among these requirements are affordability, sensitivity, specificity, ease of use, fast and dependable performance, equipment-free operation, and deliverability to people in need.<sup>280</sup> The ASSURED standards have had a significant impact on the development, validation, and regulation of POCTs for human healthcare over the last decades. In response to the advancements in wireless technology and the potential for tests to be given by untrained persons, an updated set of standards known as REASSURED was established. The requirement of streamlining sample collection and facilitating real-time communication is emphasized in this updated standard.<sup>281</sup> The REASSURED criteria are as follows: affordable (less than \$10 USD for a molecular assay); sensitive (minimizing false negatives) and specific (minimizing false positives); user-friendly (requiring only two steps and



**Table 9** The various paper-based colorimetric sensors used for the detection of pollutants found in the environment monitoring

| S. no. | Type of paper        | Analyte                             | Methods of fabrication        | Detection limit           | Ref. |
|--------|----------------------|-------------------------------------|-------------------------------|---------------------------|------|
| 1      | Chromatography paper | Explosive residue                   | Wax ink                       | 0.39 µg                   | 253  |
| 2      | Filter paper         | Br <sup>-</sup>                     | Wax printing                  | 11.25 nM                  | 254  |
|        |                      | Nd <sup>3+</sup>                    |                               | 0.65 nM                   |      |
|        |                      | As <sup>3+</sup>                    |                               | 11.53 nM                  |      |
| 3      | Filter paper         | Reactive phosphate                  | Inkjet printing               | 0.05 M                    | 255  |
| 4      | Filter paper         | Ammonia                             | Inkjet printing               | 0.8 mg N per L            | 256  |
| 5      | Filter paper         | Acid volatile sulphides             | Cutting                       | 0.1 µmol g <sup>-1</sup>  | 257  |
| 6      | Filter paper         | <i>S. chartarum</i>                 | Wax printing                  | 10 spores per mL          | 258  |
| 7      | Filter paper         | Methyl isothiocyanate               | N/R                           | 100 ppb                   | 250  |
| 8      | Filter paper         | Bisphenol A                         | Inkjet printing               | 0.28 µg g <sup>-1</sup>   | 259  |
| 9      | Filter paper         | Hypochlorite                        | N/R                           | 2 g Cl <sub>2</sub> per L | 260  |
| 10     | Filter paper         | Chromium                            | Wax printing                  | 0.12 µg                   | 261  |
| 11     | Filter paper         | Methyl-paraoxon                     | Screen printing               | 8 ng mL <sup>-1</sup>     | 262  |
|        |                      | Chlorpyrifos-oxon                   |                               | 5.3 ng mL <sup>-1</sup>   |      |
| 12     | Filter paper         | Paraaxon                            | Inkjet printing               | 0.01 ng mL <sup>-1</sup>  | 263  |
|        |                      | Trichlorfon                         |                               | 0.04 ng mL <sup>-1</sup>  |      |
| 13     | Filter paper         | Methiocarb                          | Wax printing                  | 5 ng mL <sup>-1</sup>     | 264  |
| 14     | Filter paper         | Carbofuran dichlorvos carbaryl      | Punching                      | 0.003 ppm                 | 265  |
|        |                      |                                     |                               | 0.3 ppm                   |      |
|        |                      |                                     |                               | 0.5 ppm                   |      |
|        |                      | Paraaxon                            |                               | 0.6 ppm                   |      |
|        |                      | Pirimicarb                          |                               | 0.6 ppm                   |      |
| 15     | Filter paper         | Methyl paraoxon                     | Jet printing                  | 10 µM                     | 266  |
| 16     | Art paper            | Pb <sup>2+</sup>                    | Microcontact printing         | 10 nM                     | 267  |
| 17     | Filter paper         | Pb <sup>2+</sup>                    | CO <sub>2</sub> laser cutting | 0.7 nM                    | 268  |
| 18     | Filter paper         | Ag <sup>+</sup>                     | Hand drawn                    | 10 µM                     | 269  |
| 19     | Filter paper         | Cu <sup>2+</sup>                    | Inkjet printing               | 0.08 µM                   | 270  |
| 20     | Filter paper         | Ni <sup>2+</sup>                    | Wax printing                  | 4.8 mg L <sup>-1</sup>    | 271  |
|        |                      | Cu <sup>2+</sup>                    |                               | 1.6 mg L <sup>-1</sup>    |      |
|        |                      | Cr <sup>2+</sup>                    |                               | 0.18 mg L <sup>-1</sup>   |      |
| 21     | Chromatography paper | Cr <sup>6-</sup>                    | Photolithography              | 30 ppm                    | 272  |
| 22     | Chromatography paper | Ni <sup>2+</sup>                    | Wax and screen printing       | 0.24 ppm                  | 273  |
|        |                      | Cr <sup>6-</sup>                    |                               | 0.18 ppm                  |      |
|        |                      | Hg <sup>2+</sup>                    |                               | 0.19 ppm                  |      |
| 23     | Chromatography paper | Cu, Fe, Zn                          | Wax printing                  | 0.1 ppm                   | 274  |
| 24     | Filter paper         | Fe <sup>3+</sup>                    | Laser printer and coating     | 0.2 mM                    | 275  |
|        |                      | Ni <sup>2+</sup>                    |                               | 0.4 mM                    |      |
| 25     | Filter paper         | Ca <sup>2+</sup> , Mg <sup>2+</sup> | Wax printing                  | 0.5 mM                    | 276  |
| 26     | Filter paper         | Calcium                             | Hand drawn                    | 8.3 mg L <sup>-1</sup>    | 277  |
|        |                      | Magnesium                           |                               | 1.0 mg L <sup>-1</sup>    |      |
| 27     | Filter paper         | Co(II)                              | Immersed                      | 10 mg L <sup>-1</sup>     | 278  |
| 28     | Filter paper         | Cl                                  | Wax printing                  | 1.3 mg L <sup>-1</sup>    | 279  |

minimal training); real-time connectivity (feedback for patient care and connection to surveillance systems); easy specimen collection; and environmentally friendly (non-invasive specimen collection, use of recyclable materials, and reduction of hazardous waste); fast and reliable (15–60 minutes from sample receipt to answer; withstands a range of weather and environmental conditions without refrigeration); equipment-free (or runs on solar energy or batteries); and deliverable to end users (guarantees delivery to LMIC users).<sup>282</sup> The table below displays the various point-of-care tests together with the REASSURED criteria that were evaluated in the various LMICs.

## 9. Disposal of paper sensors

The right way to dispose of paper sensors relies on a number of variables, such as the kind of sensor, any chemicals or other

materials it might contain, and local trash disposal laws. Here are a few broad recommendations:

Review the guidelines provided by the manufacturer: the manufacturer often includes disposal instructions with many paper sensors. You can find this information in the product paperwork or on the package. Use these instructions if they are accessible.

Divide components: if the paper sensors are made of several materials (paper, plastic, electronics, etc.), think about dividing them apart so that they may be disposed of or recycled using the proper techniques for each type of material.

Eliminate any batteries or electronic parts from the sensors: make sure that any batteries or electronic parts are taken out and disposed of appropriately in accordance with local laws.<sup>283</sup> Programs for recycling electronics are frequently offered for the appropriate disposal of such goods.



Look for potentially dangerous materials: certain paper sensors might have chemicals or other materials that should be handled with caution. If you have any suspicions about this, find out more about the materials the sensors are made of or speak with the manufacturer for advice on appropriate disposal techniques.<sup>284</sup>

Recycling: see whether your local recycling program can accept the paper sensors if their main constituents are recyclable materials (such as paper or some plastics). Prior to recycling, make sure that all non-recyclable parts are eliminated.<sup>284</sup>

Trash disposal: if recycling is not a possibility, dispose of the paper sensors in accordance with local waste disposal regulations by throwing them in the usual trash. Think about employing a garbage disposal company that conforms with laws regarding the safe handling of potentially dangerous substances.<sup>285</sup>

Chemical disposal: seek advice from waste management agencies or local authorities on appropriate disposal techniques if the sensors contain chemicals or materials that need special handling, such as biohazardous compounds.<sup>286</sup>

Incineration: incineration may be a viable disposal strategy in certain situations, particularly for sensors that contain biohazardous chemicals.<sup>287</sup> But only authorized facilities with the necessary equipment to handle these kinds of materials securely should be used for this.

Paper sensors, like any other electrical equipment or materials, should be disposed of with consideration for the environment and local rules. It's usually a good idea to ask the manufacturer or the appropriate municipal authorities for advice if you're unclear about the correct disposal procedure.

## 10. Conclusion: the promise, challenges, and future prospects of paper-based sensors

In a world where innovation has led to groundbreaking advancements in various fields, paper-based sensors have emerged as a beacon of hope, offering an economical, environmentally friendly, and user-friendly alternative to traditional sensing technologies. This innovative approach in sensor design leverages the simplicity of paper and the intelligence of scientific research to create versatile and accessible tools with broad applications. In this concluding section, we reflect on the significance of paper-based sensors, the challenges they face, and the promising trends that may shape their future.

### 10.1 The significance of paper-based sensors

**10.1.1 Economic viability.** Paper-based sensors have garnered immense attention and appreciation due to their economic feasibility. The simple materials, primarily paper and ink, are readily available and cost-effective. This affordability factor has made these sensors highly accessible, especially in resource-constrained settings. In developing countries where advanced sensor technology is often financially out of reach, paper-based sensors provide an affordable and reliable solution. They can empower local healthcare workers and first responders to conduct rapid on-site diagnostics and environmental monitoring without straining limited budgets.

**10.1.2 User-friendly nature.** Designed with a focus on simplicity, paper-based sensors have revolutionized the concept of user-friendliness. They are intuitive to use and require minimal training for operation. This simplicity eliminates the need for specialized technicians or elaborate instruments, thereby democratizing access to critical diagnostic tools. These sensors are particularly valuable in emergencies and scenarios where time is of the essence, such as during disease outbreaks or environmental crises.

**10.1.3 Eco-friendly attributes.** Paper-based sensors exemplify eco-friendliness in sensor technology. Unlike their electronic counterparts, which often contain hazardous materials and contribute to electronic waste, paper sensors are biodegradable. This feature aligns with global efforts to adopt more sustainable and environmentally friendly practices. The production and disposal of paper sensors are less harmful to the planet, addressing concerns about environmental impact.

**10.1.4 Rapid diagnostics in healthcare.** One of the most notable applications of paper-based sensors is in the realm of rapid diagnostics. They have transformed point-of-care testing for various diseases, from infectious diseases to chronic conditions. Speed and reliability in diagnosis can be life-saving, especially in healthcare settings where immediate action is crucial. These sensors have played a pivotal role in expanding access to diagnostics, even in remote or underserved areas, by offering fast and accurate results.

**10.1.5 Versatile applications.** Paper-based sensors have proven their versatility across different domains. Their applications span across healthcare, where they are used for disease detection and monitoring, to environmental monitoring for water quality testing and pollution detection. Additionally, they have made significant contributions to ensuring food safety by detecting contaminants and pathogens. These sensors are instrumental in addressing real-world challenges.

### 10.2 Challenges/research gaps

**10.2.1 Tensile strength and stretchability.** While paper is an ideal substrate for its cost-effectiveness, it presents challenges related to tensile strength and stretchability. Ensuring that paper-based sensors can withstand challenging conditions, such as physical stress or exposure to various environmental factors, requires ongoing research and development.

**10.2.2 Device adaptability.** Designing paper-based sensors that can adapt to diverse environments and scenarios is an evolving challenge. Ensuring that sensors function consistently under different conditions is critical. Researchers are working towards creating sensors that can be seamlessly integrated into various settings, from urban healthcare facilities to remote fieldwork.

**10.2.3 Mass production.** As the demand for paper-based sensors grows, the need for mass production methods has become evident. Balancing cost-effectiveness and quality in scaling up production processes is a challenge. Developing scalable manufacturing methods that maintain the accuracy and effectiveness of the sensors is essential to meet the increasing demand.



### 10.3 Future trends

**10.3.1 Improved sensitivity and selectivity.** Researchers are committed to enhancing the sensitivity and selectivity of paper-based sensors. This means that paper sensors will become capable of detecting even lower concentrations of analytes with precision. This development opens up new possibilities in fields like environmental monitoring<sup>288</sup> and early disease detection, where detecting minute quantities of substances is paramount.

**10.3.2 Integration with electronics.** The integration of electronic components into paper-based sensors is a promising direction.<sup>289</sup> This advancement may involve incorporating microcontrollers, wireless communication, or data analysis capabilities directly into the paper substrate. By doing so, paper sensors can become more versatile and adaptable for various applications, including remote monitoring and real-time data transmission.

**10.3.3 Multiplexing capabilities.** Future paper sensors may be engineered to detect multiple analytes simultaneously. This capability, known as multiplexing, can significantly enhance testing efficiency. It enables the simultaneous detection of various substances in a single test,<sup>290</sup> streamlining processes and enabling comprehensive analysis.

**10.3.4 Application diversification.** The applications of paper-based sensors could expand into various fields beyond healthcare and environmental monitoring. Industries such as agriculture, food quality control,<sup>291</sup> and safety, as well as wearable health monitoring devices,<sup>292</sup> stand to benefit from specialized paper-based sensors. These sensors could address unique challenges and bring cost-effective solutions to these domains.

**10.3.5 Printable electronics.** Advancements in printable electronics, including conductive inks and flexible circuits, have the potential to revolutionize paper-based sensors. Researchers are exploring the possibility of printing complex electronic components directly onto paper substrates. This innovation could enable the development of more sophisticated sensors that can perform intricate tasks and provide real-time data.

**10.3.6 Regulatory approval.** As paper-based sensors mature and gain wider acceptance, they may undergo regulatory approval processes to ensure their safety and reliability for use in critical applications. This step is vital in healthcare settings and any other context where the accuracy of results is paramount.

In conclusion, paper-based sensors have not only made their mark but have also ushered in a new era of possibility in the realm of sensor technology. As research continues to break new ground, the future is incredibly promising. Paper sensors are poised to become more accurate, adaptable, and applicable in various domains. With their affordability, accessibility, and eco-friendliness, paper-based sensors have the potential to revolutionize the way we approach diagnostics, monitoring, and data collection. In the coming years, we anticipate the emergence of novel solutions that will address global challenges and provide opportunities for further advancements in science and technology.

## Conflicts of interest

There are no conflicts to declare.

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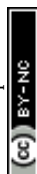
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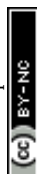
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