DE GRUYTER Reviews in Analytical Chemistry 2022; 41: 112–136

Review Article

Shibam Das, Gagandeep, and Rohit Bhatia*

Paper-based microfluidic devices: Fabrication, detection, and significant applications in various fields

https://doi.org/10.1515/revac-2022-0037
received October 12, 2021; accepted February 08, 2022

Abstract: Paper is the most abundant and inexpensive polymeric structure of cellulose available. Paper has micro-porous capillary-like networks which are responsible for flow of solvents instead of pumps or electronic power, making paper an ideally usable tool. Microfluidic paper-based analytical devices use fabricated paper devices on which hydrophilic zones are formed within hydrophobic barriers. Hydrophilic zones act as regions for actual analytical purposes, whereas hydrophobic zones act to demarcate separate zones from one another. Clinically, these devices have been proved to be excellent point-of-care-devices in diagnosis which can bypass use of costly and time-consuming laboratory techniques. In the presented review, the basic principles and components involved in the design of paper-based devices were then summarised in understandable manner. Further, various applications in different fields were also compiled in the form of text under different sections and tables. Paper-based analytical devices may serve as an excellent tool in variety of analytical works as these techniques are simple, rapid, economic, and require less human power or trainings. They have prominent applications in analysis of biological fluids, drugs/metabolites, food stuffs, colorants, biomarkers, and several other fields.

Keywords: paper, μPADs, hydrophilic zones, wax printing, biological fluids

1 Introduction

Paper has been extensively used in chemistry from very ancient times. Paper being easily available and cheap in nature can be procured with very few efforts and relatively smaller funds. Concept of use of paper in analytics emerged from use of litmus paper in chemistry, where paper was simply used as a medium for detection of pH of samples by reaction with dye retained on paper. Later, paper found many uses such as filtration aids and medium for reactions.

Paper is primarily composed of cellulosic fibres processed into sheets through various processes. Cellulosic network of paper act as micro-channels on which reagents can be retained or used to provide flow from one spot to another. These channels help to provide flow of energy, material, or both through capillary-like microfluidic channels which can be used for various purposes. Flow of reagents on paper due to cellulosic channels was then used for the purpose of separation of components. Microfluidics [1,2] refer to micro flow channels present in paper due to cellulosic fibres. These are capillary-like channels present in the structure of paper. The dimensions of these channels depend upon the type of material used for preparation of paper, such as fresh wood material, recycled papers, Whatman filters, and various others.

Microfluidic paper-based analytical devices (μPADs) are a class of "lab-on-chip" devices that are portable and easy-to-use economic devices for analytical purposes. These devices involve using Paper as the medium for analytical detection, where cellulose micropores in the paper act as channels on which reagents react with the sample for production of colour, fluorescence, or electrochemical gradient which is further used for the purpose of detection of analyte(s). Fabrication of such PADs is very easy and can be done efficiently in various ways such as wax printing [3], wax dipping [4], lacquer spraying [5], plotter printing, photolithography [6], heat embossing, etc., whose basic function is to create hydrophobic barriers

---

* **Corresponding author: Rohit Bhatia,** Department of Pharmaceutical Analysis, ISF College of Pharmacy, Ferozepur G.T. Road, Moga, Punjab-142001, India, e-mail: bhatiarohit5678@gmail.com
Shibam Das, Gagandeep: Department of Pharmaceutical Analysis, ISF College of Pharmacy, Ferozepur G.T. Road, Moga, Punjab-142001, India

for creating contained hydrophilic zones. Reaction between samples and loaded reagents occur in these hydrophilic zones only. A typical microfluidic paper-based analytical device setup is depicted in Figure 1.

For the purpose of detection, colorimetric [7], chemiluminescence [8], and electrochemical [9] techniques are used which involve detection of colour intensity/fluorescence or electrochemical gradient developed on the PAD. The present study focuses on colorimetric detection of analytes using µPADs which involves production of colour on paper and then recording the intensity of the colour produced as a function of concentration of the analyte. For recording the intensity of the colour produced, scanners, digital cameras [10], or even simple smartphone [11] cameras can be used. These recorded intensities are processed using Image Manipulation tools such as Adobe Photoshop [9,10,12] and ImageJ [13,14], where intensity of colour is noted in the channel like RGB or Cyan depending upon the colour produced. The recorded intensities for varying concentrations are used as a function for quantitation of concentration of the analyte.

These paper-based analytical devices belong to a class of “point of care” [11,13,15–17] devices used in chemical analysis. These are highly portable, lightweight, and easy to use kind of devices that require no or very less solvents as well as no specifically trained user for the operation of the device. Also, fabrication materials such as papers, waxes, printers etc., are not very costly and fabrication time is also incredibly low. This makes such devices very much cost and time effective as well as user friendly.

Recently, such devices have been employed in detection of various types of analytes in different samples such as detection of thiocyanate in saliva as biomarker of tobacco smoke exposure [18], heavy metal estimation, separation of blood into its components [19], detection of various bioactive pesticides in food and water samples, detection of dopamine in blood, estimation of creatinine levels in serum and urine, chloride level estimation in serum and sweat [20], detection of alcohol content in whiskey samples [21], colorimetric sensing of hydrogen peroxide and carbohydrates in human serum samples, estimation of total phenolic contents, etc. A self-powered rotating paper-based device has been recently introduced by Li et al. with an ability to control the fluid avoiding the complex workup. This device was successfully employed to detect thrombin following a glucose oxidase-triggered reaction [22]. Suvanasuthi et al. has developed a point of care device based on microfluidic principles to detect dengue virus serotypes. They utilised polylactic acid and wax filament to generate hydrophobic barriers on paper. This device was able to discriminate dengue viral serotypes on the basis of nucleotide sequence differences [23]. Zheng et al. also developed a microfluidic device based on Pt nanoparticles for the simultaneous detection of glucose and uric acid using smartphone for detection [24]. Zhu et al. have detected alkaline phosphatase and butyrylcholinesterase by preparing a 3D origami-based fluorimetric point of care device. They utilised pyrophosphate and thiocholine as substrates for alkaline phosphatase and butyrylcholinesterase [25]. Similarly, Chen et al. developed a smart analytical device using microfluidic

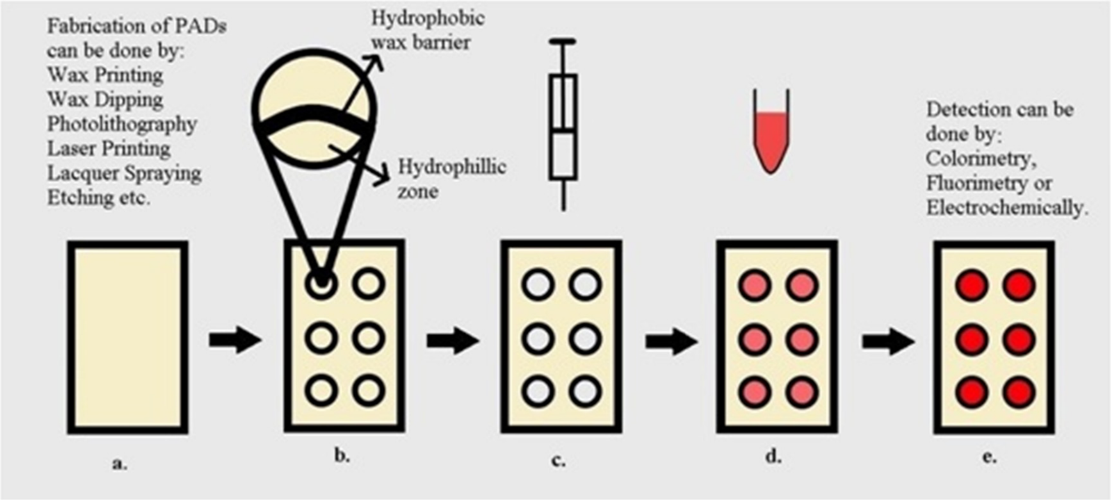

Figure 1: A typical microfluidic paper-based analytical device setup: (a) unfabricated Whatman filter paper, (b) fabricated PAD with distinct hydrophobic barriers and hydrophilic zones, (c) pre-loading of reacting reagents, (d) loading of sample on PAD, and (e) development of colour.

PMMA-paper chip for the detection of sodium-dehydroacetate in food stuffs [26].

2 Techniques of fabrication

2.1 Wax printing

Wax printing [3] is the simplest method for fabrication of paper-based analytical devices. This involves use of a computer for designing the pattern of hydrophobic barrier. Simple applications like Microsoft Word, Paint, Adobe Photoshop etc., can be used for designing the pattern. The pattern can vary from study to study depending upon the type of sample to be used, matrix in which analyte is to be detected, or the detecting reagents that are used for the production of colour or electrochemical potential. Then, the desired pattern can be printed on to the Whatman filter paper using wax printers [10,21,27,28]. The paper so obtained is then heated at about 40°C so that the wax ink permeates the paper thoroughly, thereby creating a hydrophobic barrier throughout the thickness of the paper. This makes a completely impermeable hydrophobic barrier with a hydrophilic zone enclosed inside it, shaped as per the pattern of wax printing. Finally, a clear tape [9,10] or a laminating sheet is stuck onto one surface of the paper to keep paper dry from the other side and to prevent leakage of reagents and/or sample to other side because of permeation. The general outline of wax printing technique is depicted in Figure 2.

2.2 Photolithography

Lithography has been extensively used in semiconductor industry from a long period in the past. In the past few years, lithography has also been developed for fabrication of microfluidic and micro-electric patterns. When the source of exposure used in lithography is UV radiation, it is referred to as photolithography [6]. This method for fabrication of paper devices involves use of a paper, a photoresist polymer, a photomasking agent, a developer, and etching solutions. First, desired pattern is designed for fabrication as per the demand of the study. Paper is coated with the photoresist [29–32], which in simple words is a photosensitive polymer. Photoresist can be of two types: positive and negative, i.e., if solubility increases on exposure to light, it is positive and vice-versa. Then, coating is followed by photomasking. In this step, pattern to be drawn is masked by suitable light resistant-opaque materials. Following this, UV radiation is then allowed to fall upon the setup where photoresist is exposed to light except the masked areas. Then, developer solutions are used to solubilise the photoresist and then is removed from the paper. A simple photolithographic fabrication simulation is shown in Figure 3.

2.3 Lacquer spraying

Lacquer spraying [5] is also a simple method for fabrication of PADs in relation to cost and time effectiveness. This makes use of a mould, a lacquer to be sprayed, and

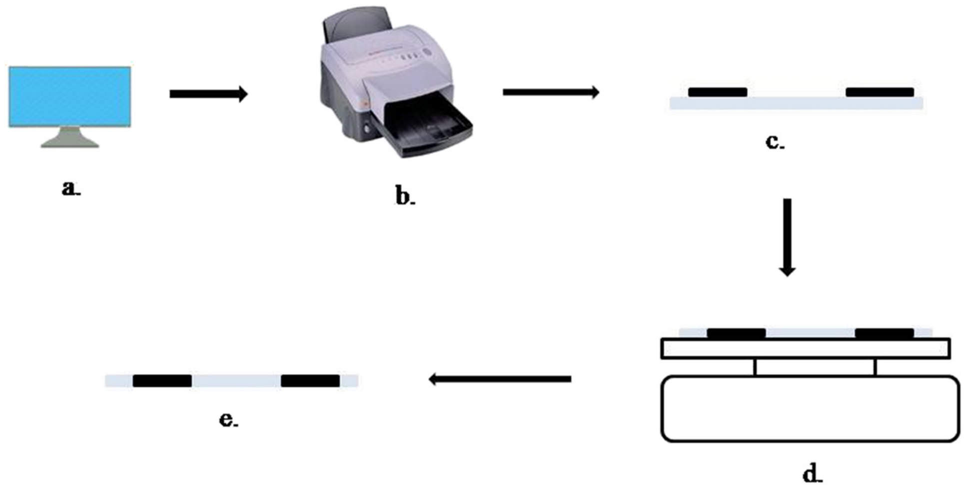

Figure 2: Technique for wax printing of PADs: (a) computer for designing the pattern, (b) wax printer for printing the pattern, (c) paper printed with wax ink, (d) paper being heated on hot plate for melting wax to make complete hydrophobic barriers, and (e) properly fabricated PAD by wax printing technique.

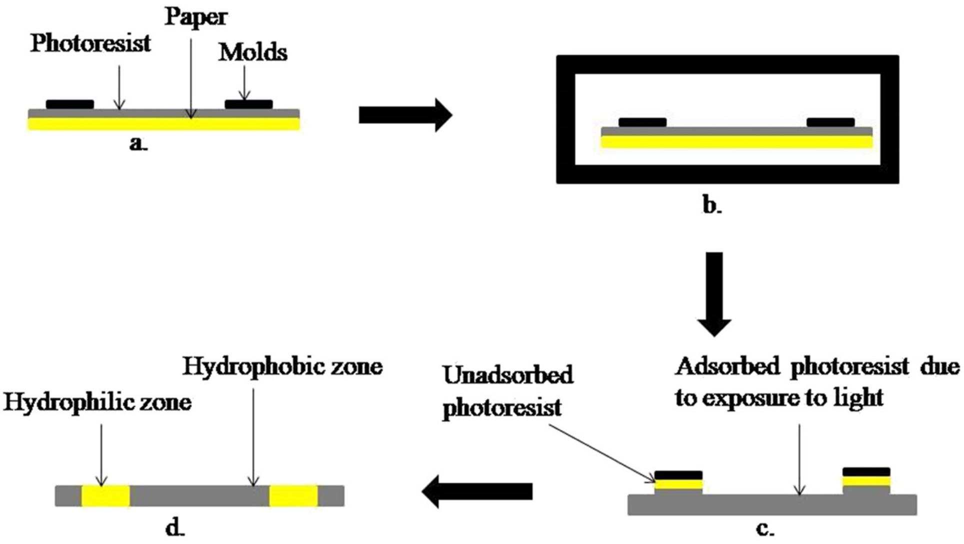

Figure 3: Photolithographic fabrication procedure: (a) paper, photoresist polymer, and moulds are held together, (b) exposure to radiation is done in contained chamber, (c) photoresist gets adsorbed in the areas exposed to radiation and unexposed region is left itself as such, and (d) completely fabricated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

paper. The mould can be a simple metal or a laminate sheet which masks the paper from being exposed to lacquer. Metal moulds are held in place using a magnet in which paper is sandwiched between the magnet and mould, whereas laminating sheets are held in place using suitable adhesive. Then, lacquer solution is sprayed uniformly over the paper using a spray bottle. Lacquers are usually hydrophobic resins by nature. The lacquer is adsorbed by the regions exposed and the desired hydrophilic zones are achieved. The paper thus obtained is dried and is ready for use for analytical purposes. Procedure for lacquer spraying is shown in Figure 4.

2.4 Metal stamping

As the name indicates, this method involves stamping [33–35] of pattern onto the paper in the desired shape of hydrophilic zone required. In this method of fabrication, first, a filter paper is completely immersed into melted wax and then taken out and dried. A stamp of metal is made in the shape of the desired pattern to be made for the hydrophobic barrier. This stamp is then heated to a temperature higher than the melting point of wax. Afterwards, wax immersed paper is placed onto another plain filter paper and stamped using hot metal

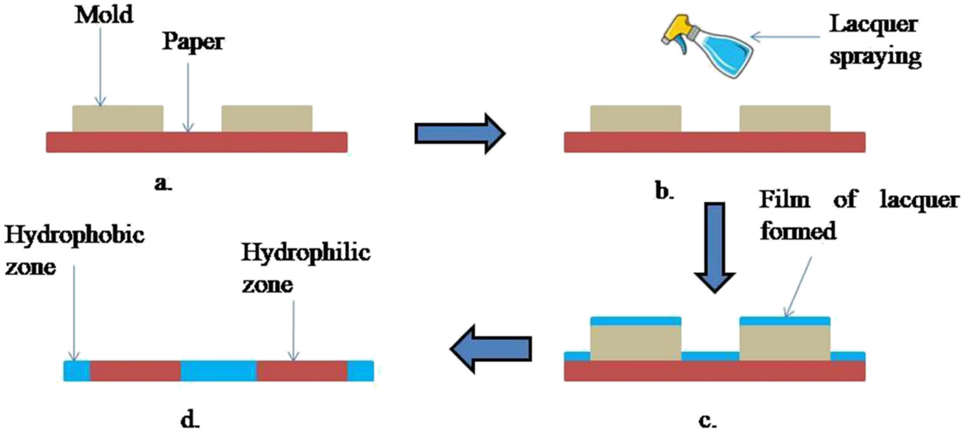

Figure 4: Lacquer spraying method for fabrication of PADs: (a) mould affixed on paper, (b) spraying of hydrophobic lacquer, (c) film of lacquer is formed over paper and mould, and (d) fabricated PAD by lacquer spraying.

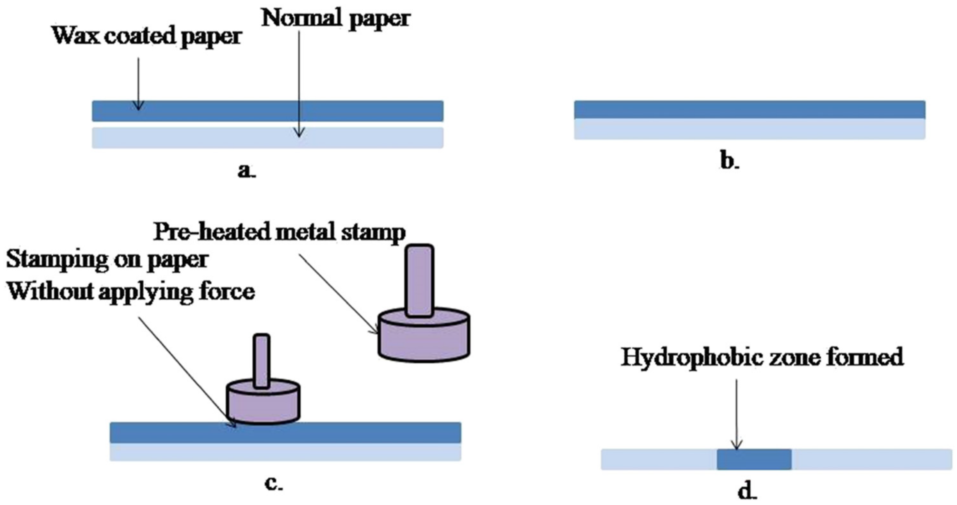

Figure 5: Metal stamping for PAD fabrication: (a) aligning wax coated and simple paper, (b) placing wax coated paper over the normal paper, (c) pre-heated stamp is taken and placed over the wax paper, and (d) hydrophobic barrier is formed in the shape of the pattern on metal stamp.

stamp. This causes melting of wax from wax immersed paper and permeation into second paper without any application of force. The wax in the shape of the desired pattern gets deposited on the paper with complete hydrophobic barriers throughout the thickness of the paper due to micro-porous channels present in the structure of the paper. A simple setup for metal stamping of hydrophobic barriers is depicted in Figure 5.

2.5 Wax dipping

Dipping was initially used for preparation of thin layer chromatography (TLC) plates for coating with silica slurries in the plates. In past few years it has also been used in the fabrication of paper devices. But unlike coating of TLC plates, where two plates are held together back-to-back for coating, a different approach is used in the fabrication of the paper microfluidic devices by wax dipping [4,19]. Metallic moulds are prepared in the shape of pattern of hydrophobic barrier to be drawn on the paper device. Mould is placed over the paper and held into place using strong permanent magnets. Then, the whole setup is immersed into the molten wax. Magnets should be strong enough to prevent seepage of molten wax under the metallic mould. Once completely immersed, setup is taken out and excessive wax is scrapped off. Then, metal moulds are removed from the paper and paper is heated to temperature of about 40°C or so. Thus, the wax melts and completely permeates into the paper. The paper is taken and dried, which then can be used for analytical purposes. A simple setup of wax dipping is shown in Figure 6.

2.6 2-dimensional (2D) plotting

This technique requires a 2D plotter for fabrication of PADs. A 2D plotter is a plotting device that can print or plot objects on a plane in two dimensions. Variation between plotting or printing can be brought about by changing the type of plotter head used. A spray nozzle that sprays a stream of ink is primarily used for fabrication of PADs. Hydrophobic ink is sprayed by a 2D plotter on the paper placed in the plotter. Pattern of spray can be pre-determined or controlled by computing systems. After plotting, paper may or may not be required to be heated depending upon the viscosity of the ink and how much it permeates into paper at various temperatures.

2.7 Laser printing

In future, it would be difficult to buy wax printers, this is where laser printing with hydrophobic inks comes into the scene of PAD fabrication. This technique simply requires a laser printer which can print with hydrophobic inks. Most of the procedure of laser printing [36] of PADs is similar to that of wax printing. The major advantage is that these printers are updated version of technology as per modern times unlike wax printers which are severely

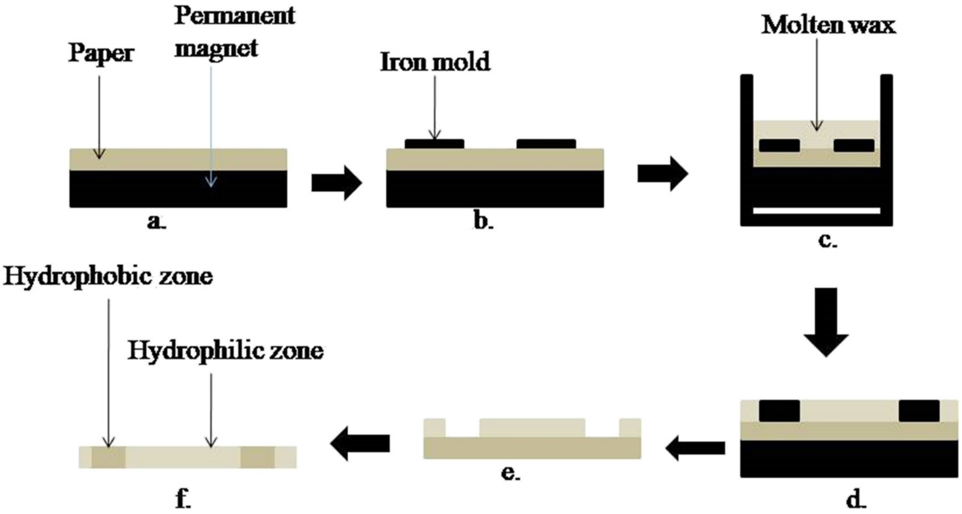

Figure 6: Setup for wax dipping fabrication of PADs: (a) paper setup on magnet, (b) iron moulds placed over paper, (c) setup placed in molten wax, (d) setup removed from wax coating formed, (e) moulds removed from paper and paper is heated, and (f) permeation of wax into micro-porous channels of paper forming distinct hydrophobic and hydrophilic zones.

outdated type of printers because of their little use and wax blocks being used as ink blocks instead of cartridges.

3 Methods of detection

3.1 Colorimetric detection

Colorimetric method of detection or as commonly called colorimetric analysis is a technique in which the concentration of the desired analyte is determined with the help of a colouring agent which may or may not be produced during the basic detection reaction. This technique can be classified into two groups depending upon use of enzymes. These are:
- enzymatic methods
- non-enzymatic methods.

3.1.1 Enzymatic methods

In this type of methods, analyte specific enzyme(s) are selected for conversion of the analyte into compound which is either coloured or liberate compounds that are responsible for the production of the colour. These are usually used for biomolecules or similar analytes that have specific enzymes for metabolism. Common examples of such colorimetric reactions are:

$$\text{Urea} + \text{Water-(enzyme urease)} \rightarrow \text{Ammonium carbonate} \tag{1}$$

$$\text{Ammonium Carbonate} + \text{Phenol} + \text{Hypochlorite} \rightarrow \text{Colored complex} \tag{2}$$

The above reaction uses urease enzyme to convert urea into ammonium carbonate which forms a coloured complex in the presence of phenol and hypochlorite [37].

$$\text{Glucose} + \text{Oxygen} + \text{Water-(enzyme glucose oxidase)} \rightarrow \text{Gluconate} + \text{Hydrogen peroxide} \tag{3}$$

$$\text{Hydrogen peroxide} + \text{ABTS} - \text{(enzyme peroxidase)} \rightarrow \text{Colored complex} \tag{4}$$

In the above reaction, glucose oxidase enzymes aerobically hydrolyse glucose to form gluconate and H_2O_2. The H_2O_2 so formed reacts with ABTS to form a coloured complex [38].

3.1.2 Non-enzymatic methods

These methods usually involve complexation principle to form coloured complexes with the analytes of interest. But since these compounds are not as specific as enzymes, this method becomes less specific and reliable than enzymatic ones. Common examples include [38,39]:

$$\text{Copper} + \text{Bathocuproin disulfonate} \rightarrow \text{Colored complex} \tag{5}$$

$$\text{Creatinine} + \text{Picrate} \rightarrow \text{Colored complex} \quad (6)$$

The colour thus produced is then detected using a simple colorimeter. But in recent practices, instead of colour, intensity of the colour produced is used as a parameter. This gives much more reliable and efficient data. Also, with increasing concentration of the analyte, the intensity of the colour is largely affected, thus in the analytical detection of biomolecules, colorimetry can be used effectively.

3.2 Electrochemical detection

Electrochemical detection, when compared to colorimetric detection, has better sensitivity and selectivity due to the electrode materials, detection systems, and measurement technologies used. ePADs may be kept portable, while still providing great performance because of the downsizing of the electronic components required for the measurements [40], as Henry and colleagues first described the use of ePADs in 2009 [41]. A variety of electrodes, paper platforms, and detectors have been combined for the investigation of biological, environmental, and other samples. Furthermore, because of paper's flexibility, electrodes may be easily patterned or printed on the surface of the substrate for low-cost detection. Because a wide range of drug compounds are electrochemically active, electrochemical detection is a viable option for direct detection [42,43]. A three-electrode setup comprising working, reference, and counter electrodes is typically used for the electrochemical detection. The working electrode is particularly significant because it is responsible for the majority of ePAD systems' analytical performance and functionality [44]. In ePADs, carbon paste electrodes (CPEs) are extensively employed, including those that target drug analytes [45,46]. These electrodes, on the other hand, may have little electrochemical activity. Modifiers such as metal nanoparticles, nanocomposites, and/or graphene are reportedly utilized in CPEs to boost catalytic activity and detection sensitivity [47].

3.3 Luminescence

Luminescence detection techniques rely on a substance emitting light after being stimulated by light, a chemical reaction, or ionising radiation. Chemiluminescence (CL), fluorescence (FL), bioluminescence (BL), electrochemiluminescence (ECL), and thermochemiluminescence (TCL) are the types of luminescence [48–50]. Because of its simplicity, low cost, and great sensitivity, luminescence-based detection has been frequently used with microfluidic devices, including paper microfluidics [51].

FL detection is based on emission of light from a material that has absorbed light or other electromagnetic energy. The interaction of analytes with fluorescent dyes (fluorophores) is the basis of FL detection in μPADs [52].

CL is a luminescence technique based on chemical reaction with a large dynamic range and excellent sensitivity due to the low noise level caused by the lack of excitation light [10]. CL detection instrumentation is also easier and less expensive to construct than FL because no excitation source is required [51].

BL, ECL, and TCL detection for drug analysis utilising μPADs are yet to be reported, but they have the potential to be deployed in the field. In case of BL, the activity of enzymes or photoproteins obtained from living organisms is responsible for the creation of light [51]. The key advantage of BL reactions over CL reactions is that they have greater quantum yields, allowing ultrasensitive detection down to the attomole scale [52]. An electrochemical process produces the light emission in ECL. However, unlike CL, it has the advantage of being able to regulate the signal captured by the applied potentials [48]. The detection in TCL is based on the emission of light caused by a molecule's thermal decomposition [53].

4 Applications in different fields

4.1 Analysis of biological fluids

4.1.1 Detection of creatinine in urine

Creatinine is one of the most important biomarkers present in the body. Determination of creatinine in urine helps in determining kidney functions efficiently. Creatinine is formed in the body as an end product of creatine metabolism. The reason behind using creatinine as biomarker for kidney function determination is that it is excreted from body mostly through the glomerular filtration at constant rate. When there is any abnormality in the pattern of creatinine excretion such as increased or decreased levels, impairment of kidney functions can be detected. Additionally, concentration of creatinine present in urine also works as an index for urine dilution in body. Originally, creatinine assays were done in laboratories using Jaffe's reaction, but it lacks specificity. So, Talalak et al., in 2015, [54] proposed a low-cost enzymatic paper-based method for detection of creatinine in urine. This method involved generation of H_2O_2 because of enzymatic action of creatininase, creatinase, and sarcosine oxidase on creatinine present in urine sample. H_2O_2 hence produced reacts with 4-aminophenazone and HTIB giving pink red quinoneimine dye which was detected colorimetrically. This method showed linearity range between 2.5 and 25 $mg \cdot dL^{-1}$ of creatinine ($r^2 = 0.983$), and limit of detection (LOD) was found to be 2.0 $mg \cdot dL^{-1}$. Although, this method is 10–20 times less sensitive than conventional methods, but unlike conventional methods, sophisticated instruments, trained analysts, tedious sampling techniques etc., are not required and it also offers portability and time and cost effectiveness. This proposed method thus provides us with low-cost detection method for creatinine to be used for low-income and developing countries.

4.1.2 Detection of thiocyanate in saliva

Thiocyanate is an endogenous molecule present in the physiological fluids due to consumption of thiocyanate-containing foods such as milk, cheese, glucosinolates containing vegetables, etc. But when the human body is exposed to tobacco smoke, the thiocyanate ion levels in the body are increased abruptly because of the hydrocyanic acid present in the smoke of tobacco. The reason behind this is production of thiocyanate as a detoxification of cyanide ion in the body is carried out by the reaction of cyanide with thiosulphates in the liver. Thiocyanate ion concentration in body can be determined from any physiological fluid but in cases of smoking, these levels are pronounced in saliva. Various reported methods for determination of thiocyanate ion in the body involved UV-Vis spectrophotometry, fluorimetry, atomic absorption spectroscopy (AAS), surface-enhanced Raman Spectroscopy (SERS) etc., but all these methods were time consuming and non-economic, producing large amounts of waste products with consumption of high volume of solvents. Thus, Pena-Pereira et al., in 2016, [18] devised a paper-based analytical method for detection of thiocyanate ion in human saliva. This study proposed a paper-based device of about credit card size with 40 test zones on which detection was carried out with Fe(III) reagent solution as a preloaded agent. The matrix used in the study was Whatman No. 1 filter paper. The detection reaction involves colour formation by production of iron(III)–thiocyanate complex which is red coloured in nature. The detection was done using HP-4500 desktop scanner/printer for recording of images and using blue channel for recording intensities. Channel blue was selected after carefully comparing results in grayscale, red, green, and blue channels. The proposed study shows LOD of 0.06 mM for thiocyanate with RSD of 3%. Recovery values in recovery studies were found to be in the range of 96.1–103.6%. These results were compared to reported UV spectrophotometric method and differences were found to be not significant analytically. Thus, this method provides analytically reliable results as compared to previously reported methods and at the same time proves to be a time and cost-effective method by consuming very few solvents and producing reproducible and reliable results in noticeably short period.

4.1.3 Detection of IL-5, HBsAg, and IgG as biomarkers

Interleukin (IL)-5, HBsAg, and IgG are important biomarkers in our body which are indicative of various diseases. IL-5 is primarily produced in $CD4^+$ T cells and functions to regulate growth, differentiation, activation, and survival of eosinophils in the body. Its increased levels in the body act as a critical biomarker for bronchial asthma. Similarly, HBsAg is an important qualitative biomarker for hepatitis B virus infection in the body which is the major reason behind hepatic damage and hepatocarcinoma worldwide. IgG acts as biomarker for detection of Neuromyelitis optica, an inflammatory condition characterised by demyelination of optic nerve and spinal cord. Originally, various highly sophisticated methods were used for determination of these biomarkers such as heterogenous immunoassays, chemiluminescence, thermal lens microscopy, etc. Although these methods were very much sensitive and selective, but being highly sophisticated and tedious, these methods proved to be expensive and non-portable. In 2009, Yu et al. [55] proposed a dextran-based PDMS-fabricated microfluidic device for the determination of these biomarkers. This device was fabricated using soft lithographic techniques and allowed on-line real time detection of biomarkers in ELISA. This proposed method used a series of reaction involving dextran, PDMS, sodium periodate, hydrochloric acid, and hydrogen peroxide to produce luminescent complex that was detected colorimetrically. The study shows LOD in the range of nanograms of analyte and hence proves to be sufficiently sensitive method. The levels of IL-5 were found to be 90.04, 98.67, and 94.34 $ng \cdot mL^{-1}$ when spiked with 100 $ng \cdot mL^{-1}$ of standard. Similar reproducible results were also observed for HBsAg and IgG. This proved the proposed method to be more time efficient, greener, and portable in which detection devices cost was almost negligible as compared to the traditional instrumental methods.

4.1.4 Detection of glucose in blood as biomarker of diabetes

Diabetes is one of the major causes behind deaths among all the diseases. It is the most common cause of blindness, renal failure, strokes, heart attacks, limb amputations, etc. According to WHO, diabetes cost 1.6 million lives directly in 2016. Such dangerous disease should be detected at as early stages as possible. One of the most important biomarkers for detection of diabetes is blood glucose levels. Normal fasting blood glucose level is 100 $mg{\cdot}dL^{-1}$ and within 2 h of eating, normal glucose levels are up to 140 $mg{\cdot}dL^{-1}$. If glucose levels rise above normal, then the person might be suffering from diabetes. One of the easiest diagnostic parameters of diabetes is blood glucose level, for which Ornatska et al., in 2011, [56] proposed an instrument free, paper-based analytical device based upon the principle of colorimetry for detection of blood glucose levels. This study devised a method involving microfluidic device coated with ceria nanoparticles that change colour in the presence of H_2O_2 evolved out in the reaction between glucose from serum and glucose oxidase enzyme pre-loaded on the paper device. The device was first optimised using various concentrations of H_2O_2 and then glucose and their calibration curves were prepared. The r^2 value for calibration curves were found to be 0.965 and 0.985 for H_2O_2 and glucose, respectively. For recovery studies, 100 mM glucose was added and recovered values were calculated to be 102.2 (±4.4) mM for 11 runs with RSD of 4.3%. For analytical purposes, linearity curve of glucose was constructed between 0 and 35 mM concentration ($r^2 = 0.963$) and glucose content was determined to be 3.71 mM in non-processed, no additives human serum sample (normal level = 3.6–5.8 mM glucose). Thus, this method proved to be sensitive and efficient when analysing blood glucose levels. Also, in the study, reusability of nano-ceria coated test strips was checked, and strips were found to be re-usable for at least ten repeated assays.

4.1.5 Detection of G6PD in blood

Haemolytic anaemia is a quite common disorder characterised by destruction of red blood corpuscles at a rate higher than their rate of generation. A most common phenomenon that can contribute to haemolytic anaemia is deficiency of glucose-6-phosphate-dehydrogenase or G6PD enzyme. Although G6PD deficiency is a genetic disorder, it is usually triggered when any drug causing oxidative stress to cells is administered. Under such conditions of oxidative stress, excessive haemolysis starts to occur in the body of G6PD deficit person causing haemolytic anaemia which can also prove fatal if use of drug is not stopped or anaemia is not controlled. For such patients, checking G6PD levels becomes necessary before administration of any drug that can cause stress to cells. For this purpose, various testing methods such as biochemical G6PD activity test, methaemoglobin reduction test, cytochemical test, fluorescent spot test, etc., are available but these tests are very costly and require sophisticated instruments, trained personnel, and are not portable. So, in 2016, Kaewarsa et al. [10] proposed a method of G6PD detection based on paper-based analytical devices which is a lab-on-chip kind of device that can be used as portable, solvent friendly, cost effective, and time economic method for prevention of acute haemolytic crisis. In this study, the group fabricated a device on Whatman Filter paper No. 4, by using wax printing technique. The fabricated device was pre-loaded with reagents like Glucose-6-phosphate, $NADP^+$, and TNBT which produced red coloured formazan complex detected by using desktop scanner and recording intensities from the colour observed. The calibration curve for varying concentrations of G6PD was prepared and r^2 value of 0.973 was observed. 151 samples of blood were tested for G6PD levels and results were compared to the gold standard biochemical G6PD activity test and precision was found to be 5.38% and 11.91% for G6PD normal and G6PD deficit samples, respectively. Although the proposed method is not as much sensitive as compared to the gold standard test, but it gives reliable results in relation to G6PD levels and can be used to detect G6PD levels at the spot in minimal time.

4.1.6 Detection of dopamine in blood

All the functions in the human body are controlled by different neurotransmitters such as acetylcholine, adrenaline, dopamine, etc. Among these, dopamine has very important role in the nervous system in functions of behaviour, mood, circadian rhythm, cognitions etc., of a person. Normal concentrations of dopamine are in nanomolar to micromolar ranges (from 10 $nmol{\cdot}L^{-1}$ to 1 $\mu mol{\cdot}L^{-1}$) of extracellular fluids of body. Even minute changes in this concentration of dopamine can result in various psychological disorders such as schizophrenia, attention deficit hyperactivity disorder (ADHD), and Parkinson's disease. Conventionally, levels of dopamine in body have been determined using various methods such as enzymatic assays, chromatographic techniques, and capillary electrophoresis. These methods are extremely sensitive but at the same time require high precision, costly solvents, trained professionals, and tedious instrumentation for testing. To overcome this, Liu et al. in 2018 [27] reported a paper-based analytical device-based method for easy, portable, time economic, and cost-effective detection of dopamine in blood samples. The device was fabricated on Whatman Filter paper No. 1 using solid ink printer with consequent heating for effective formation of distinct hydrophobic and hydrophilic zones. The fabricated device was loaded with ferric chloride and phenanthroline solutions. On addition of dopamine, ferric ions are converted into ferrous form which reacts with phenanthroline giving red coloured complex. This red coloured complex is detected using iPhone 5 camera and the intensities were recorded using Adobe Photoshop software. The recorded intensities were processed as a function of concentration of dopamine in the sample. The study shows a detection limit of 0.37 $\mu mol \cdot L^{-1}$ of dopamine with r^2 value = 0.999 for the calibration curve of the same. The LOD of the analyte in this study was found to be similar to that of an already reported study performed using a highly sophisticated instrument, hence proving a sensitive and effective method. The recovery using the proposed method was found to be 99.4–99.6%. Thus, the proposed study helps with convenient, disposable, and time economic alternate of traditional analytical methods.

4.1.7 Detection of H_2O_2 and glucose using starch-iodide-gelatin μPAD

Glucose is one of the most important metabolic products in the body. It also acts as biomarker for diseases affecting glucose metabolism and in some cancers such as Islet cell tumours. Thus, detection of glucose with high sensitivity, selectivity, and efficiency is one of the prime needs in biochemical detection methods. Microfluidic paper-based analytical devices have emerged as important technique for such detections using relatively simpler solvents, being very time and cost effective and highly portable methods. In a study by Liu et al., in 2019 [57], a starch-iodide-gelatin-based microfluidic paper device was proposed. This study used a device fabricated using starch, potassium iodide, and gelatin mixture. H_2O_2 was detected using starch, potassium iodide, and gelatin, whereas glucose was detected using starch, potassium iodide, gelatin, and glucose oxidase system. Analytes were tested in concentration ranges of 0.5–6 mM for H_2O_2 and 0.5–5 mM for glucose, and their LOD was found to be 0.1 and 0.05 mM, respectively. For real time studies, real human serum samples were tested for Glucose and recovery values were found to be in the range 95.7–97%.

4.1.8 Detection of lactoferrin in human body fluid

Lactoferrin is one of the glycoproteins present in our body and it is also known as biomarker for various diseases. Various traditional or conventional methods are present which are based on immunoassay and show selectivity and high sensitivity but is not suitable for point-of-care testing. Kudo et al. [58] in 2019, developed a colorimetric detection method using μPADs. In this method, the color intensity can be judged by naked eye and also using smartphone for colorimetric sensing for quantitative lactoferrin analysis due to high affinity of lactoferrin to ferric ions. Lactoferrin concentration is estimated according to colour change in complex-encapsulated particles formed by displacement of indicator from a calorimetric by lactoferrin. This method shows the LOD to be 110 $\mu L \cdot mL^{-1}$. This proposed method can be used for routine analysis of lactoferrin in blood.

4.1.9 Detection of SARS-CoV-2 humanised antibody

SARS-CoV-2 is a 30 kb single-stranded positive-sense RNA that is more than 89% identical to SARS-CoV [59]. The symptoms of infected people range from mild flu-like symptoms to severe bilateral pneumonia and death [60]. Thus, diagnosis of COVID-19 infected patients is urgently needed to execute the quarantine procedure to isolate them from the rest of the population, limiting the spread of the virus. A timely and accurate testing method is required for the diagnosis of SARS-CoV-2 infection. The World Health Organisation (WHO) currently recommends reverse transcription polymerase chain reaction (RT-PCR) as one of the most accurate and sensitive methods for determining SARS-CoV-2 infection because it directly detects viral RNAs in the sample [61]. However, collecting the sample and performing this diagnosis techniques necessitate highly trained medical personnel and advanced instruments, as well it is an expensive procedure. A relatively quick, sensitive, and low-cost platform could aid in alleviating the aforementioned challenges, particularly in underdeveloped nations. Kasetsirikul et al. [62] created a paper-based ELISA for the detection of SARS-CoV-2 humanized antibodies. Because of its low cost, ease of use, and small sample volume, paper-based ELISA is promising for diagnostic applications. The paper device was coated with recombinant SARS-CoV-2 nucleocapsid antigen and laminated for easy sample handling. In the presence of 3,3′,5,5′-tetramethylbenzidine substrate, this assay used a colorimetric reaction followed by a horseradish peroxidase (HRP) conjugated detecting antibody (TMB). By using digital image processing, the colorimetric readout allows for naked eye inspection and quantifiable data. According to the results, this assay can detect SARS-CoV-2 humanized antibody at 9 $ng \cdot L^{-1}$ (0.122 $IU \cdot mL^{-1}$) with a percent RSD of less than 10%. In contrast, the LOD and repeatability of commercial ELISA kits on the market are 5 IU·mL^{-1} with a percent RSD of 15–20%.

4.1.10 Detection of biomarkers of bladder cancer

Bladder cancer is a tumour of the urinary system that is more common in men and has a high incidence of recurrence [63]. In clinics, cystoscopy is the gold standard for detecting and tracking bladder cancer recurrence [64]. This method, however, is inconvenient and less sensitive to early detection. Other diagnostics, such as fluorescent *in situ* hybridisation (FISH) [65], quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR) [66], circulating tumour cells (CTCs) [67], and ImmunoCyt, however, require expensive apparatus and skilled operators, limiting their utility. Jiang et al. [68] have conducted a study employing a microfluidic paper-based analytical device fabricated by wax printing to detect bladder cancer biomarkers named nuclear matrix protein 22 (NMP22) and bladder cancer antigen (BTA) from urine samples. The detection is screened by ELISA using a pair of antibodies that can combine with NMP22 and BTA antibodies, with phosphate-buffered saline as the control. An antibody from the pair is fixed at the detection line, while the other is coupled to the colloidal gold, based on the idea of an antigen-antibody immune response (creation of a double-antibody sandwich structure). As experiment and control groups, urine samples were taken from 11 bladder cancer patients and 10 healthy people. Out of which only one is a false negative, with eight being double-positive and two being only positive for NMP22. For the urine samples from the ten healthy subjects, no colour was seen in the detection line. These findings suggested that the PAD can be used to detect bladder cancer in urine samples successfully. As a result, the data revealed an astonishing checkout efficiency of up to 90.91%. Meanwhile, this technology allows for home-based self-detection from urine samples in less than 10 min for the entire procedure, providing a new tool for tumour diagnosis, prognosis evaluation, and drug response evaluation that is quick, inexpensive, and simple. The significant results of applications of μPADs devices in bio-fluid analysis have been compiled in Table 1.

4.2 Analysis of foods and food products

4.2.1 Detection of pesticides in vegetables

With the rapid expansion of population, food demands of the economy have risen a lot due to which use of chemical agents in agricultural practices has seen an abrupt increase. With the increased use, chances of presence of pesticides as contaminants in foods and vegetables have thus been raised multifold. Pesticides are usually chemical toxins responsible for improved growth, better disease prevention, and great pest resistance, but at the same time, are more dangerous for human health when consuming foods with pesticide contaminations. Among all the pesticides, organophosphates (OP) are the most common class which are effectively used in agriculture. Thus, better, sensitive, and effective analytical tools are required for their detection in food products. There have been many conventional methods used for the detection of OPs in food products such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS)/MS, enzyme linked immunosorbent assay (ELISA), etc., but all these techniques have their own merits and demerits. A relatively simple and cheap but reliable method is proposed by Nouanthavong et al. in 2016 [69] based on nanoceria-coated paper-based analytical device. The principle behind detection of pesticides in this study was using acetylcholine esterase and choline oxidase activity reduction and sensing using evolved H_2O_2 for colour change in ceria particles pre-loaded on the paper. For analytical purposes, two main OP pesticides methyl-paraoxon and chlorpyrifos-oxon were tested and their detection limits were found to be 18 and 5.3 ng·mL^{-1}, respectively. For actual samples, spiked cabbage (vegetable) and dried green mussel (food) were tested. Recovery values of tested OPs in these tests were found to be ~95% which were very similar to those of LC-MS/MS analysis, thereby giving reliable and sensitive results.

4.2.2 Detection of chlorpyrifos by competitive inhibition

Use of pesticides have increased rapidly over the last few years to meet food demands in the expanding population. To detect such pesticides present as contaminants in the food products have thus become utmost important in respect to human health. For this purpose, Kim et al., in 2018 [70], developed a paper-based analytical device based on principal competitive inhibition for detection of chlorpyrifos. The study used indoxyl acetate and acetylcholine esterase as pre-loaded agents to be loaded on the fabricated paper. Here indoxyl acetate converts into blue coloured indigo dye if there is any inhibitor of Acetylcholinesterase (AChE) present. When pesticide-contaminated sample is added, pesticide inhibits activity of AChE hence producing colour in indoxyl acetate. In this study, the colour thus produced is recorded by imaging using Canon Powershot G15 digital camera and intensities are recorded

Table 1: Applications of μPADs in bio-fluid analysis

No.	Analyte	Biological matrix	Type of device	Detection technique	End point	R^2	LOD	Scanner	Ref.
1	Creatine	Urine	Enzymatic paper-based device	Colorimetric	Pink red colour	0.983	$2.0\ \text{mg}\cdot\text{dL}^{-1}$	Inkjet	[54]
2	Thiocyanate	Saliva	Paper-based analytical device	Colorimetric	Red colour		0.06 mM	HP-4500 printer/scanner	[18]
3	IL-5, HBsAg, and IgG	Eosinophil and hepatic	Dextran-based PDMS-fabricated microfluidic device	Colorimetric	Luminescent complex	Range of ng of analyte	—	—	[55]
4	Glucose and H_2O_2	Blood	Microfluidic device coated with cera nanoparticles	Colorimetric		0.985 and 0.965	—	—	[56]
5	G6PD	Blood	Lab-on-chip kind device	Colorimetric	Red colour	0.973	—	Desktop scanner	[10]
6	Dopamine	Blood	PAD	Colorimetric	Red	0.999	$0.37\ \mu\text{mol}\cdot\text{L}^{-1}$	Adobe	[27]
7	H_2O_2 and glucose	Human serum	Starch-iodine-gelatin-based microfluidic paper	Colorimetric	—	—	0.1 mM and 0.05 mM	—	[57]
8	Lactoferrin	Blood	μPAD	Colorimetric	—	—	$110\ \mu\text{L}\cdot\text{mL}^{-1}$	—	[58]
9	SARS-CoV-2-humanised antibody	Human serum	Paper-based ELISA	Colorimetric	Blue colour	—	$9\ \text{ng}\cdot\mu\text{L}^{-1}$	MATLAB R2018b	[62]
10	Nuclear matrix protein-22 and bladder cancer antigen	Urine	Dual channel μPAD	Colorimetric	—	—	—	Image J	[68]

using image manipulation software. The detection limit for chlorpyrifos was found to be between 8.6 and 12.46 ppm. The r^2 value for calibration curve was found to be 0.96.

4.2.3 Bioactive paper sensor for organophosphates and carbamates detection

Food contamination due to pesticides require rigorous and effective control on limits with safe, efficacious, reliable, and reproducible methods with extreme sensitivity. Organophosphates and carbamates are among the most toxic chemical agents to be used as pesticides with severe fatal and sometimes lethal outcomes. Badawy and El-Aswad in 2014 [71], developed a paper-based analytical biosensor device for detection of organophosphate and carbamate pesticides. Profenofos and methomyl were the main pesticides used as analyte in this study among the organophosphate and carbamate classes, respectively. Biosensor paper was fabricated by spreading the reagents on paper using a painting brush. The spread of reagents included mixture of chitosan immobilised with acetylcholine esterase by glutaraldehyde in the presence of DTNB. When the analyte containing sample was introduced on the paper, the pesticides in the sample caused a reduction in the hydrolytic activity of AChE on acetylthiocholine as a substrate. The yellow colour produced was noted for its intensity and recorded for analytical purposes. Detection limits for profenofos and methomyl were found to be 0.27 mM and 6.16×10^{-4} mM, respectively.

4.2.4 Detection of nitrite and nitrate in food samples

Sodium and potassium salts of nitrite are used in various food products as preservatives and also to increase the colour and flavour in various vegetables. As intake of these causes health concern, to overcome these Thonkam et al., in 2020 [72], have proposed a method for simultaneous estimation of nitrate and nitrite in food products. In this method they used a µPAD, which uses beeswax as a new hydrophobic material. Beeswax emulsion was applied onto the filter paper with the help of screen printing to form a hydrophobic barrier and thus defining hydrophilic areas in the paper as it permeates in a portion of the paper and it measures simultaneously the nitrite and nitrate using colorimetric detection based on Griess method. Then, the results are measured by measuring the colour intensity using ImageJ of the resultant colour. Nitrite can be detected by derivatising with sulphanilamide and *N*-(1-naphthyl)ethylenediamine, it is detected on the left side of the paper and in case of nitrate it must be first reduced to nitrite by vanadium(III) salt and then Griess reaction can proceed, after measuring the colour intensity by using ImageJ and after comparing the two values, the concentration of nitrite and nitrate can be measured. This method has good reproducibility, efficient resolution, and does not require organic solvent or complicated instrument, and the results obtained by the µPAD was closely related with those obtained from the spectrophotometric method. The detection limit of nitrite and nitrate was found to be 0.1 and 0.4 $\mathrm{mg{\cdot}L^{-1}}$, respectively, and RSD of nitrite and nitrate was found to be 1.8–2.9% (inter-day) and 2.1–3.1% (intra-day), respectively. The proposed method can be used for routine analysis of nitrate and nitrite in food product.

4.2.5 Detection of norfloxacin residues in food

With the growth of large-scale animal agriculture has come a marketable increase in the farmers' use of veterinary drugs or veterinary medicinal products to prevent illness, treat illness, improve feed efficiency, and augment animal growth [73]. This increase in antibiotic use has unfortunately resulted in an increase in antibiotic-resistant strains of bacteria, a decrease in overall fitness of the animals, and a serious public health risk because these residues frequently pass through the food supply chain and end up in our diets [74]. Antibiotic use on a regular basis can result in unexpected bioaccumulation of these antibiotics in our bodies. Antibiotic resistance, allergenic response, carcinogenicity, mutagenicity, teratogenicity, and disruption of normal intestinal gut flora can all result from such accumulation to a certain level [75]. The fluoroquinolone class is one of the most commonly used antibiotic families in animal agriculture, distinguished by its broad spectrum of action. Norfloxacin, also known as 1-ethyl-6-fluoro-4-oxo7-piperazin-1-yl-1*H*-quinoline-3-carboxylic acid, is a common member of this family used in animal agriculture. It works by inhibiting bacterial DNA replication. It is a fluorinated third-generation quinolone antibiotic with fluorination at position 6. The complex food matrix, which might impair sensitivity and so necessitates lengthy sample pre-treatment steps, is one of the challenges in detecting antibiotics in meat products. Antibiotic monitoring systems such as spectrofluorometry, HPLC-MS, and ELISA that have been used in the past are labour and resource intensive, necessitate highly trained personnel, are costly, and take a long time. A method for detecting norfloxacin in four different food matrices has been proposed by Trofimchuk et al. [76] using the coffee-ring effect with paper-based microfluidic analytical devices. The sensitivity of norfloxacin determination was improved in this study. In this study, μPADs were created by printing wax channels onto cellulose-based filter paper and then heating the paper to allow the wax to enter the paper. The colorimetric reaction between norfloxacin and the added iron(III) nitrate nonahydrate in 5 mM ammonia in each reaction chamber was monitored to determine norfloxacin content in the food samples. A noticeable colour shift was caused by the metal ion–antibiotic combination. Following the colorimetric reaction, pictures were captured and analysed using ImageJ software to estimate the relative pixel intensity, which was then used to calculate norfloxacin concentration. The analytical sensitivity of this device was determined to be as low as 50 ppm when studying the inner-ring reaction and as low as 5 ppm while analysing the outer coffee ring, providing for a cheaper, faster, and more user-friendly technique to detect norfloxacin than current methods.

4.2.6 Detection of pesticides residue in food using organic solvents

Assays of AChE inhibition have received a lot of attention as a good way to quickly screen pesticide residues in food safety assessment [77]. The detection is typically predicated on the basis of toxicity mechanism that organophosphate and carbamate pesticides from samples block the activity of AChE selectively, suppressing the AChE catalysed probe molecule hydrolysis [78]. Most organophosphate and carbamate insecticides are well recognised for their low water solubility. As a result, aqueous solution extraction efficiency of pesticide residues in food samples is typically low, resulting in poor responsiveness and incorrect findings. However, most organic solvents, despite their greater extraction capabilities, would poison AChE, and therefore, affect detection accuracy if they were present in the enzyme reaction system [79,80]. Jin et al. [78] proposed a new protocol incorporating AChE inhibition assay with organic-solvent-based sample extraction for the rapid and sensitive detection of organophosphates (phoxim, chlorpyrifos, triazophos, and methamidophos) and carbamates (carbaryl and carbofuran) pesticides in food, which was developed on microfluidic paper-based devices fabricated using wax printing technique. Instead of being pre-immobilised onto the chip substrate, AChE was applied after the sampling and solvent evaporation steps. As a result, enzyme toxicity from sample pre-treatment solvents was totally avoided. From this study it was found that the pesticides chlorpyrifos, phoxim, carbaryl, triazophos, carbofuran, and methamidophos had detection limits of 0.77, 0.39, 0.25, 1.29, 0.006, and 1.39 $mg{\cdot}L^{-1}$, respectively.

4.2.7 Detection of *S. aureus* and *E. coli* and their antibiotic resistant strains in milk

Milk is an important part of the human diet and is included in many nations' official dietary standards. It contains nutrients that are difficult to come by in dairy-free diets, including amino acids, vitamins, and minerals [81]. However, milk is not only healthy for people and animals, but it is also a favourable development medium for a variety of bacteria [82]. *Escherichia coli* and *Staphylococcus aureus* are important milk-borne pathogenic bacteria that cause a variety of illnesses in people and animals, including food poisoning and gastroenteritis. One of the main factors for the development of antibiotic resistance bacterial strains is the frequent and extended use of antibiotics in feedstock animals. The ability to design effective preventive and treatment plans requires the identification of infections and their antibiotic sensitivity. Asif et al. [83] have conducted research in which a variety of chromogenic substrates were used to test microfluidic paper-based devices fabricated by wax printing for presumptive identification of *E. coli* and *S. aureus* and their antibiotic-resistant strains in milk samples. *E. coli* and *S. aureus* are detected using chromogenic substrates such as CPRG and BCIP, respectively. Traditional and extended lactam resistant bacteria were identified using the chromogenic substrates nitrocefin and HMRZ-86, respectively. At *E. coli* concentration of $\geq 3.6 \times 10^6$ $cfu{\cdot}mL^{-1}$, significant change in colour from yellow to red-violet when reacted to CPRG was observed. In case of *S. aureus*, an identifiable change in colour from colourless to mauve/purple was noticed at concentration exceeding 3.6×10^6 $cfu{\cdot}mL^{-1}$. The change in colour at a concentration $\geq 1.3 \times 10^6$ $cfu{\cdot}mL^{-1}$ confirmed the presence of β-lactam-resistant strains of *S. aureus*. According to the study, the paper devices had a sensitivity of 90% and selectivity of 100% on 640 milk samples. The important outcomes of analysis of food stuffs through these techniques are summarised in Table 2.

Table 2: Results of food analysis through μPADs

No.	Analyte	Biological matrix	Type of device	Detection	End point	R^2	LOD	Scanner	Ref.
1	Pesticide (methyl paraoxon and chlorpyrifos-oxon)	Vegetables	Nano ceria-coated PAD	Colorimetric	–	–	18 and 5.3 ng·L^{-1}	–	[69]
2	Chlorpyrifos	Food products	PAD	Colorimetric	Blue coloured indigo dye	0.96	8.6–12.46 ppm	Cannon power shot G15 digital camera	[70]
3	Organophosphate and carbamate pesticide (profenofos and methomyl)	Food products	PAD	Colorimetric	Yellow colour	–	0.27 mM and 6.16 mM		[71]
4	Nitrite and nitrate	Food products	PAD	Colorimetric	–	–	0.1 mg·L^{-1} and 0.4 mg·L^{-1}	Image J	[72]
5	Norfloxacin	Meat and fish	μPAD	Colorimetric	Yellow orange colour	–	50 ppm per 5 ppm	Image J	[76]
6	Chlorpyrifos, phoxim, carbaryl, triazophos, carbofuran, and methamidophos	Tea, head lettuce, and apple juice	μPAD	Colorimetric	–	–	0.77, 0.39, 0.25, 1.29, 0.006, and 1.39 mg·L^{-1}	Photoshop software	[78]
7	*S. aureus* and *E. coli*	Milk	μPAD	Colorimetric	Yellow to red violet (*E. coli*) and colourless to mauve/purple (*S. aureus*)	–	≥3.6 × 10^6 cfu·mL^{-1} (*E. coli*) and 3.6 × 10^6 cfu·mL^{-1} (*S. aureus*)	Image J	[83]

4.3 Analysis of water samples

4.3.1 Determination of reactive phosphates in water samples

Water is one of the most important aspects for sustenance of all life forms on the planet. While 71% of the Earth is covered with water, the amount of potable water is very less as compared to the total volume because almost 97% of water is held in ocean bodies. Amidst all this, water pollution is a major reason behind the crisis of potable water availability. Thus, a check on such reasons is a very crucial issue in the present times. For the same, various methods and tests are available including pH tests, clarity tests, biological oxygen demand (BOD) value, as well as sophisticated instrumental methods like HPLC/UPLC etc., are also available. But these require time and expertise for a reproducible and reliable testing. In 2012, Jayawardane et al. [84] devised a paper-based analytical method for detection of reactive phosphate species involving colorimetric detection of analytes based on the formation of phospho-molybdenum blue due to phosphate ion species in water. Technique employed was wax printed fabricated device for analytical purposes. The reported device was characterised in the working concentration range of 0.2–10 mg·L^{-1} of phosphorus with LOD and LOQ of 0.05 and 0.16 mg·L^{-1} of P, respectively. The observed results were reproducible and reliable as compared to the standard testing methods with extremely low requirement of solvents, equipment, and expert professionals.

4.3.2 Tetrazine-based nitrite determination

Nitrite is an especially important component in chemistry playing an imperative role in many synthetic pathways in addition to physiological, food, and environmental functions. Conventionally, nitrite analysis was done in many ways such as spectrophotometry, potentiometry, amperometry, and luminescence, but all these techniques had few demerits like controlling pH and temperatures or using carcinogenic agents. To overcome these, Ortiz-Gomez et al., in 2016 [85], reported a microfluidic paper-based analytical device involving detection and quantitation of nitrite based on formation of dye on reaction with tetrazines. The study employs acidic medium to convert nitrite into nitrous acid responsible for reaction with s-dihydrotetrazine producing pink colour. With optimisation of parameters like pH, sample volume, and reaction time, the detection limit for this method was found to be 1.30 μM. The method was found to be highly reproducible and reliable and was also applied for detection of nitrite ions in water and compared to standard procedures.

4.3.3 Determination of water hardness, total phenol, and pH

Water is one of the most important resources in our life, therefore its quality control is very important, there are many problem regarding the quality of water like water hardness, acidity, and the presence of toxic organic species. The dissolved calcium and magnesium ions in water define the total water hardness, water hardness less than 60 mg·L^{-1} is soft, 61–120 mg·L^{-1} level is considered moderate hard, 121–180 mg·L^{-1} is considered hard, and above 180 mg·L^{-1} is considered very hard. The determination of water hardness is carried out by complexometric titration with EDTA. Da Silva et al., in 2020 [86], proposed a device based on colorimetric measurements using a smartphone camera and a μPAD. In this method, the samples are added on the μPAD containing the indicator and then the images are captured by smartphone and lastly the treatment based on the generated histogram. The hardness is determined by reaction between EBT and Mg^{2+} and Ca^{2+} and the formation of complex between them and is compered using the colour contrast. The pH determination is observed by change in colour between the protonated structures. The method found the linearity to be in range of 20–560 mg·L^{-1} for Ca^{2+} and Mg^{2+} (hardness), 0.20–16 mg·L^{-1} for catechol, and pH from 4.7 to 12, and limits of detection of 0.083 mg·L^{-1}, 0.124 mg·L^{-1}, and 0.262 for water hardness, catechol, and pH, respectively. This proposed method can be used for routine analysis of water quality.

4.3.4 Detection of phosphate in water

Water is one of the most important things in our life. Groundwater is contaminated in various ways and high concentration of contaminants like phosphates can impact our health. So, to address this problem, various methods are proposed like microelectrode integrated with microfluidics system, but this method is too complicated, large quantities of samples are required, and was also difficult to monitor. So Waghwani et al., in 2019 [87], proposed a method which uses μPAD for quantification of phosphates in water by colorimetric assay and RGB value of colour produced quantifies the phosphate concentration. It detects the phosphates in water by the use of reagent stored in paper. The μPAD is printed with the help of rubber stamp PDMS material and kept in the oven to parch and form the hydrophobic barrier and then the chemical are applied on the paper. The test is carried out by applying a single drop of water and results are obtained. The results obtained by ImageJ is specific and more precise. This method is very excellent because it uses low volume of fluid, has high specificity, and low cost. The concentration range of 120 mg·mL^{-1} of phosphate can be detected for which only 1 mL of water is required. This proposed method can be used for routine analysis of water.

4.3.5 Detection of bacterial sample in water

Microfluidic paper-based device is used in various applications and to analyse complicated biological samples. Lin et al., in 2019 [88], proposed a method for fabrication of μPADs with water-based polyurethane acrylate. In this method, the filter paper is coated with water-based polyurethane acrylate and then covered by a mask with designed patterns. It is then exposed to UV lamp and then rinsed with water. Water-based polyurethane acrylate is used as it can withstand surfactant solution and organic solvent. This method is used as it are useful for analysing complicated biological samples. Assay of *E. coli* was done by dropping the bacteria lysate onto the colorimetric substrate of the hollowed circles and after some time the colour intensity was measured by seeing the colour change from yellow to red-violet. The LOD was found to be 3.7×10^3 cfu·mL^{-1} and linear response was found to be in the range of 10^4 to 10^9 cfu·mL^{-1}, R^2 was found to be 0.065 and at varied concentrations of 10^5, 10^6, 10^7, and 10^8 cfu·mL^{-1}, the RSD of colour intensity were found to be 3.15%, 3.62%, 2.26%, and 2.78%, respectively. This method can be used for routine analysis of bacterial detection like *E. coli* and other bacterial samples in water.

4.3.6 Detection of nitrate in water

Nitrate is an ion that occurs naturally as part of the nitrogen cycle [89]. It is also an important ingredient for plant growth, but when present in high amounts, it contributes significantly to water nutrient pollution [90]. Nitrate in water comes from a variety of sources, including fertilisers or manure runoff, air deposition, agricultural sources, septic tanks, and wastewater treatment plants.

Because nitrate is the most stable form of nitrogen in oxygenated conditions, all other nitrogen-containing molecules in water can likewise serve as a source of dissolved nitrate [91]. Drinking nitrate-rich water raises the risk of colorectal cancer, thyroid disorders, and birth abnormalities in the central nervous system. There are numerous methods for measuring nitrate levels in water that are currently in use. These traditional procedures, on the other hand, necessitate costly instruments and time-consuming analysis. They also necessitate special sample handling and preparation, which necessitate the use of qualified staff. Charbaji et al. [90] has developed a microfluidic paper-based device, fabricated via wax printing, to detect nitrate in water. It was a simple paper-based design with folding architecture. The detection zone's quality and uniformity, as well as the detection limit, were increased by folding the detection zone over the reagent pad. In water, the proposed nitrate device has detection and quantification limits of 0.53 and 1.18 ppm, respectively.

4.3.7 Determination of pesticides in water

Pesticide use in agriculture has increased, posing a risk of pesticide accumulation in soil, agrofood products, and water, resulting in pollution that is hazardous to living things and human health [92]. Pesticide residues, even at low levels, can have a significant impact on live animals' neurological systems if they enter the food chain [93]. Furthermore, pesticide residues are accumulated in rivers and ponds as a result of rain or irrigation runoff near areas where pesticides are widely used, resulting in water pollution. As a result, a simple, portable, and easy-to-use analytical device for determining pesticides is still necessary. Fernández-Ramos et al. [94] proposed a study presenting a bioactive microfluidic paper device, fabricated using laser cutting technique, for the determination of organophosphorus and carbamate pesticides in water, using chlorpyrifos and carbaryl as models, respectively. Enzyme immobilised original and effective paper design as the sensor platform is employed for detection. The signal transduction is caused by the inhibited enzyme hydrolysing the substrate, which causes local pH changes in the sol–gel entrapped indicator, as well as a colour change, which is measured with a photographic camera using colour coordinates as the analytical parameter for pesticide quantification. The suggested approach was used to successfully determine carbaryl and chlorpyrifos with a LOD of 0.24 and 2.00 $g{\cdot}L^{-1}$, and a repeatability of 4.2% and 5.5%, respectively.

4.3.8 Detection of ammonia in industrial wastewater

Ammonia (NH_3) is a chemical that is widely used and produced in a variety of industries. In wastewater, it is the most prevalent nitrogenous contaminant [95]. Because of its toxicity, the presence of ammonia in wastewater is a major concern. Ammonia toxicity can be harmful to fish and other aquatic animals, resulting in dissolved oxygen depletion and eutrophication [96]. It has already been documented that aquatic species can die at concentrations as low as 0.2 ppm ($mg{\cdot}L^{-1}$). Nxumalo et al. [96] has proposed a method for colorimetric identification and quantification of ammonia in industrial wastewater using μPAD. The device was fabricated by wax printing. This study has produced a low-cost monitoring system for ammonia detection in wastewater, a modified version of the colorimetric approach employing Nessler reagent integrated with microfluidic technologies. The method can detect ammonia in the range of 0–5 ppm and has a detection limit of 3.34 ppm.

4.3.9 Detection of bisphenol A (BPA) in water

US Environmental Protection Agency (EPA) defined endocrine-disrupting compound as "an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process" [97]. Compounds such as dioxin and dioxin-like compounds, polychlorinated biphenyls, pesticides, and plasticisers are examples of endocrine-disrupting substances, which are classified as emerging pollutants because they can disrupt human hormonal functions [98]. BPA, an endocrine-disrupting compound, which is used in the industrial manufacturing of epoxy resins, polycarbonate plastics, and lacquer coating, to mention a few, has drawn significant attention from the scientific community due to its toxicity and presence in the environment [99]. Several studies have linked BPA exposure to diabetes, heart disease, obesity, breast and prostate cancer, sperm quality problems, neurotoxicity, and polycystic ovarian syndrome [100]. For BPA quantification, variety of analytical techniques are available, for instance, HPLC, ELISA, gas chromatography-mass spectrometry, and molecular imprinted sensor. However, the abovementioned techniques are time consuming and require costly instruments. The rapid and sensitive detection technique of BPA is thus an important issue in analytical chemistry for quickly assessing the safety of water and food samples, as well as the pollution of surface waterways. Jemmeli et al. [98] have proposed a highly sensitive paper-based electrochemical sensor for an enzymatic-free detection of BPA method. For a reagent-free analytical tool, this sensor includes the full electrochemical cell printed on filter paper as well as the reagents for the measurement loaded in the cellulose fibre network. The working electrode was printed with carbon black-modified ink, and the paper was fabricated by wax printing technique. The combination of low-cost nanomaterial carbon black with cheap filter paper enables the construction of a low-cost, high-sensitivity sensor for BPA measurement at the microgram level in river and drinking water samples. The results of water analysis in different samples are depicted in Table 3.

5 Techniques to increase sensitivity of µPAD

There are various sampling and pre-concentration techniques available that have been reported to increase the sensitivity of µPAD. They are elaborated below.

5.1 Isotachophoresis (ITP)

ITP is a sample focusing and separation technique that uses varying ion mobility zones [101]. In ITP, a leading electrolyte (LE), a trailing electrolyte (TE), and a sample analyte sandwiched between the two are used. ITP demands that LE and TE have a common counterion but separate coions when it comes to their composition. Coions (counterions) have the same (opposite) electric polarity as charged analytes. Analytes should have a higher ionic mobility than TE coions but a lower ionic mobility than LE coions. A low (high) electrical field in LE (TE) is formed when an electric potential is supplied across the entire zone. Analyte ions in the TE travel quicker than TE coions, whereas analytes in the LE move more slowly. The difference in analyte velocity between LE and TE causes analytes to focus on the LE/TE interface. Kondratova et al. were the first to demonstrate successful ITP extraction of nucleic acids from complex biological samples in agarose gel rods, including human plasma/serum and urine, though this required extensive sample preparation, such as overnight incubation with proteinase K/SDS and dialysis [102,103]. Since then, ITP has been used to produce pure nucleic acids from a variety of biological materials, including urine, milk, and blood,

Table 3: Outcomes of water analysis through µPADs

No.	Analyte	Biological matrix	Type of device	Detection	Endpoint	R^2	LOD	Scanner	Ref.
1	Reactive phosphate species	Water	PAD	Colorimetric	Phospho-molybdenum blue	—	0.05 mg·L^{-1}	—	[84]
2	Nitrite	Water	PAD	Colorimetric	Pink colour	—	1.30 µM	—	[85]
3	Water hardness, total phenol, and pH	Water	PAD	Colorimetric	—	—	0.083, 0.12, and 0.262 mg·L^{-1}	—	[86]
4	Phosphate	Water	PAD	Colorimetric	—	—	1 mg·mL^{-1}	Image J	[87]
5	Bacterial sample	Water	PAD	Colorimetric	Red violet colour	0.065	3.7×10^3 cfu·mL^{-1}	—	[88]
6	Nitrate	Water	µPAD	Colorimetric	Coloured azo dye		0.53 ppm	Canon TS6020	[90]
7	Chlorpyrifos and carbaryl	Water	µPAD	Colorimetric	—	—	Chlorpyrifos: 2 g·L^{-1} and Carbaryl: 0.24 g·L^{-1}	Image J	[94]
8	Ammonia	Industrial wastewater	µPAD	Colorimetric	Brown or intense yellow		3.34 ppm	Image J	[96]
9	Bisphenol A	Water	µPAD	Electrochemical	—	—	0.3 µM	—	[98]

in both microchannel and paper-based formats [104–106]. Sullivan et al. have demonstrated nucleic acid sample preparation in paper-based microfluidic device using ITP from whole blood [107].

5.2 Field amplified sample stacking

Field-amplified sample stacking (FASS) is a popular method for preconcentrating analytes before capillary electrophoresis separation [108]. The sample is prepared in a lower conductivity solution than the running buffer solution in FASS. FASS may attain concentration factors of 100–1,000 folds; however, it necessitates the use of several buffers [109]. However, On-line concentration sampling, such as using FASS grafted onto a paper chip with array channels, has not yet been reported. Moving reaction boundary and field-amplified sample stacking (M-FASS) is a hyphenated technique described by Ouyang et al. for the separation, concentration, and visualisation of multi-component metal ions on a paper chip with six micron wide channels. Using a hybridised stacking technique called M-FASS, researchers were able to produce a 160-fold increase in sample concentration in paper channels with micro-widths in just 6 min. The Fe^{3+}–I^- oxidation–reduction reaction was shown to enhance the Fe^{3+} detection limit, which was as low as 0.34 ng for samples containing multiple metal ions [110].

5.3 Ion concentration polarisation

Ion concentration polarisation is another preconcentration technique. To improve detection sensitivity, using an electrokinetic pre-concentrator based on ion concentration polarisation (ICP) is a viable approach. A nanoporous cation exchange membrane (e.g., nafion) and modest amounts of electricity, which can be delivered by a battery, are required for this process [111]. Gong et al. and Yang et al. have confirmed that ICP preconcentration in paper-based systems is feasible [112,113]. Based on the ICP phenomena, Han et al. developed a paper-based biomolecule pre-concentrator. Using nafion-patterned tape and wax-printed paper, they designed simple ICP pre-concentrators. Fluorescent dyes and proteins were continually delivered to the concentration zone by maintaining the lateral fluid flow with an adsorbent pad, resulting in a 1,000-fold increase in 10 min [114].

5.4 Solid phase extraction (SPE)

The basic principles of SPE are similar to liquid–liquid extraction. Both methods involve the distribution of dissolved species between two phases. However, SPE involves the dispersion of the analyte between a liquid (sample medium) and a solid (adsorbent) phase instead of the two liquid phases which cannot be mixed together as in liquid–liquid extraction. This technique allows the enrichment and purification of the analytes on a solid adsorbent through adsorption from the solution [115]. Wu et al. [116] developed a μPAD that was used in conjunction with a home-made SPE column to determine the copper(II) ion in a selective and quantitative manner. SPE-combined PAD greatly improved the selectivity and sensitivity for copper(II) ion.

6 Recent advancements

6.1 Paper-based pump free magnetophoresis

The phenomenon of migration of particles under the influence of magnetic field is known as magnetophoresis. Positive and negative magnetophoresis are two types of the phenomenon. The migration of magnetic particles in a diamagnetic medium is known as positive magnetophoresis. The migration of diamagnetic particles in a magnetic medium is known as negative magnetophoresis [117]. Paper-based microfluidic devices, unlike traditional microfluidics, do not require any external pumps to generate flow. However, particles and cells become stuck in the paper fibres, and the fluid velocities created in PADs are not fast enough for continuous separation, hence they are not currently used for magnetophoresis. The capacity of newly designed fast-flow microfluidic paper-based analytical devices (ffPADs) to perform magnetophoresis has been disclosed by Call et al. [118]. ffPADs are made by stacking two layers of wax-printed paper over a laser-cut double-sided adhesive to form channels or gaps. Fast flow is possible due to the passageways between the layers of paper, which prevent particles from becoming trapped. They show that ffPADs can separate magnetic particles in a μPAD with a neodymium permanent magnet and investigate critical performance parameters.

6.2 Development of 3D fabrication

For lateral flow assays, analytical separations, flow-based analysis, or other analytical applications, the PAD fabrications frequently include the construction of hydrophobic barriers for determining the boundaries of spot test or detection of microzones for chemical reactions, as well as fluidic channels. Photolithography, stamping, wax printing, lacquer and glue spraying, inkjet printing, laser printing, flexographic printing, 3D wax printing, and screen printing are all typical methods for creating these barriers. However, the abovementioned fabrication processes necessitate the usage of additional tools such as a printer, heat plate, oven, and so on for printing, heating, or spraying. Besides traditional fabrication processes, solutions based on pen-on-paper (PoP) have developed as easy and innovative methodologies for fabricating conductive tracks and hydrophobic barriers on paper substrates. Although it has been claimed that ordinary pens may be used to create hydrophobic barriers on paper, most PoP methods still require extra steps such as heating. Inked materials also have a low chemical compatibility with typical organic solvents and surfactants, as well as a lower mechanical resistance. For the first time, Sousa et al. [119] reported the use of a 3D pen for PADs' fabrication. The technique entails utilising an acrylic resin to create a 3D pen sketch of the desired design on paper. The polymer deposited by the pen is cured using a portable flashlight, and it takes less than a minute to make a single device. When subjected to acid and alkaline solutions, surfactants, and organic solvents (excluding ethanol), the barriers produced with the 3D pen showed high chemical resilience. The proposed approach was capable of producing spot tests with a diameter of 2 mm and channels with a width of 3 mm. Furthermore, this manufacturing process does not require any extra stages like heating or printing, which reduces the number of instruments required. Lateral flow assays specific to the detection of glucose and nitrite in saliva, as well as iron, nitrite, and copper in ambient samples, were used to effectively demonstrate the viability of the suggested devices.

By stacking layers of patterned paper and double-sided adhesive tape, Whitesides et al. proposed a method for manufacturing 3D microfluidic devices. Microfluidic capabilities in paper-based 3D microfluidic devices are challenging to achieve with traditional open-channel microsystems comprised of glass or polymers. Three-dimensional (3D) paper-based devices, in particular, wick fluids and disperse microliter quantities of samples from single inlet points into arrays of detection zones. They convey fluids both horizontally and vertically, and they allow fluid streams to cross without mixing. Capillary wicking is used in these devices to distribute fluids into complicated arrays of tens to thousands of sensing zones in seconds to minutes [120]. Liu et al. presented a method for building 3D paper microfluidic devices based on origami (paper folding) principles. In a single photolithographic step, the complete 3D device is constructed on a single sheet of flat paper. It is put together by folding the paper by hand [121].

6.3 Development of atom stamp printing and paper-based solid–liquid extraction device

μPADs are made by hydrophobically treating paper substrates to create discrete hydrophobic and hydrophilic regions, which restrict and guide fluid flow. Photolithography, wax printing, paper cutting, sketching, inkjet printing, laser cutting, and stamping are just a few of the technologies that may be utilised to make μPADs right now. Guan and Sun [122] have devised a novel atom stamp printing (ASP) method for fabricating μPADs. Atom stamps (ASs), also known as machine-etched penetrating stamps, are engraved manually or using a laser engraving equipment. The cheap cost, simplicity of operation, high production efficiency, and great resolution of this innovative technology (the minimum widths of the hydrophobic barriers and hydrophilic channels are 312 and 328 μm, respectively) set it apart from previous technologies. Since ASP uses PDMS as hydrophobic solvent, which is a clear liquid, this technique may be utilised in almost any circumstance, preventing the possibility of the hydrophobic substance contaminating the hydrophobic channels. μPADs fabricated with the ASP method were used to detect different concentrations of Cu^{2+} (heavy metal) via a colorimetric method to check the efficiency of this new fabrication technique, and the approach was found to be capable of detecting Cu^{2+} at a concentration of $1\,mg{\cdot}L^{-1}$ when combined with a distance-based detection method.

A novel paper-based solid–liquid extraction device (PSED) was also developed by Guan and Sun [122], which is based on a 3D PAD with a "3 + 2" structure and a recyclable extraction mode. Specifically, the device performed numerous extraction and filtering phases from classic solid–liquid extraction process with great efficiency by utilising the features of paper filtration and capillary force.

6.4 Development of a morphological colour image processing algorithm for PADs

The method of signal readout, as well as the detecting mechanism, is critical to the viability of PADs. Digital image processing (DIP) or the naked eye may easily notice the colour change. Because the results of signal readout by the naked eye varies for various people, DIP techniques are mostly used as an alternative to avoid such mistakes and verify that the procedure is done correctly. DIP-based approaches may also transform images into various colour spaces including NTSC, YCbCr, and HSV, as well as offer more quantitative descriptions of the system. The use of mathematical morphology recognition for automated regions of interest selection can be a viable approach to bolster DIP. Hamedpour et al. [123] proposed combining digital image processing with a model PAD to simplify and improve the signal readout method. The suggested algorithm is based on mathematical morphology recognition and reduces the mistakes that might occur while manually selecting a region. This is the first research on the design of an image processing method for PADs that allows for automatic circular and square form recognition, blank correction, and visual outlier detection. Further chemometric studies, such as outlier identification, calculating LOD, and creating regression (prediction) models, may be readily conducted using the given numerical matrices and image profiles. The proposed algorithm's signal readout results compared to ImageJ software show substantial increase in analysis speed, repeatability, accuracy, and colour values. As a result, the suggested algorithm appears to be a viable technique for practical applications.

7 Challenges and future aspects

Current µPAD technologies cannot, without a doubt, replace traditional instrumental analytical procedures for regular medication quality monitoring in the pharmaceutical industry. The difficulty in automating the platform for processing a high number of samples, as well as the absence of method validation to establish their analytical figures of merit over conventional techniques, is the problem that µPAD applications face in the pharmaceutical industry. Low sensitivity and selectivity are common problems with PAD, which creates additional hurdles for trace detection of medicinal compounds in complicated sample matrices. Incorporating on-device sample preparation that can selectively extract and enrich the analyte to increase both the selectivity and sensitivity, while reducing user procedures to a minimal is one technique that might be investigated in the future. Using more sensitive optical detection technologies such as chemiluminescence and its variants (e.g., BCL and ECL) over colorimetry, as well as developing a robust and portable detector for the applications, might be an approach to increase detection limits for drug trace analysis. The capacity to detect drug enantiomers selectively is another issue in drug analysis. Integrating chiral recognition components into µPADs might be a way to increase the application of paper microfluidics for drug analysis in the future [124]. Intrinsic variability of paper adds indeterminate mistakes into every application of paper-based devices, which is the major drawback of paper-based microfluidic devices. The accuracy of the hydrophobic barriers patterned into paper, the wicking rates of fluids in the devices, and the findings of paper-based assays can all be affected by these variations. The development of more consistent paper-making processes might significantly enhance the accuracy of paper-based microfluidic devices. The coffee ring effect, in which solutes prefer to congregate along the outer borders of test zones as solutions dry out on paper. This effect causes non-uniform distributions of analytes, reagents, or reaction products throughout the test zones, which can reduce assay accuracy and precision. The shelf life of reagents kept on paper-based microfluidic devices is another constraint. Many chemicals, including enzymes, kept on paper-based devices denature quickly. However, the introduction of different nanomaterials in µPADs has improved detection limits by up to 1,000 times, overcoming the difficulties of low sensitivity and selectivity [125].

8 Conclusion

µPAD techniques are attractive and economic “lab on chip” type devices which involve use of paper as analytical media. Hydrophobic barriers are introduced on to the paper making hydrophilic zones on the paper for containment of reaction media. For detection, the most common method used is colorimetric sensing where intensity of colour produced is used as a function of concentration of analyte. Recently, this technique has been widely used in qualitative and quantitative detection of various analytes in different samples such as detection of thiocyanate in saliva, nitrites in water, creatinine in urine, dopamine in blood, etc. These techniques have also been successfully applied in the analysis of food stuffs and water. This technique shows advantages over others as being time and cost effective. Only a very few reagents are required in this technique thereby showing green approaches. This technology also does not require any specialised training and hence can be very useful for all types of users. Future scopes of this technique involves development of analytical methods in bulk and marketed formulations, for quick estimation of drugs in different matrices and sensitive detection of various analytes from food samples, etc.

Acknowledgements: The authors are grateful to ISF College of Pharmacy, Moga for the timely guidance and encouragement.

Funding information: Authors state no funding involved.

Author contributions: Shibam Das: writing – original draft and methodology; Gagandeep: writing – review and editing; Rohit Bhatia: project administration, supervision, and validation.

Conflict of interest: Authors state no conflict of interest.

References

[1] Bragheri F, Vázquez RM, Osellame R. Microfluidics. Three-dimensional microfabrication using two-photon polymerization. Netherlands: Elsevier; 2020. p. 493–526.

[2] Carstens F, Gamelas JA, Schabel S. Engineering microfluidic papers: determination of fibre source and paper sheet properties and their influence on capillary-driven fluid flow. Cellulose. 2017;24(1):295–309.

[3] Carrilho E, Martinez AW, Whitesides GM. Understanding wax printing: a simple micropatterning process for paper-based microfluidics. Anal Chem. 2009;81(16):7091–5.

[4] Songjaroen T, Dungchai W, Chailapakul O, Laiwattanapaisal W. Novel, simple and low-cost alternative method for fabrication of paper-based microfluidics by wax dipping. Talanta. 2011;85(5):2587–93.

[5] Nurak T, Praphairaksit N, Chailapakul O. Fabrication of paper-based devices by lacquer spraying method for the determination of nickel(II) ion in waste water. Talanta. 2013;114:291–6.

[6] Burrow GM, Gaylord TK. Multi-beam interference advances and applications: nano-electronics, photonic crystals, metamaterials, subwavelength structures, optical trapping, and biomedical structures. Micromachines. 2011;2(2):221–57.

[7] Lappin G, Clark L. Colorimetric method for determination of traces of carbonyl compounds. Anal Chem. 1951;23(3):541–2.

[8] Liu W, Kou J, Xing H, Li B. Paper-based chromatographic chemiluminescence chip for the detection of dichlorvos in vegetables. Biosens Bioelectron. 2014;52:76–81.

[9] Mani V, Kadimisetty K, Malla S, Joshi AA, Rusling JF. Paper-based electrochemiluminescent screening for genotoxic activity in the environment. Environ Sci Technol. 2013;47(4):1937–44.

[10] Kaewarsa P, Laiwattanapaisal W, Palasuwan A, Palasuwan D. A new paper-based analytical device for detection of glucose-6-phosphate dehydrogenase deficiency. Talanta. 2017;164:534–9.

[11] Paterson AS, Raja B, Mandadi V, Townsend B, Lee M, Buell A, et al. A low-cost smartphone-based platform for highly sensitive point-of-care testing with persistent luminescent phosphors. Lab A Chip. 2017;17(6):1051–9.

[12] Busa LSA, Mohammadi S, Maeki M, Ishida A, Tani H, Tokeshi M. Advances in microfluidic paper-based analytical devices for food and water analysis. Micromachines. 2016;7(5):86.

[13] Smith S, Oberholzer A, Korvink JG, Mager D, Land K. Wireless colorimetric readout to enable resource-limited point-of-care. Lab A Chip. 2019;19(19):3344–53.

[14] Puangbanlang C, Sirivibulkovit K, Nacapricha D, Sameenoi Y. A paper-based device for simultaneous determination of antioxidant activity and total phenolic content in food samples. Talanta. 2019;198:542–9.

[15] Majors CE, Smith CA, Natoli ME, Kundrod KA, Richards-Kortum R. Point-of-care diagnostics to improve maternal and neonatal health in low-resource settings. Lab A Chip. 2017;17(20):3351–87.

[16] Wang S, Tasoglu S, Chen PZ, Chen M, Akbas R, Wach S, et al. Micro-a-fluidics ELISA for rapid CD4 cell count at the point-of-care. Sci Rep. 2014;4(1):1–9.

[17] Yetisen AK, Akram MS, Lowe CR. Paper-based microfluidic point-of-care diagnostic devices. Lab A Chip. 2013;13(12):2210–51.

[18] Pena-Pereira F, Lavilla I, Bendicho C. Paper-based analytical device for instrumental-free detection of thiocyanate in saliva as a biomarker of tobacco smoke exposure. Talanta. 2016;147:390–6.

[19] Songjaroen T, Dungchai W, Chailapakul O, Henry CS, Laiwattanapaisal W. Blood separation on microfluidic paper based analytical devices. Lab A Chip. 2012;12(18):3392–8.

[20] Cinti S, Fiore L, Massoud R, Cortese C, Moscone D, Palleschi G, et al. Low-cost and reagent-free paper-based device to detect chloride ions in serum and sweat. Talanta. 2018;179:186–92.

[21] Nogueira SA, Lemes AD, Chagas AC, Vieira ML, Talhavini M, Morais PA, et al. Redox titration on foldable paper-based analytical devices for the visual determination of alcohol content in whiskey samples. Talanta. 2019;194:363–9.

[22] Li Q, Xu Y, Qi J, Zheng X, Liu S, Lin D, et al. A self-powered rotating paper-based analytical device for sensing of thrombin. Sens Actuators B: Chem. 2022;351:130917.

[23] Suvanasuthi R, Chimnaronk S, Promptmas C. 3D printed hydrophobic barriers in a paper-based biosensor for point-of-care detection of dengue virus serotypes. Talanta. 2022;237:122962.

[24] Zheng J, Zhu M, Kong J, Li Z, Jiang J, Xi Y, et al. Microfluidic paper-based analytical device by using Pt nanoparticles as highly active peroxidase mimic for simultaneous detection of glucose and uric acid with use of a smartphone. Talanta. 2022;237:122954.

[25] Zhu Y, Tong X, Wei Q, Cai G, Cao Y, Tong C, et al. 3D origami paper-based ratiometric fluorescent microfluidic device for visual point-of-care detection of alkaline phosphatase and butyrylcholinesterase. Biosens Bioelectron. 2022;196:113691.

[26] Chen KH, Liu CC, Lu SY, Chen SJ, Sheu F, Fu LM. Rapid microfluidic analysis detection system for sodium dehydroacetate in foods. Chem Eng J. 2022;427:131530.

[27] Liu C, Gomez FA, Miao Y, Cui P, Lee W. A colorimetric assay system for dopamine using microfluidic paper-based analytical devices. Talanta. 2019;194:171–6.

[28] Nery EW, Kubota LT. Evaluation of enzyme immobilization methods for paper-based devices—a glucose oxidase study. J Pharm Biomed Anal. 2016;117:551–9.

[29] Xia Y, Whitesides GM. Soft lithography. Annu Rev Mater Sci. 1998;28(1):153–84.

[30] Lipomi D, Martinez R, Cademartiri L, Whitesides G. 7.11: soft lithographic approaches to nanofabrication. Polym Sci Compr Ref. 2012;10:211–31.

[31] Xiang N, Yi H, Chen K, Wang S, Ni Z. Investigation of the maskless lithography technique for the rapid and cost-effective prototyping of microfluidic devices in laboratories. J Micromech Microeng. 2013;23(2):025016.

[32] Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE. Soft lithography in biology and biochemistry. Annu Rev Biomed Eng. 2001;3(1):335–73.

[33] Curto VF, Lopez-Ruiz N, Capitan-Vallvey LF, Palma AJ, Benito-Lopez F, Diamond D. Fast prototyping of paper-based microfluidic devices by contact stamping using indelible ink. RSC Adv. 2013;3(41):18811–6.

[34] de Tarso Garcia P, Cardoso TMG, Garcia CD, Carrilho E, Coltro WKT. A handheld stamping process to fabricate microfluidic paper-based analytical devices with chemically modified surface for clinical assays. RSC Adv. 2014;4(71):37637–44.

[35] Chiang C-K, Kurniawan A, Kao C-Y, Wang M-J. Single step and mask-free 3D wax printing of microfluidic paper-based analytical devices for glucose and nitrite assays. Talanta. 2019;194:837–45.

[36] Ghosh R, Gopalakrishnan S, Savitha R, Renganathan T, Pushpavanam S. Fabrication of laser printed microfluidic paper-based analytical devices (LP-µPADs) for point-of-care applications. Sci Rep. 2019;9(1):1–11.

[37] Fawcett J, Scott J. A rapid and precise method for the determination of urea. J Clin Pathol. 1960;13(2):156–9.

[38] Werner W, Rey H-G, Wielinger H. Über die Eigenschaften eines neuen Chromogens für die Blutzuckerbestimmung nach der GOD/POD-Methode. Fresenius' Z Analytische Chem. 1970;252(2):224–8.

[39] Hawk PB. Practical physiological chemistry. Philadelphia: P. Blakiston's Son & Company; 1916.

[40] Ainla A, Mousavi MPS, Tsaloglou MN, Redston J, Bell JG, Fernández-Abedul MT, et al. Open-Source Potentiostat for Wireless Electrochemical Detection with Smartphones. Anal Chem. 2018;90(10):6240–6.

[41] Dungchai W, Chailapakul O, Henry CS. Electrochemical detection for paper-based microfluidics. Anal Chem. 2009;81(14):5821–6.

[42] Primpray V, Chailapakul O, Tokeshi M, Rojanarata T, Laiwattanapaisal W. A paper-based analytical device coupled with electrochemical detection for the determination of dexamethasone and prednisolone in adulterated traditional medicines. Anal Chim Acta. 2019;1078:16–23.

[43] Costa-Rama E, Nouws HPA, Delerue-Matos C, Blanco-López MC, Fernández-Abedul MT. Preconcentration and sensitive determination of the anti-inflammatory drug diclofenac on a paper-based electroanalytical platform. Anal Chim Acta. 2019;1074:89–97.

[44] Yang Y, Noviana E, Nguyen MP, Geiss BJ, Dandy DS, Henry CS. Paper-based microfluidic devices: emerging themes and applications. Anal Chem. 2017;89(1):71–91.

[45] Nantaphol S, Kava AA, Channon RB, Kondo T, Siangproh W, Chailapakul O, et al. Janus electrochemistry: Simultaneous electrochemical detection at multiple working conditions in a paper-based analytical device. Anal Chim Acta. 2019;1056:88–95.

[46] Petroni JM, Lucca BG, da Silva Júnior LC, Barbosa Alves DC, Souza Ferreira V. Paper-based electrochemical devices coupled to external graphene-Cu nanoparticles modified solid electrode through meniscus configuration and their use in biological analysis. Electroanalysis. 2017;29(11):2628–37.

[47] Narang J, Malhotra N, Singhal C, Mathur A, Chakraborty D, Anil A, et al. Point of care with micro fluidic paper based device integrated with nano zeolite–graphene oxide nanoflakes for electrochemical sensing of ketamine. Biosens Bioelectron. 2017;88:249–57.

[48] Fu LM, Wang YN. Detection methods and applications of microfluidic paper-based analytical devices. TrAC – Trends Anal Chem. 2018;107:196–211.

[49] Roda A, Guardigli M. Analytical chemiluminescence and bioluminescence: Latest achievements and new horizons. Anal Bioanal Chem. 2012;402(1):69–76.

[50] Singh AT, Lantigua D, Meka A, Taing S, Pandher M, Camci-Unal G. Paper-based sensors: emerging themes and applications. Sens (Switz). 2018;18(9):2838.

[51] Mirasoli M, Guardigli M, Michelini E, Roda A. Recent advancements in chemical luminescence-based lab-on-chip and microfluidic platforms for bioanalysis. J Pharm Biomed Anal. 2014;87:36–52.

[52] Akyazi T, Basabe-Desmonts L, Benito-Lopez F. Review on microfluidic paper-based analytical devices towards commercialisation. Anal Chim Acta. 2018;1001:1–17.

[53] Thouand Gérald RM. Gérald RM, editors. Bioluminescence: fundamentals and applications in biotechnology – volume 2 thouand. Berlin Heidelberg: Springer; 2014.

[54] Talalak K, Noiphung J, Songjaroen T, Chailapakul O, Laiwattanapaisal W. A facile low-cost enzymatic paper-based assay for the determination of urine creatinine. Talanta. 2015;144:915–21.

[55] Yu L, Li CM, Liu Y, Gao J, Wang W, Gan Y. Flow-through functionalized PDMS microfluidic channels with dextran derivative for ELISAs. Lab A Chip. 2009;9(9):1243–7.

[56] Ornatska M, Sharpe E, Andreescu D, Andreescu S. Paper bioassay based on ceria nanoparticles as colorimetric probes. Anal Chem. 2011;83(11):4273–80.

[57] Liu M-M, Lian X, Liu H, Guo Z-Z, Huang H-H, Lei Y, et al. A colorimetric assay for sensitive detection of hydrogen peroxide and glucose in microfluidic paper-based analytical devices integrated with starch-iodide-gelatin system. Talanta. 2019;200:511–7.

[58] Kudo H, Maejima K, Hiruta Y, Citterio D. Microfluidic paper-based analytical devices for colorimetric detection of lactoferrin. Slas Technology: Translating Life Sci Innov. 2020;25(1):47–57.

[59] Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265–9.

[60] Wu JT, Leung K, Leung GM. Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study. Lancet. 2020;395(10225):689–97.
[61] Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. Clin Infect Dis. 2020;71(16):2027–34.
[62] Kasetsirikul S, Umer M, Soda N, Sreejith KR, Shiddiky MJ, Nguyen N-T. Detection of the SARS-CoV-2 humanized antibody with paper-based ELISA. Analyst. 2020;145(23):7680–6.
[63] Lin Y-H, Chen Y-J, Lai C-S, Chen Y-T, Chen C-L, Yu J-S, et al. A negative-pressure-driven microfluidic chip for the rapid detection of a bladder cancer biomarker in urine using bead-based enzyme-linked immunosorbent assay. Biomicrofluidics. 2013;7(2):024103.
[64] Kelley ZD, Rogers DT, Littleton JM, Lynn BC. Microfluidic capillary zone electrophoresis mass spectrometry analysis of alkaloids in Lobelia cardinalis transgenic and mutant plant cell cultures. Electrophoresis. 2019;40(22):2921–8.
[65] Hentschel AE, Nieuwenhuijzen JA, Bosschieter J, van Splunter AP, Lissenberg-Witte BI, van der Voorn JP, et al. Comparative analysis of urine fractions for optimal bladder cancer detection using DNA methylation markers. Cancers. 2020;12(4):859.
[66] Hanke M, Hoefig K, Merz H, Feller AC, Kausch I, Jocham D, et al., editors. A robust methodology to study urine microRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer. Urologic oncology: seminars and original investigations. Netherlands: Elsevier; 2010.
[67] Feil G, Zumbrägel A, Päulgen-Nelde HJ, Hennenlotter J, Maurer S, Krause S, et al. Accuracy of the ImmunoCyt assay in the diagnosis of transitional cell carcinoma of the urinary bladder. Anticancer Res. 2003;23(2A):963–7.
[68] Jiang Q, Han T, Ren H, Aziz AUR, Li N, Zhang H, et al. Bladder cancer hunting: a microfluidic paper-based analytical device. Electrophoresis. 2020;41(16–17):1509–16.
[69] Nouanthavong S, Nacapricha D, Henry CS, Sameenoi Y. Pesticide analysis using nanoceria-coated paper-based devices as a detection platform. Analyst. 2016;141(5):1837–46.
[70] Kim HJ, Kim Y, Park SJ, Kwon C, Noh H. Development of colorimetric paper sensor for pesticide detection using competitive-inhibiting reaction. BioChip J. 2018;12(4):326–31.
[71] Badawy ME, El-Aswad AF. Bioactive paper sensor based on the acetylcholinesterase for the rapid detection of organophosphate and carbamate pesticides. Int J Anal Chem. 2014;2014:536823.
[72] Thongkam T, Hemavibool K. An environmentally friendly microfluidic paper-based analytical device for simultaneous colorimetric detection of nitrite and nitrate in food products. Microchem J. 2020;159:105412.
[73] Crawford L. The impact of residues on animal food products and human health. Rev Sci Et Tech (Int Office Epizootics). 1985;4(4):669–723.
[74] Dayan A. Allergy to antimicrobial residues in food: assessment of the risk to man. Veterinary Microbiol. 1993;35(3–4):213–26.
[75] Beyene T, Tesega B. Rational veterinary drug use: Its significance in public health. J Veterinary Med Anim Health. 2014;6(12):302–8.
[76] Trofimchuk E, Nilghaz A, Sun S, Lu X. Determination of norfloxacin residues in foods by exploiting the coffee-ring effect and paper-based microfluidics device coupling with smartphone-based detection. J Food Sci. 2020;85(3):736–43.
[77] Amine A, Arduini F, Moscone D, Palleschi G. Recent advances in biosensors based on enzyme inhibition. Biosens Bioelectron. 2016;76:180–94.
[78] Jin L, Hao Z, Zheng Q, Chen H, Zhu L, Wang C, et al. A facile microfluidic paper-based analytical device for acetylcholinesterase inhibition assay utilizing organic solvent extraction in rapid detection of pesticide residues in food. Anal Chim Acta. 2020;1100:215–24.
[79] Arduini F, Ricci F, Bourais I, Amine A, Moscone D, Palleschi G. Extraction and detection of pesticides by cholinesterase inhibition in a two-phase system: a strategy to avoid heavy metal interference. Anal Lett. 2005;38(11):1703–19.
[80] Montesinos T, Pérez-Munguia S, Valdez F, Marty J-L. Disposable cholinesterase biosensor for the detection of pesticides in water-miscible organic solvents. Anal Chim Acta. 2001;431(2):231–7.
[81] Górska-Warsewicz H, Rejman K, Laskowski W, Czeczotko M. Milk and dairy products and their nutritional contribution to the average polish diet. Nutrients. 2019;11(8):1771.
[82] Oliver SP, Jayarao BM, Almeida RA. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodbourne Pathog Dis. 2005;2(2):115–29.
[83] Asif M, Awan FR, Khan QM, Ngamsom B, Pamme N. Paper-based analytical devices for colorimetric detection of S. aureus and E. coli and their antibiotic resistant strains in milk. Analyst. 2020;145(22):7320–9.
[84] Jayawardane BM, McKelvie ID, Kolev SD. A paper-based device for measurement of reactive phosphate in water. Talanta. 2012;100:454–60.
[85] Ortiz-Gomez I, Ortega-Muñoz M, Salinas-Castillo A, Álvarez-Bermejo JA, Ariza-Avidad M, de Orbe-Payá I, et al. Tetrazine-based chemistry for nitrite determination in a paper microfluidic device. Talanta. 2016;160:721–8.
[86] Da Silva VAOP, De Freitas RC, De Oliveira PR, Moreira RC, Marcolino-Júnior LH, Bergamini MF, et al. Microfluidic paper-based device integrated with smartphone for point-of-use colorimetric monitoring of water quality index. Measurement. 2020;164:108085.
[87] Waghwani B, Balpande S, Kalambe J. Development of microfluidic paper based analytical device for detection of phosphate in water. 2019;8:592–5.
[88] Lin D, Li B, Qi J, Ji X, Yang S, Wang W, et al. Low cost fabrication of microfluidic paper-based analytical devices with water-based polyurethane acrylate and their application for bacterial detection. Sens Actuators B: Chem. 2020;303:127213.
[89] Organization WH. Nitrate and nitrite in drinking-water: background document for development of WHO guidelines for drinking-water quality. Geneva: World Health Organization; 2003.

[90] Charbaji A, Heidari-Bafroui H, Anagnostopoulos C, Faghri M. A new paper-based microfluidic device for improved detection of nitrate in water. Sensors. 2021;21(1):102.

[91] Silva CG, Pereira MF, Órfão JJ, Faria JL, Soares OS. Catalytic and photocatalytic nitrate reduction over Pd-Cu loaded over hybrid materials of multi-walled carbon nanotubes and TiO2. Front Chem. 2018;6:632.

[92] Yan X, Li H, Su X. Review of optical sensors for pesticides. TrAC Trends Anal Chem. 2018;103:1–20.

[93] Taiwo AM. A review of environmental and health effects of organochlorine pesticide residues in Africa. Chemosphere. 2019;220:1126–40.

[94] Fernández-Ramos MD, Ogunneye A, Babarinde N, Erenas M, Capitán-Vallvey LF. Bioactive microfluidic paper device for pesticide determination in waters. Talanta. 2020;218:121108.

[95] Lee, Cg, Fletcher, TD, Sun. G. Nitrogen removal in constructed wetland systems. Eng Life Sci. 2009;9(1):11–22.

[96] Nxumalo NL, Madikizela LM, Kruger HG, Onwubu SC, Mdluli PS. Development of a paper-based microfluidic device for the quantification of ammonia in industrial wastewater. Water SA. 2020;46(3):506–13.

[97] Diamanti-Kandarakis E, Bourguignon J-P, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev. 2009;30(4):293–342.

[98] Jemmeli D, Marcoccio E, Moscone D, Dridi C, Arduini F. Highly sensitive paper-based electrochemical sensor for reagent free detection of bisphenol A. Talanta. 2020;216:120924.

[99] Michałowicz J. Bisphenol A–sources, toxicity and biotransformation. Environ Toxicol Pharmacol. 2014;37(2):738–58.

[100] Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). Reprod Toxicol. 2007;24(2):139–77.

[101] Gebauer P, Boček P. Recent progress in capillary isotachophoresis. Electrophoresis. 2002;23(22–23):3858–64.

[102] Kondratova VN, Serd'uk OI, Shelepov VP, Lichtenstein AV. Concentration and isolation of DNA from biological fluids by agarose gel isotachophoresis. Biotechniques. 2005;39(5):695–9.

[103] Kondratova VN, Botezatu IV, Shelepov VP, Lichtenstein AV. Isotachophoresis of nucleic acids in agarose gel rods. Biochem. 2009;74(11):1285–8.

[104] Persat A, Marshall LA, Santiago JG. Purification of nucleic acids from whole blood using isotachophoresis. Anal Chem. 2009;81(22):9507–11.

[105] Bercovici M, Kaigala GV, MacH KE, Han CM, Liao JC, Santiago JG. Rapid detection of urinary tract infections using isotachophoresis and molecular beacons. Anal Chem. 2011;83(11):4110–7.

[106] Moghadam BY, Connelly KT, Posner JD. Isotachophoretic preconcenetration on paper-based microfluidic devices. Anal Chem. 2014;86(12):5829–37.

[107] Sullivan BP, Bender AT, Ngyuen DN, Zhang JY, Posner JD. Nucleic acid sample preparation from whole blood in a paper microfluidic device using isotachophoresis. J Chromatogr B Anal Technol Biomed Life Sci. 2021;1163:122494.

[108] Burgi DS, Chien RL. Optimization in sample stacking for high-performance capillary electrophoresis. Anal Chem. 1991;63(18):2042–7.

[109] Palmer J, Munro NJ, Landers JP. A universal concept for stacking neutral analytes in micellar capillary electrophoresis. Anal Chem. 1999;71(9):1679–87.

[110] Ouyang L, Liu Q, Liang H. Combining field-amplified sample stacking with moving reaction boundary electrophoresis on a paper chip for the preconcentration and separation of metal ions. J Sep Sci. 2017;40(3):789–97.

[111] Kim SJ, Song YA, Han J. Nanofluidic concentration devices for biomolecules utilizing ion concentration polarization: theory, fabrication, and applications. Chem Soc Rev. 2010;39(3):912–22.

[112] Gong MM, Zhang P, MacDonald BD, Sinton D. Nanoporous membranes enable concentration and transport in fully wet paper-based assays. 18th International Conference on Miniaturized Systems for Chemistry and Life Sciences MicroTAS2014; 2014. p. 1595–7.

[113] Yang RJ, Pu HH, Wang HL. Ion concentration polarization on paper-based microfluidic devices and its application to preconcentrate dilute sample solutions. Biomicrofluidics. 2015;9(1):014122.

[114] Han Il S, Hwang KS, Kwak R, Lee JH. Microfluidic paper-based biomolecule preconcentrator based on ion concentration polarization. Lab Chip. 2016;16(12):2219–27.

[115] Keçili R, Büyüktiryaki S, Dolak I, Hussain CM. The use of magnetic nanoparticles in sample preparation devices and tools. Handb Nanomater Anal Chem Mod Trends Anal. Netherlands: Elsevier; 2019. p. 75–95.

[116] Wu Q, He J, Meng H, Wang Y, Zhang Y, Li H, et al. A paper-based microfluidic analytical device combined with home-made SPE column for the colorimetric determination of copper(II) ion. Talanta. 2019;204:518–24.

[117] Munaz A, Shiddiky MJ, Nguyen N-T. Recent advances and current challenges in magnetophoresis based micro magnetofluidics. Biomicrofluidics. 2018;12(3):031501.

[118] Call ZD, Carrell CS, Jang I, Geiss BJ, Dandy DS, Henry CS. Paper-based pump-free magnetophoresis. Anal Methods. 2020;12(43):5177–85.

[119] Sousa LR, Duarte LC, Coltro WK. Instrument-free fabrication of microfluidic paper-based analytical devices through 3D pen drawing. Sens Actuators B: Chem. 2020;312:128018.

[120] Martinez AW, Phillips ST, Whitesides GM. Three-dimensional microfluidic devices fabricated in layered paper and tape. Proc Natl Acad Sci. 2008;105(50):19606–11.

[121] Liu H, Crooks RM. Three-dimensional paper microfluidic devices assembled using the principles of origami. J Am Chem Soc. 2011;133(44):17564–6.

[122] Guan Y, Sun B. Detection and extraction of heavy metal ions using paper-based analytical devices fabricated via atom stamp printing. Microsyst Nanoengineering. 2020;6(1):1–12.

[123] Hamedpour V, Oliveri P, Malegori C, Minami T. Development of a morphological color image processing algorithm for paper-based analytical devices. Sens Actuators B: Chem. 2020;322:128571.

[124] Noviana E, Carrão DB, Pratiwi R, Henry CS. Emerging applications of paper-based analytical devices for drug analysis: a review. Anal Chim Acta. 2020;1116:70–90.

[125] Nishat S, Jafry AT, Martinez AW, Awan FR. Paper-based microfluidics: simplified fabrication and assay methods. Sens Actuators B: Chem. 2021;336:129681.