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Review

# Paper-based microfluidic devices for food adulterants: Cost-effective technological monitoring systems

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ABSTRACT

Analytical sciences have witnessed emergent techniques for efficient clinical and industrial food adulterants detection. In this review, the contributions made by the paper-based devices are highlighted for efficient and rapid detection of food adulterants and additives, which is the need of the hour and how different categories of techniques have been developed in the past decade for upgrading the performance for point-of-care testing. A simple strategy with an arrangement for detecting specific adulterants followed by the addition of samples to obtain well-defined qualitative or quantitative signals for confirming the presence of target species. The paper-based microfluidics-based technology advances and prospects for food adulterant detection are discussed given the high-demand from the food sectors and serve as a valued technology for food researchers working in interdisciplinary technological frontiers.

## 1. Introduction

Food is an absolute necessity to support life on earth. The highest quality of food must be reaching the needs of the society to express its nutritional effects, which otherwise may cause deleterious effects, including diseases and toxicity. One such practice that has raised concerns within and beyond the food industry is that of food adulteration, also called food fraud. A food adulterant is defined as any substance, edible or inedible, intentionally added to food products to provide them more bulk or weight or look more attractive. The addition of adulterants to food items is commonly associated with making the final product more harmful to health, cheaper and inferior. However, this often deprives the consumers of actual valuable nutrients, which have been removed to some extent to be replaced with adulterants. Overall, adulterants are extraneous matter that is not naturally present in the food items but is also added.

Food adulteration can be classified into several categories, such as metallic adulteration, which involves contaminating the food substances with metals or metallic compounds such as arsenic, cobalt, and cadmium in water, fruits, and juices or drinks. The most common method of food fraud is physical mixing, which involves the addition of substances such as sand, stones, and marble chips to food grains and exhausted tea leaves to tea. Simultaneously, biological adulteration is emerging as a grave issue, which includes contamination of pure forms of food products with foreign organic substances, such as rancid oils, low-quality species of wheat, milk, and meat, to name a few. The infusion of microbes facilitates another method of food contamination, such as *Bacillus cereus* in cereal products, *Clostridium perfringens* in improperly processed milk, etc., which can occur during almost any food processing stage during production, storage, processing or distribution. Some types of adulterants might enter the food products unintentionally. For example, pesticide residues have a risk of being present in all kinds of foods, while

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fluorides are usually present in drinking water (Bansal et al., 2017). Some of the other common examples include adding urea, starch, and extra quantities of water to milk, curd, butter, and ghee products, thereby having a negative effect on the nutritional value of the milk (Khan & Chittora, 2017).

Fats and oils have often been adulterated with foreign ingredients such as soyabean oil (Li et al., 2015), lard (Basri et al., 2017) and mixing of high-quality edible oils with the ones of lower price and quality such as olive oil and the adulterations are becoming more sophisticated these days (Przykaza et al., 2021). Foodgrains such as cereals suffer from adulteration by adding melamine to increase their apparent protein content (Ehling et al., 2007). Other commonly used food additives include chilli powder mixed with red brick powder, tea leaves mixed with used tea leaves, dried papaya seeds to pepper and the widespread emergence of artificial (synthetic sweeteners) in products such as honey, etc. (Choudhary et al., 2020). The above practices have led to a diverse range of impacts, both on the producers and the consumers.

From the producer's perspective, there can be a tremendous loss in sales of the adulterated products and other products of the company. Most importantly, customer confidence and the brand value are lost, which ultimately damages the business. Farmers, being the weakest link in the chain, have to suffer from the problems of rising cost and milk, cow shortage and most importantly, the consumers face numerous health risks and conditions such as diarrhoea, abdominal pain, nausea, vomiting, eye-sight problems, headache, cancer, anemia, insomnia, muscular paralysis and brain damage (Fig. 1 and Fig. 2). Gastrointestinal issues and liver and kidney infections have also become common (McGrath et al., 2021; Mohammadi & Jafari, 2020). More elaborately, physical and biological mentioned previously contribute to cancers, loss of essential vitamins, and damage to the digestive tract. Incidentally, present contaminants such as pesticides and polycyclic compounds are linked to skeletal, neurological, and gastrointestinal organ impairment. Other substances such as metals can lead to muscular convulsions, paralysis, cardiac disturbances, and other health issues. Biological contaminants such as bacteria and fungi lead to several health-related complications such as nausea, vomiting, diarrhoea, fever, chills, and food infection, to highlight a few (Bansal et al., 2017).

The aforementioned problems associated with food adulterants demand the urgent need for robust and rapid methods of their detection and separation (if needed). Conventionally methods such as high-performance liquid chromatography(HPLC), spectrofluorimetric, ultraviolet-visible spectrophotometry, enzyme-linked immunosorbent assays (Lin et al., 2015), Fourier-transform infrared spectroscopy and chemiluminescence have been used to quantify the food products (Gao et al., 2018). Though these methods have proven to be efficient and accurate in the past, they are often associated with the drawbacks of needing complex pre-treatment, a long preparation time, expensive equipment (Lin et al., 2015) and time-consuming separation procedures (Chen et al., 2014), thereby not being ultimately suitable for in-situ measurement and analysis.

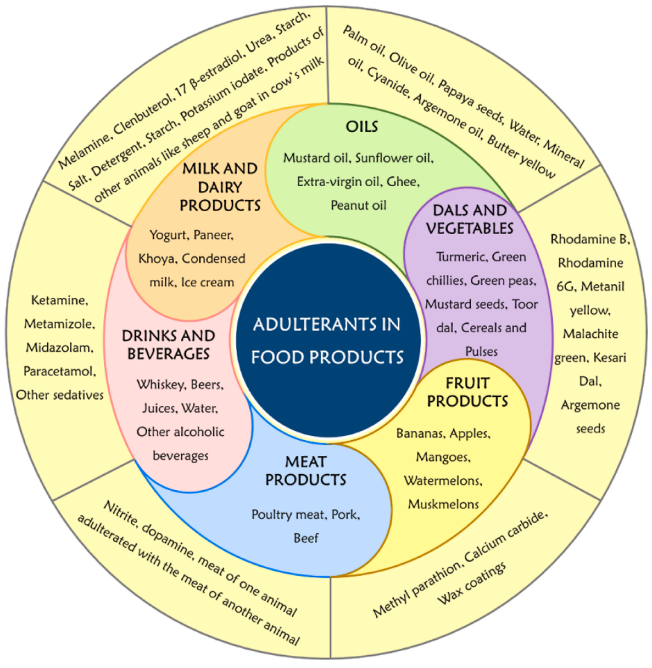


**Fig. 1.** Types of foods with their comprehensive list of adulterants.

To overcome these problems, newer and previously less explored techniques, such as microfluidics, have now gained much attention to extend their prominence in chemical and biological sciences, pharmaceuticals, clinical diagnostics and environmental sensing areas (Hasandka et al., 2022; Kelkar et al., 2022; Govindarajalu et al., 2019; Mani et al., 2013). These comprise a new type of sensors or detection approaches, which are being used lately for a variety of analytical studies, one of them being food safety testing such as the detection of pesticides (Xu et al., 2020), adulterants (Weng & Neethirajan, 2017) and heavy metals (Zhang et al., 2015). Their usage is supported by the fact that they are small, portable, cost-effective and need fewer sample amounts and relatively simple instruments for the analysis but still offer the same level of results (Deng et al., 2018). Realizing their frugal nature, researchers have been focusing specifically on paper-based microfluidic devices to detect adulterants to apply these techniques for point-of-care testing (POCT).

Paper' can be applied to materialize many detection approaches such as chemical, optical and hybrid sensing systems (Yehia et al., 2020). Paper-based devices offer the advantages of being commonly available, compatible with many chemicals, being biodegradable and disposable and using only capillary action for the transport of solutions without using any external forces (Hasandka et al., 2021; Li et al., 2020; Mani et al., 2019, 2020; Prabhu et al., 2020a; Singhal et al., 2021). One of the first essential aspects of performing paper-based experiments is the choice of paper to be used. Filter paper is one of the more commonly used materials due to its wicking ability. Particularly, Whatman® cellulose paper is preferred, and there are various categories of paper under this, differentiated by the essential parameters such as porosity, particle retention, and flow rate. For attaining the medium particle retention and flow rate, Whatman® filter paper #1 is commonly utilized, whereas #4 type of material is employed when higher liquid penetration is required. Apart from the widely used filter paper, membranes made up of nitrocellulose provide desirable non-specific interactions with biomolecules such as deoxyribonucleic acids (DNA), proteins, and enzymes due to their hydrophobic properties. It is, thus, essential to design the paper with some modifications on its surface for performing studies with molecules of interest.

The paper surface modification is usually achieved by fabricating the paper-based devices by either some chemical modification or physical deposition or by physical blocking of its pores (Dou et al., 2015) to tune the paper material to have the desired properties to make it suitable for direct usage in critical experiments/applications. Some commonly used methods to perform modifications are photolithography, wax printing, etching, plasma treatment, paper cutting, screen printing, and laser treatment. This fabrication results in a hydrophobic barrier on the paper's surface, which will confine the samples/reagents/fluids in a hydrophilic area and materialize the experiments such as sample preparation, purification, and multiple bioreactions at the micro-level (Dou et al., 2015). One example of a paper microfluidic device in action was developed by Martinez et al. (2007). A hydrophobic SU-8 polymer had been lithographically deposited on paper in the shape of a three-branch tree pattern. A liquid sample was introduced into the hydrophilic area confined by the barrier, which then travelled through the matrix of the paper due to capillary action and then split into three different directions at the branching point to reach three different types

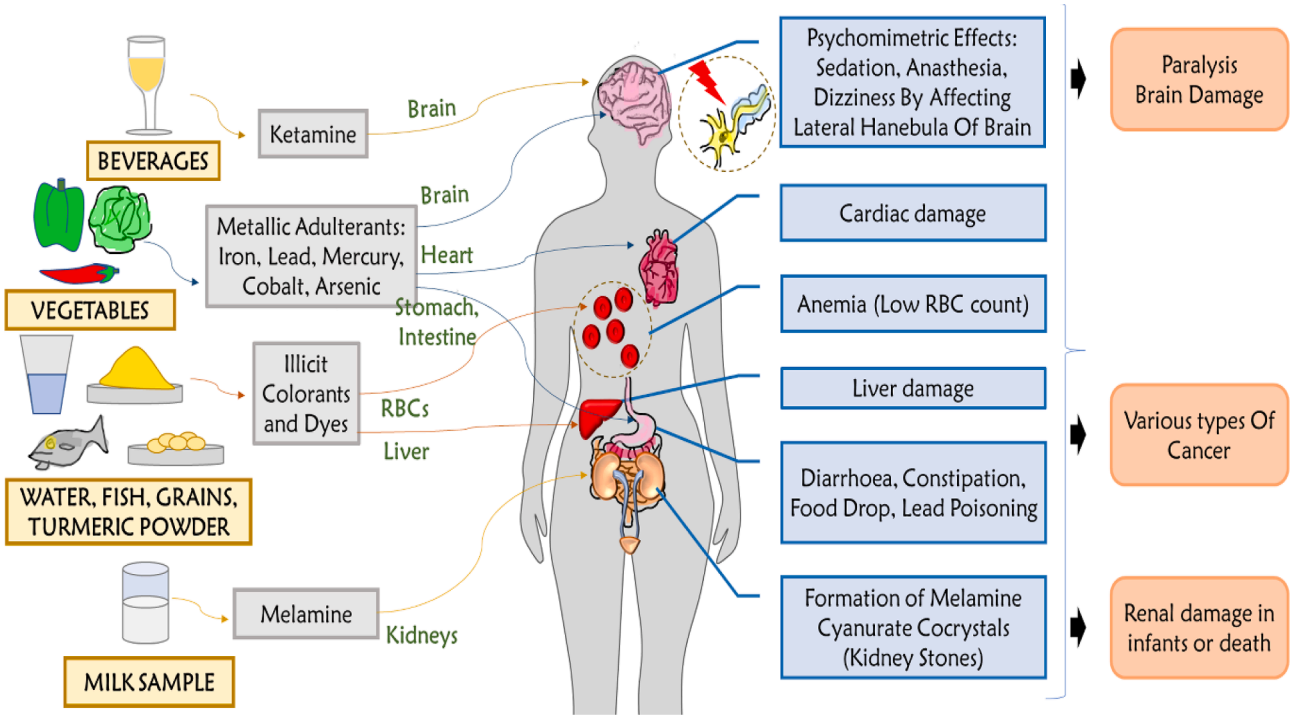


**Fig. 2.** Adulterants and their effect on human health.

reagents for executing chemical reactions.

One of the crucial parameters contributing to the success of paper microfluidic devices is the accurate manipulation/control of fluid flow. The flow rate in these channels depends on the fluid's viscosity, the radius of the capillary channel, fluid surface tension, etc. Many tools have been developed recently to adjust the fluid flow by (i) changing the fluid velocity within a channel relative to the conventional porous channel, as seen in operations of the open channels in omniphobic (a surface that repels virtually any liquid) paper and (ii) functioning as valves to start or stop the flow within a porous channel as seen in the use of temperature to control the solubility of surfactants in the paper (Fu & Downs, 2017). Additionally, measuring the flow rate within such channels is also a significant aspect of the operation of paper-based devices. One such a study was conducted wherein microgram quantities of paraffin wax were deposited on the defined regions in hydrophilic surfaces of paper, which served as a convenient method to meter fluid flow in three-dimensional (3D) micro-pads (μPADs) where the deposited wax delayed the flow of fluid and aided in its measurement (Noh & Phillips, 2010).

Apart from food adulterants, there have been applications in immunoassay tests, chromatographic separations, gas analysis and other vital detections (Dossi et al., 2013). Considering the existing costlier, non-compatible food detection technologies coupled with cumbersome pretreatment procedures, the present review emphasizes the great accolade of industrial-verse, less-costlier paper-based microfluidics detection approaches for the frugal detection of food adulterants in a cradle to the grave manner by putting forth different strategic approaches towards sustainable, cost-effective future adulterants detection platform for the food industry.

## 2. Detection of food adulterants using paper-based microfluidic devices

Considering the negative implications and importance of frugal and timely detection of adulterants (Fig. 2), the specific use of paper-based microfluidic devices has garnered attention, supported by the simple and convenient features of the material itself. The paper's biodegradable and biocompatible nature makes it easier to operate in a laboratory environment with the least amount of damage to the ecosystem. The most valuable property of the paper utilized in these studies is the fact that it possesses an intrinsic wicking system, which allows fluids understudy to flow through its hydrophilic cellulosic channels solely under the influence of capillary action, thereby eliminating the use of any complex instruments for controlling the flow of materials being loaded (Xie et al., 2019).

The application of paper substrates in analytical and diagnostic experiments is facilitated by fabricating the platform with a suitable hydrophobic layer to act as a barrier for confining the samples under study. The choice and robustness of each fabrication approach directly contribute to the overall efficacy of the device and hence, is a factor to consider. We have highlighted several fabrication methods, some of which include molecular imprinting of polymers onto the paper surface (Xiao et al., 2017), coating a layer of metal nanoparticles (Gao et al., 2018), the printing of wax layers (Chen et al., 2019), pressure-based ball pen writing (Li et al., 2015) and other approaches that have been successfully applied in studies conducted in recent past; these are preferred due to the simple strategy involved coupling with the design of a susceptible device (Fig. 3). Various methods are summarized in their critical applications, advantages, limitations, and future improvements in Table S1.

### 2.1. SERS-based detection

The set of studies involved using surface-enhanced Raman spectroscopy (SERS). Despite offering numerous advantages such as high sensitivity, portability and rapid detection up to a single molecular level, they have not been used as much for some critical studies (Table 1). The method involves using a Raman spectrometer from which laser beams of a specific wavelength are incident on the SERS substrates coated with the sample under study. The resultant vibration in the sample molecules gets translated into signals and is displayed as Raman peaks in a graph, uniquely corresponding to the molecular structure under consideration. The substrate used in these studies is a nanostructured plasmonic material that aids in enhancing the SERS signals by exclusively emitting the

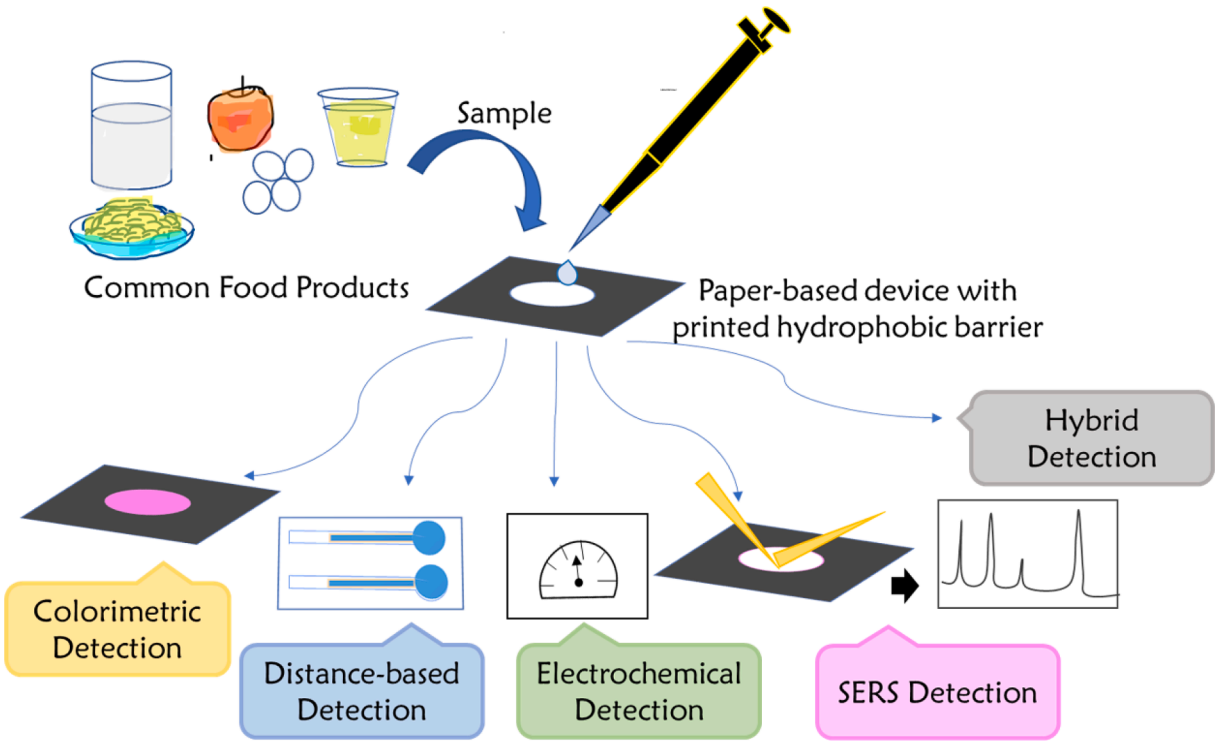


**Fig. 3.** Paper-based devices for detecting adulterants using various detection mechanisms.

same from the adsorbed molecular layer closest to the surface. Mainly, paper-based SERS studies involve nonmaterial such as silver and are designed to be low-cost- point-of-care in nature (Kumar & Santhanam, 2019). SERS-based techniques have garnered significant attention due to their flexible integration with paper-based devices and portability. They facilitate single-molecule detection of the analyte of interest and are compatible with a range of substrates (Kumar & Santhanam, 2019). It also involves fewer pre-treatment steps and less result generation time while reducing the interference in signals due to fluorescence compared to the traditional Raman methods. Its stability and reproducibility have also been documented where low RSD (relative standard deviation) has been noted after multiple usages. Moreover, its integration with NPs has increased its stability, optical responses, and biocompatibility (Sha et al., 2020). One of the ground-breaking developments in SERS techniques has been its fruitful integration with filter paper to design paper-based SERS substrates reducing the cost and making them suitable for POC (point-of-care) applications (Xie et al., 2020). Utilizing the capillary action of the paper has facilitated the transfer and concentration of samples in desired areas of the substrate (Zhu et al., 2015). This method has also been praised for its high recovery rates, narrow spectral bandwidth, and ability to overcome water interference (Lin et al., 2015).

An early study used this approach to fabricate a triangular filter paper-based device with silver nanoparticles (AgNP) and functionalized with negatively charged poly (sodium 4-styrene sulfonate) or PSS for the detection of common food dyes such as sunset yellow and lemon yellow in a way to form the charge gradient to have the most nominal charge at the tip and hence, migrate the negatively charged food dyes to the tip by repulsion, thereby detecting them using the laser. The sensor showed LOD values as low as $10^{-5}$ M in real samples such as drinks (Zhu et al., 2015). A similar approach was also used for the detection of Rhodamine B (RB), another common food dye, where AgNPs were coated onto a filter paper and on applying the prepared RB solutions at various concentrations, characteristic SERS peaks were observed at 1355 $cm^{-1}$, 1504 $cm^{-1}$ and 1644 $cm^{-1}$ by the laser; using this to real samples, peaks at the same wavenumbers were observed, supporting the practical applicability of the sensor with a LOD of $10^{-6}$ g/g (Lin et al., 2015).

Another research group reported the development of an AgNP coated paper swab-based sensor (Fig. 4**A**) with a portable spectrometer for the detection of food dyes like metanil yellow (MY) and malachite green (MG). The device was coupled with an orbital raster scanning (ORS) technology to reduce measurement's relative standard deviation (RSD) values. MY was efficiently detected in "spiked" dal samples with characteristics Raman peaks at 1148 $cm^{-1}$ and 1404 $cm^{-1}$ with a detection limit of 1 μM. Green peas and green chillies were also used to detect MG that showed a prominent peak at 1370 $cm^{-1}$. Negligible responses were observed at concentrations below 10 μM, confirming its LOD (Kumar & Santhanam, 2019). A more recent study was used for the detection of Rhodamine B and Rhodamine 6G (R6G) dyes in food samples, where an AgNP-coated cellulose paper-based device was designed, which efficiently detected the RB in nanomolar concentrations, where the defined peaks were observed at 1194.5 $cm^{-1}$, 1287.2 $cm^{-1}$ 1357.3 $cm^{-1}$, 1512.8 $cm^{-1}$, 1560.1 $cm^{-1}$ and 1650.9 $cm^{-1}$ due to interactions of the laser with bonds undergoing stretching and bending. R6G was detected even more sensitively up to picomolar concentrations, giving unique peaks at 613.1 $cm^{-1}$, 771.4 $cm^{-1}$, 1188.1 $cm^{-1}$, 1314.1 $cm^{-1}$. The sensor was also successfully analyzed for its reproducibility and stability and its catalytic activity using the reduction of 4-nitrophenol as a model (Das et al., 2019).

Apart from food dyes, the SERS strategy was also used to detect the drugs in beverages such as estazolam (EST). The combined merits of both Ag and Au (gold) nanoparticles created the paper-based sensor. EST in prepared aqueous solutions showed distinct peaks at 687 $cm^{-1}$ and 1000 $cm^{-1}$. The results were reproduced with less prominence in real "spiked" juice samples, probably due to the background interference from the changing environment. The study reported a LOD of 10 mg/l (Sha et al., 2020). SERS technology was also extended to the detection of pesticides in food samples (Fig. 4**B**), such as organophosphorus pesticide methyl parathion in fruits, where an AuNP-fabricated paper-based device detected the target analyte at three prominent SERS peaks of 848 $cm^{-1}$, 1104.1 $cm^{-1}$ and 1336.7 $cm^{-1}$ in fruit samples with a low LOD of 0.011 μg/$cm^3$. The sensor was also checked successfully for its robustness against the laser damage (Xie et al., 2020).

**Table 1**
List of adulterants and their source, detection modes and detection limits.

| Adulterant | Source of Analysis | Detection Method | Limit of Detection | Ref. |
|---|---|---|---|---|
| Surface Enhanced Raman Spectroscopy (SERS) | | | | |
| Sunset yellow and lemon yellow (synthetic dyes) | Drinks | AgNP-based paper-based substrate functionalized with polyelectrolytes poly(allylamine hydrochloride) (PAH) and poly(sodium 4-styrenesulfonate) (PSS) facilitating the separation, preconcentration and detection of colorants through a surface gradient and an electrostatic interaction | 10^-5 M | (Zhu et al., 2015) |
| Rhodamine B | Chilli powder | Paper-based substrate fabricated using liquid–liquid interface mediated self-assembly technique for obtaining the characteristic SERS peaks of target analyte using based on density functional theory (DFT) | 10^-6 g/g | (Lin et al., 2015) |
| Metanil yellow and Malachite green | Toor dal and green chillies/peas respectively | Paper-based SERS substrates designed by inkjet-printed thin films of silver nanoparticles coupled with a portable Raman spectrometer utilizing orbital raster scanning(ORS$^{TM}$) to obtain detection signals of analytes | 1uM and 10uM, respectively | (Kumar and Santhanam, 2019) |
| Rhodamine B and Rhodamine 6G | Vegetables | Paper-SERS substrate designed using 'silver mirror' reaction for loading of AgNPs for the production of characteristic Raman peaks for target species | in nanomoles and picomoles, respectively | (Das et al., 2019) |
| Estazolam | Water and beverages | Fast and simple acting filter paper-based substrate coated with Au-Ag core–shell nanoparticles integrated with density functional theory (DFT) to obtain characteristic vibrational modes of target adulterants | 10 mg/l | (Sha et al., 2020) |
| Methyl parathion | Fruit | AuNP-fabricated paper-devices for detecting contaminants from surface of fruits by 'press'peel-off' approach, laser application to obtain unique SERS peaks from used substrate | 0.011 μg/cm$^2$ | (Xie et al., 2020) |
| Colorimetric Techniques | | | | |
| 17 beta-estradiol | Milk samples | Molecular imprinted polymer(MIP)-coated paper analytical device for detection of 17 beta-estradiol by colorimetric signal production through competition between the former and 17 beta-estradiol-horse radish peroxidase(HRP) for binding to the MIP | 0.25ug/l | (Xiao et al., 2017) |
| Ketamine (Rape drug) | Alcoholic beverages | Paper-based microfluidic device(uPAD) coated for the detection of ketamine through a coloured complex formation with indicator bromocresol green(BCG) on the substrate | 0.001 M | (Narang et al., 2018) |
| Clenbuterol | Milk samples | uPAD fabricated with antibodies specific to clenbuterol for aa competitive ELISA between the former and HRP-tagged clenbuterol(indicator) reaction and subsequent colour reduction on entrapment of target analyte by the coated antibodies | 0.2 ppb | (Ma et al., 2018) |
| Melamine | Milk samples | Triton-X-100-citrate-trapped AuNPs coated paper-based substrate, wine red-to-blue colour change due to interaction between melamine and the AuNPs and the subsequent destabilization and aggregation of the latter | 5.1 nM | (Gao et al., 2018) |
| Urea, starch, salt, detergent | Milk samples | uPAD fabricated with polydimethoxysilane as a hydrophobic barrier to confine reagents specific to the target analyte, addition of the target adulterant to the spot followed by a chemical reaction to produce a coloured compound to confirm the detection | 5 mg urea, 17 mg starch, 29 mg salt and 20 mg detergent in 10 ml of milk | (Salve et al., 2018) |
| Milk Adulterants | Milk samples | Milk carton integrated with uPAD having wax-printed hydrophobic barrier, simultaneous production of unique coloured complexes at different detection zones, each due to a different chromogenic reaction with a coated chemical specific to the adulterant in milk | NA | (Fan et al., 2018) |
| Benzoic acid (preservative) | Commercial food samples | Paper-based microfluidic analytical device for the colorimetric detection of benzoic acid by utilizing the Janovsky reaction on a portable detection zone of the substrate to produce coloured anionic complexes with NaOH(sodium hydroxide) | Multiple values for the various samples | (Liu et al., 2018) |
| Iron fortificant | Wheat flour, infant formula, nixtamalized corn flour | Chromogenic reaction between Ferrozine reagent and iron present in the samples(Ferrozine reaction) for the detection of iron fortificants by producing magenta coloured compound, followed by determination of analyte concentrations by image capturing and analysis by smartphone app | 3.691ug/ml | (Waller et al., 2019) |
| Starch | Milk samples | Detection of starch through reaction with iodine solution on the paper-based analytical device to produce bluish-black compound, colour intensity measured and correlated to analyte concentration by smartphone app algorithms | NA | (Govindarajalu et al., 2019) |
| Melamine | Milk samples | Folded paper-based device with sandwiched chromatographic paper, polydimethoxysilane and AuNPs for producing visible colour change on interaction with melamine in the samples and subsequent aggregation of the AuNPs | 0.1 ppm | (Xie et al., 2019) |
| Sulfite | Food samples | Paper-based thin film microextraction of sulfite for its reduction to $SO_2$by Fe(III), 1,10-phenanthroline indicator to produce red colour complex, images captured and quantitatively analysed by smartphone app | 0.04ug/l | (Shahvar et al., 2019) |
| Mercury(Metallic adulteration) | Fish(salmon) | AuNP-fabricated paper-based substrate functionalized with functionalized with N,N'-bis(2-hydroxyethyl)dithiooxamide to | 15 nM | (Shariati and Khayatian, 2020) |

(*continued on next page*)

**Table 1** (*continued*)

| Adulterant | Source of Analysis | Detection Method | Limit of Detection | Ref. |
|---|---|---|---|---|
| | | produce visible colour change on interaction with AuNPs and their aggregation | | |
| Nitrite and nitrate | Food products | Beeswax-coated paper-based analytical device, Griess reaction with sulfanilamide and N-(1-naphthyl) ethylenediamine on the hydrophilic test spot to produce red coloured azo dye, colour intensity measured by software | 0.1 and 0.4 mg L-1 resp. | (Thongkam and Hemavibool, 2020) |
| Borax, Salicylic acid, Nitrite and Nitrate | Food samples | Paper-based analytical device(PAD) printed with wax as the hydrophobic boundary, Griess reaction of sulfanilamide and N-(1-naphthyl) ethylenediamine with nitrite and nitrate and reaction between FeCl3 and curcumin and salicylic acid and curcumin respectively to produce characteristic coloured compounds | 10, 35 and 0.4 mg/l resp. | (Ratnarathorn and Dungchai, 2020) |
| Nitrite | Meat samples | uPAD imprinted with wax material, reaction between Griess reagent in the test zone and nitrite in pork samples to produce coloured complexes in 15 min | 1.1 mg/kg of meat | (Trofimchuk et al., 2020) |
| Tartrazine and Indigo carmine | Jelly, candy and 4 types of drink samples | Three-dimensional paper-chromatography used as a microfluidic platform, separation of the two dyes due to differences in the solubility in the stationary and mobile phases and their quantification by image capturing and analysis by MATLAB software | 0.620 and 0.060 g/l respectively | (Gharaghani et al., 2020) |
| Electrochemical Techniques | | | | |
| Palm oil | Sunflower oil samples | Cellulosic paper-based lab-on-a-chip device, reaction between fatty acids in palm oil and phenolphthalein indicator in the detection zone to produce a visible colour change from white to pink, image analysis by smartphone app | 10–50% palm oil adulteration | (Muthukumar et al., 2018) |
| Ascorbic acid and Sunset yellow | Mixture of the two dyes present in food samples | Paper-based device with pencil-drawn three electrochemical cells, wax-based ink created hydrophobic boundaries, separation of the two dyes due to differences in their solubilities in the running medium and adsorption onto the polar cellulosic fibres on applying a potential of 0.9 V | Good separation of peaks of the two analytes | (Dossi et al., 2013) |
| Melamine | Lab-prepared samples | Paper-based device coated with ink by pressure-assisted ball pen, differential pulse voltammetry(DPV) applied to promote reaction with melamine and uric acid(indicator) followed by reduction of peak DPV currents of uric acid | 1uM | (Z. Li et al., 2015) |
| Ketamine (Rape drug) | Alcoholic beverages | Paper substrate fabricated withzeolites nanoflakes and graphene-oxide nanocrystals (Zeo-GO), electro-oxidation of ketamine on applying voltage which induced changes in its sensing signal on the testing zone | 0.001 nM/ml | (Narang et al., 2017) |
| Metamizole, paracetamol and midazolam maleate | Whiskey samples | Graphite-pencil coated electrodes on a paper-based device, voltage applied to produce cyclic voltammograms which show differences in electrical resistances of the analytes | 20 mg/l, 45 mg/l and 5 mg/l, respectively | (Dias et al., 2018) |
| Distance-based Techniques | | | | |
| Potassium iodate | Milk samples | Microfluidic paper-based analytical device coated with novel wax valves for confining samples, followed by breakdown of the valve on adding organic solvents and movement of sample through capillary action to promote a colorimetric reaction with starch at the detection zone | 0.05 mM | (Chen et al., 2019) |
| Lead | Century egg samples | Distance paper-based analytical device (dPAD) modified with polyethylenimine (PEI), correlating the reduction in distance travelled by carminic acid on the PEI-coated substrate due to increasing concentrations of lead in the samples | 12.3ug/ml | (Katelakha et al.) |
| Sudan 1 | Aqueous media used in the lab | Self-assembled conjugated polymer/ carboxymethyl chitosan grafted poly (p-dioxanone)nanomicelles(PFO/CMCs-g-PPDO) coated on a paper-substrate, selective detection of Sudan 1 through fluorescence produced by reaction with PFO/CMCs-g-PPDO | Sensing constant = 1.74*10^7 /M | (Chen et al., 2014) |
| Hybrid Detection Approaches | | | | |
| Brillinant blue and amaranth food dyes | Mixture of the two dyes | Paper-based analytical device which utilizes field amplified sample stacking (FASS) effect, stacking of the two dyes due to lowering of electric field followed by their separation due to differences in their electrophoretic mobilities | NA | (Wu et al., 2017) |
| Carmine and Sunset yellow | Mixture of the two dyes | Thread-based microfluidic device, application of well-decided potentials at the various terminals to separate the dyes from the mixture based on their electrophoretic mobilities | NA | (Xu et al., 2018) |
| Milk Adulterants | Milk samples | Wax-printed uPADs for the detection of target species from food samples through reactions with corresponding reagents at the detection zones and subsequent colour production | Multiple LOD values for various adulterants | (Younas et al., 2019) |
| DNA of cow, sheep and goat | Yogurt | DNA probes immobilized on the paper-based substrate for binding with PCR-amplified biotin-bound DNA of the targeted animals from milk samples, visual detection of the analytes by AuNPs conjugated with streptavidin by tis reaction with biotin | 0.01% milk adulteration | (Bougadi and Kalogianni, 2020) |
| Beta-agonists (illegal feed additives) | Swine hair samples | Novel paper-based analytical device coupled with chemiluminescence(CL) which utilizes the reduction in CL values due to oxidation of beta-agonists by $K_3[Fe(CN)_6]$ | 0.00000002 M | (Li et al., 2020) |

(*continued on next page*)

**Table 1** (*continued*)

| Adulterant | Source of Analysis | Detection Method | Limit of Detection | Ref. |
|---|---|---|---|---|
| Ketamine (Rape drug) | Beverages | Wax-printed ion-sensing PAD for applying voltage at the sampling zone using a USB plug, followed by simultaneous detection of ketamine by colorimetric, fluorimetric and potentiometric reactions | 3.2*10^-6 mol/l | (Yehia et al., 2020) |

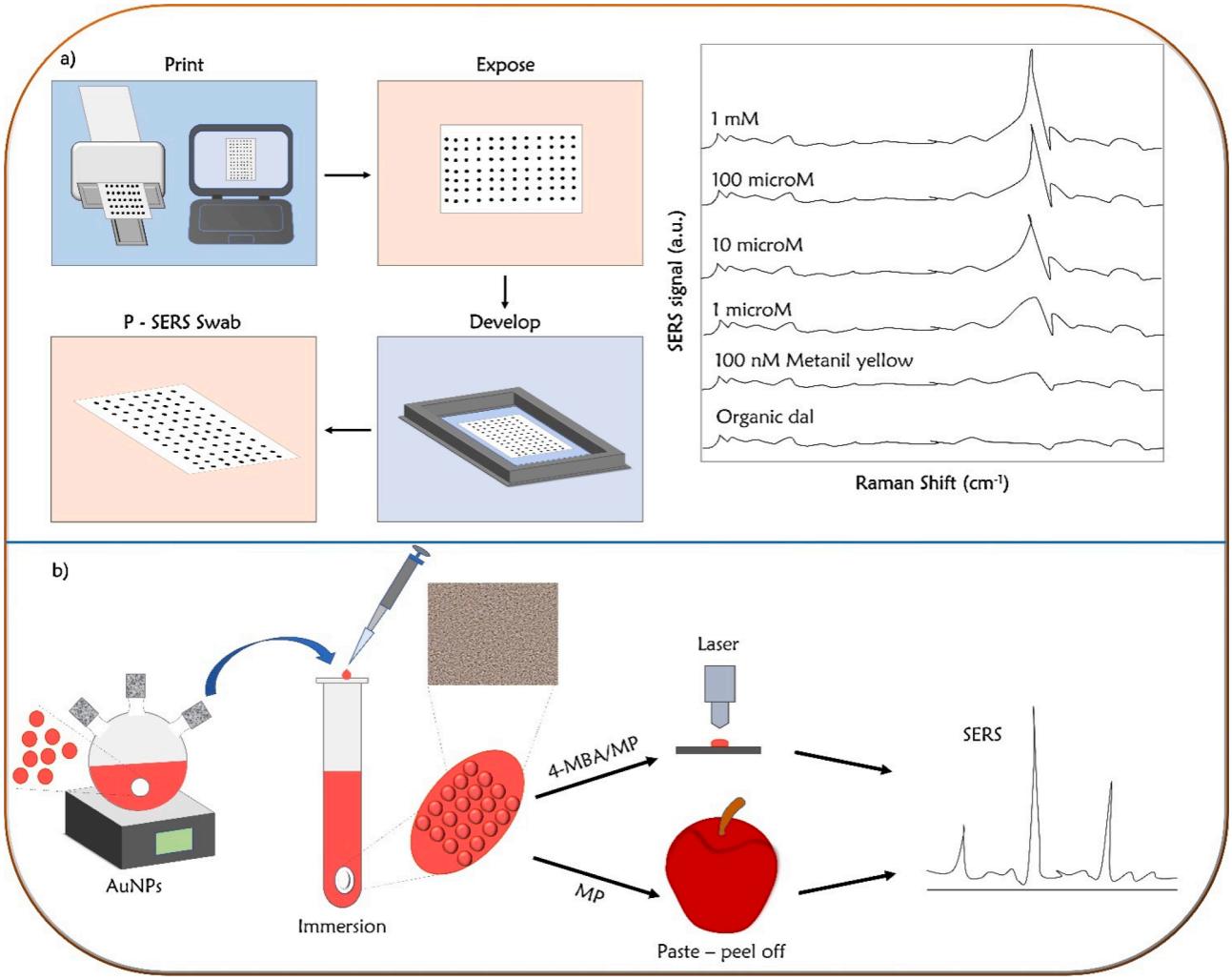


**Fig. 4. a)** Process involved in fabricating Paper-SERS (left) (Modified and adapted from Das et al., 2019). Dal samples spiked with Metanil Yellow and their respective SERS spectra (Right). **b)** Schematic illustration for detecting methyl parathion on the fruit peels surface using paper-based SERS substrates (Modified and adapted from Xie et al., 2020). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Even though SERS has been effectively used in integration with paper microfluidics in all the studies mentioned above, some challenges remain related to either the method individually or when used as a paper-based hybrid platform. For example, silver-based SERS substrates have a shelf-life of only 3–6 weeks due to the degeneration of the activity of silver as a result of tarnishing under environmental conditions. Moreover, packaging and storing them in an inert condition is expensive. Additionally, RSD values range from 10 to 20% due to the spectrometer throughput limitations due to losses (caused by the spreading of light) (Kumar & Santhanam, 2019).

In SERS-based studies, diluting the sample of interest with a solvent such as water might affect the test results due to the interference by other molecules in the solution, which can reduce the signal enhancement by the NPs (Sha et al., 2020). In the study involving the detection of methyl parathion, the intensity of Raman signals lowered rapidly during the first six days of the storage, attributed to the fact that a few NPs initially adsorbed onto the surface of the paper fell later due to weak physical binding and is thus, a limitation initially encountered, which was later stabilized (Xie et al., 2020). In experiments employing the catalysts, such as in the detection of Rhodamine B and Rhodamine 6G in food samples, the catalyst should be easy to synthesize, scale-up, and free from the hassles of separation for reuse because the fabrication and separation of such molecules involve a tedious multi-step process, often impeding the rapid operation (Das et al., 2019). It is also worth mentioning that except for integrating with paper-based platforms, developing a low-cost, portable SERS device is still a significant challenge. Another restriction is that it is challenging to exclude interference and target the compound of interest when working with the complex samples (Zhu et al., 2015).

### 2.2. Colorimetric-based detection

Paper-based analytical instruments have also been applied to detect illegal food additives' colorimetric detection (Table 1). The success of these tests relies on the occurrence or generation of color on the device/platform due to a specific reaction between the desired analyte and an indicator reagent, which can be analyzed qualitatively by visual monitoring and quantitatively by using tools to measure the intensity of the color produced. The observed change can indicate the desired analyte's presence or absence depending on its type. The test is performed for detection; these reactions are commonly known as colorimetric reactions. This method is widely regarded as the most suitable for integration with μPADs due to its simplicity and compatibility with low-cost signal read-out devices such as scanners and smartphones. Colorimetry has been used in environmental and other point-of-care applications and colorimetry-based μPADs in forensic analysis. Integrating these devices with chemometric tools can make it possible to screen a large number of compounds within minutes by employing them in a single device. The production of colorimetric outputs has been materialized through several methods such as nanoparticles, redox, pH indicators, dyes, and enzymes (Morbioli et al., 2017).

A study was conducted to detect 17 beta-estradiol (17 beta-$E_2$), an illegally abused steroid hormone in food products, using a molecularly imprinted polymer-grafted (MIP) paper-based method, where (3-aminopropyl) triethoxysilane (APTES) was used as the polymer and tetraethyl orthosilicate (TEOS) as the cross-linking agent. The principle of competitive binding for APTES between 17 beta-$E_2$-horseradish peroxidase (HRP) applied onto the polymer as a template molecule first, followed by 17 beta-$E_2$ from spiked milk samples was used as the basis of the reaction (Fig. 5**A**). A substrate was added that would yield a colored product on catalysis by 17 beta-$E_2$-HRP and the intensity of this color reduced almost linearly as the more of the 17 beta-$E_2$ from spiked milk samples were added. The LOD of the sensor was reported to be 0.25 μg/l, which was selective to 17 beta-$E_2$ more than the other steroidal samples tested (Xiao et al., 2017).

The rape-drug ketamine was successfully detected by another research group, where the authors (Narang et al., 2018) coated a paper-based strip with bromocresol green (BCG). On the addition of ketamine from the prepared samples, a complexation reaction between the dye and the drug due to protonation/deprotonation of hydroxyl groups on phenols of the former led to the change of color on the strips that were captured by a smartphone; MATLAB program was used to create the database of optical densities of ROIs of all the samples. Then, real samples like Pepsi, rum and whiskey spiked with ketamine were applied for the detection and using the same mechanism; the MATLAB program compared the ROI values to those in this database; if the concentration

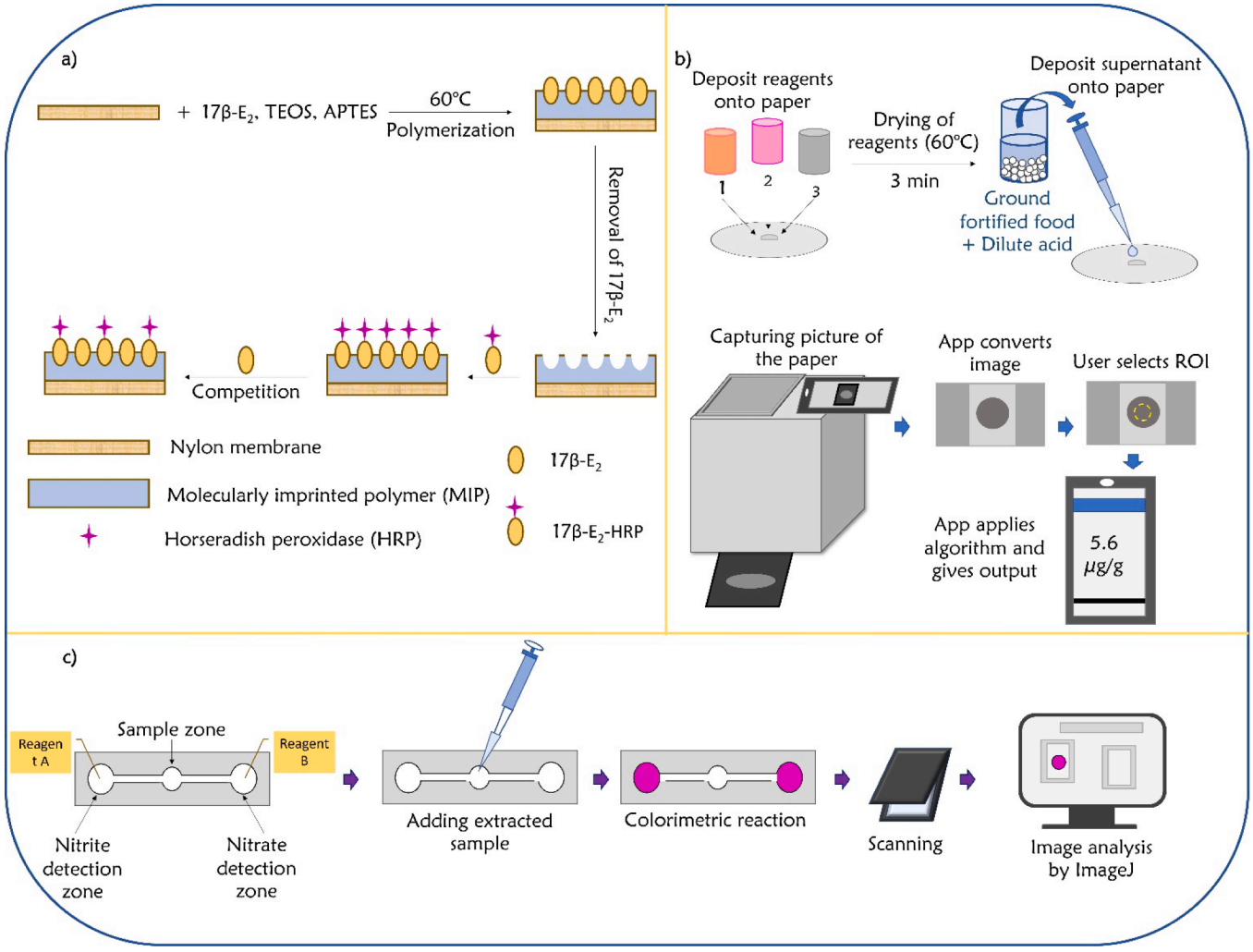


**Fig. 5. a)** Schematic representation of the detection mechanism for 17β-estradiol based on competition between 17 β-estradiol from the sample and the 17 β-estradiol tagged with horse radish peroxidase (HRP) for binding to the molecularly imprinted polymer (Modified and adapted from Xiao et al., 2017). **b)** Detection of fortificants using paper devices and smart-phone (Modified and adapted from Waller et al., 2019). **c)** Colorimetric paper-based devices for detecting nitrite and nitrate (Modified and adapted from Thongkam & Hemavibool, 2020).

was beyond safe limits, then a warning was directly sent to the user's mobile not to consume the drink, thereby providing on-spot analysis. The study attained a LOD of 0.001 M (Narang et al., 2018).

A significant interest was developed in analyzing milk and milk products for quality testing. For example, a study was performed to detect clenbuterol, an illicitly used feed-additive for animals, using a microfluidic paper-based ELISA platform. The device was functionalized by immobilizing antibodies for clenbuterol and in a region coated by printing wax on the paper. The milk samples spiked with varying amounts of clenbuterol were applied on the platform, followed by incubation and the addition of HRP-conjugated clenbuterol later. This has resulted in a competitive binding on the added antibodies between the two substrates. When substrate TMB was added, it reacted with the HRP enzyme to produce a color change and the intensity of the color was inversely related to the concentration of clenbuterol in the real samples as it was based on a competitive ELISA principle. The images were captured and analyzed using the Adobe Photoshop software and the method showed a LOD of 0.2 ppb (Ma et al., 2018).

As previously discussed, the use of NPs for analytical purposes has also progressed in the recent past. A team (Gao et al., 2018) used AuNPs to detect melamine in milk, where the NPs were first stabilized and dispersed on a paper-based platform using negatively charged citrate ions, followed by further stabilization by Triton X-100. The dispersed arrangement was disturbed by adding melamine, causing the NPs to be aggregated, which was translated to color change from wine-red to blue, measured under 630 nm using UV–visible spectroscopy. The color intensity was directly related to the amount of melamine in the samples (prepared or real). The sensor achieved a detection limit of 5.1 nM (Gao et al., 2018). A modification of this approach was used by another research team, where a folded paper coated with poly-dimethoxy silane was used which was pressed and heated on hydrophilic chromatographic paper from both sides to transfer the hydrophilic part to the folded paper and the rest of the process was same as that of the previous study. This research achieved 0.1 ppm and the study proved how melamine was able to react with AuNPs due to its three functional amine ($–NH_2$) groups (Xie et al., 2019).

A study by Salve et al., 2018, focused on detecting multiple adulterants (urea, starch, salt and detergent) in milk. The authors used a paper-based microfluidic device (uPAD), onto which a circular hydrophobic barrier was created using poly-dimethylsiloxane to be used as the test spot. Urea was detected by using its reaction with *para*-dimethylaminobenzaldehyde (DMAB), which produced a yellow color with a LOD of 5 mg/10 ml of adulterated milk. For detecting starch, iodine was applied on the test spot, which produced an indigo color upon the reaction and yielded a LOD of 17 mg/10 ml. The detection of salt was done by adding silver nitrate and potassium dichromate, which reacted with the same to produce a yellow color. The LOD was found to be 29 mg/10 ml. Finally, the amount of detergent in milk was found by adding a phenolphthalein indicator, which displayed pink color in the presence of detergent and was able to detect it in amounts as low as 20 mg/10 ml. All the spot images were captured and analyzed using a milk analyzer android application, thereby providing point-of-care testing (Salve et al., 2018).

A highly efficient and automated method was used to detect adulterants like urea, protein and nitrite almost simultaneously in milk by integrating the uPAD on the surface of the milk carton itself to allow rapid resting. A wax barrier was printed onto the milk carton. The milk sample was applied to the central samples spot, which then was driven by the capillary force in three directions, each of which led to a detection zone having a substrate to react with a certain adulterant in the sample; urea reacted with DMAB to form a visible yellow complex, protein reacted with $CuSO_4$ and NaOH by a reaction between nitrogen atoms from peptide bonds and $Cu^{2+}$ to produce a color shift from blue to violet and nitrite reacted with sulfanilamide and N-(1-naphthyl)-ethylene diamine dichloride to form a red-violet azo dye. Moreover, the analysis of the color produced was done efficiently and rapidly by a designed mobile app (Fan et al.,2018).

A portable detection device was also developed to nullify the location constraints. One such study involved the using a uPAD lab-on-a-chip platform to detect benzoic acid in milk. On the section of the device coated printed with a wax layer, benzoic acid from the samples was made to react with potassium nitrate and sulfuric acid to produce 3,5-dinitrobenzoic acid through a nitration reaction, followed by transferring it to a hot plate for inducing a Janovsky reaction with NaOH and the resulting change in color intensity was captured by a CMOS camera. A software app studied the smartphone's red, blue, and green (RGB) color intensities for a quick analysis. The sensor produced results with a deviation of 6.6% and was successfully applied to 21 commercial food samples (Liu et al., 2018). The use of paper-based devices has also been extended to detecting fortificants in food items for quality control (Fig. 5**B**).

A smartphone-compatible paper-based sensor was used to detect iron fortificants in food samples. A Ferrozine colorimetric reaction occurred between the iron-containing sample loaded on the detection zone and the Ferrozine reagent, turning the paper magenta. This reaction was not affected by temperature and the images were captured using Nu3px mobile app. The color intensities were then measured using Fiji software and correlated to the iron concentration. The sensor was found to suffer interference when tested using Zn, Se, Cu and Co, among other interferents and was able to yield a LOD of 3.691 μg/ml. This method was considered novel because no other paper-based method had previously been used to study ground fortified foods (Waller et al., 2019).

A study described the fabrication of a wax-coated sensor for the detection and measurement of detergent, urea and carbonates in milk samples, highlighting the effects of time (t), temperature (T) and initial width (I) of wax layer on the results. The sample was loaded at the starting point of the central channel by a micropipette, which then flowed by capillary action to three different zones: one with bromocresol purple solution, which gave prominent purple color on reacting with detergents in milk, and the other with DMAB reagent which reacted with urea to give yellow color and the third zone with rosolic acid solution which reacted with carbonates/bicarbonates to give a rose-red color. A control was also used to verify the results. The device reported the satisfying results with low errors, with the only limitation of being a qualitative sensor with 'Yes/No' results (Younas et al., 2019). Further studies have reported the successful on-spot detection of illicitly used starch in milk samples. One such experiment used the formation of tri-iodide from potassium iodide (KI) and iodine, followed by the addition of the adulterated milk samples. The linear structure of tri-iodide facilitated its entry into the helical structure of starch molecules, thereby producing a dark bluish color whose images were captured using a smartphone camera under good lighting conditions and later, the grey-scale intensities were measured using the designed equations (Govindarajalu et al., 2019).

A recent study involved the use of thin-film microextraction (TFME) to detect sulfite in food products, which was able to convert sulfite into $SO_2$, and then adsorbed onto a paper impregnated with $Fe^{3+}$ and 1,10-phenanthroline using TFME. The adsorbed $SO_2$ reduced $Fe^{3+}$ to $Fe^{2+}$, which reacted with 1,10-phenanthroline to form a red-colored complex captured images using a smartphone and later, RGB analysis was done using an analyzer software for the android. The sensor could detect as low as 0.04 μg/l and successfully tested for the selectivity with interferences caused only due to nitrite and sulfide in the samples (Shahvar et al., 2019). Apart from detecting synthetic analogs of food ingredients, paper-based devices were hazardous contaminants such as heavy metals to the food items. A study aimed to detect mercury in food samples using uPADs coated with AuNPs in which AuNPs have been modified with N, N'-bis (2-dihydroxyethyl) dithiooxamide (HEDTO) and coated on hydrophobic-hydrophilic barrier created by triethoxymethylsilane and upon addition of $Hg^{2+}$ containing samples, HEDTO-AuNPs formed aggregation that was transduced into color change from red to the blue of the uPAD. A smartphone camera captured images

of these results. Color intensities were measured by a free software program Adobe Photoshop CS6 and obtained linear relationships between color intensity and analyte concentration. This sensor was tested using real samples of three types of salmon fishes commonly contaminated by mercury. The design offered a LOD of 15 nM and was highly selective toward target species (Shariati & Khayatian, 2020).

Some other studies based on the Griess reaction were helpful for the detection of nitrites and nitrates in food samples (Fig. 5**C**). The detection was done using uPAD. The sample was loaded onto a central sampling zone, then travelled to two simultaneous detection zones: to the left for nitrite and to the right for nitrate by the force of the capillary action. The nitrite that participated in the Griess reaction was derivatized with sulfanilamide and N- (1-naphthyl)-ethylenediamine, forming a red-pink azo dye. On the other hand, the nitrate was reduced to nitrite by a vanadium salt and then, through Griess reaction, produced the same compound, but was delayed by 5 min. The images were analyzed for color intensity using the Image J software to compare both results. The sensor was further checked for its resolution, stability, selectivity and reproducibility for its successful practical application and was demonstrated using the baby food, hotdogs, ham, bacon and sausage (Thongkam & Hemavibool, 2020).

A modified approach utilized the the coating of hydrophilic channel with zinc dust surrounded by a wax-printed hydrophobic channel to detect nitrite, nitrate, borax and salicylate in food samples. Nitrite and nitrate were detected via Griess reaction, where nitrite formed purple color compounds upon reacting with sulfanilamide and N-(1-naphthyl)-ethylene diamine dichloride. Still, nitrate yielded the same only after further addition of zinc dust. For the detection of borax, curcumin was applied, which reacted with boron borax formed a red complex. Finally, salicylate was detected by adding $FeCl_3$, whose $Fe^{3+}$ ion reacted with two oxygen atoms in salicylate to give a purple color. Its application was demonstrated using real samples such as meatball, pork ball, chicken ball, pork sausage, chicken sausage, pickled mango, pickled tamarind, pickled green mustard and pickled bamboo in which these compounds are generally present. The sensor showed LOD values of 5 mg/l, 30 mg/l and 50 mg/l for nitrite, nitrate, borax and salicylate, respectively (Ratnarathorn & Dungchai, 2020).

Studies for the detection of nitrite have been continued by considering the potential and existing side-effects of the preservative in meat. Focussing on these aspects, a study (Trofimchuk et al., 2020) was done using a uPAD fabricated with wax and the Griess reaction to detect nitrite in meat samples with the lowest detection capacity 1.1 mg/kg of the meat. Apart from the primary reaction, the authors also demonstrated the coffee-ring effect resulting from the evaporation of the analytes across the surface and colorimetry was used to detect the commonly used food dyes. A sensor was designed to detect and separate tartrazine and indigo carmine efficiently in food items such as jelly, candy and drink samples. For this, a 3D microfluidic device was constructed by folding the paper into many strips in a zig-zag fashion and stacking them to create a pile. A central hydrophilic channel was created in each strip, while the surrounding area was made hydrophobic using a laser printer. Samples were loaded onto the central channel, causing the analytes to flow through it and separating them based on the interaction with the stationary and mobile phases. Indigo carmine was stopped at the second layer, while tartrazine was stopped at the eighth layer; hence, their color intensities were detected prominently at the distinct layers with the well-separated peaks. The device was optimized for solvent composition, channel diameter and pH and the LODs of 0.620 g/l and 0.060 g/l for tartrazine and indigo carmine, respectively (Gharaghani et al., 2020).

A simple titration was used for the visual detection of palm oil in sunflower oil by fabricating a lab-on-a-chip microfluidic device. Samples containing varying concentrations of palm oil in sunflower oil were added to the loading zone of a rectangular paper strip, which was then driven by the capillary action towards the detection zone containing KOH and propanol with phenolphthalein as the indicator. A visible transition from colorless to pink was observed upon reacting with palm oil, which was captured by a smartphone camera and analysed by UV spectrophotometry. Palm oil was detected up to 10–50 % (v/v) in sunflower oil samples (Muthukumar et al., 2018).

Some potential challenges must be considered for the triumphant integration of colorimetry with paper-based devices. For example, the conditions chosen for enzyme-based colorimetric assays simultaneously for two or more biological tests might be suitable for just one of the many samples used, which might affect the results of other tests. Hence, it is essential to optimize/check the system before developing such an assay to avoid building a dysfunctional system. Additionally, the quantities of both the enzyme and the redox indicator should be adequate to prevent low signal readout and saturation of the signal, respectively. Concerning the nanoparticle-based colorimetric tests, some of the common limitations are complex and sometimes lengthy procedures to synthesize them, as can be seen in cases of AgNPs and latex-based NPs, low signal readouts due to the large size of the particles as seen in the case of carbon dots. However, some colorimetric methods cannot even be coupled with paper-based products due to heating or organic solvents, which are incompatible with fabrication methods such as those commonly encountered during wax printing (Morbioli et al., 2017).

### 2.3. Electrochemical-based detection

Apart from the aforementioned techniques, electrochemical analysis is another major approach that has been used particularly for the quantitative detection of food adulterants (Table 1). These methods involve replicating a three-electrode system consisting of the counter, reference, and working electrodes, which are deposited onto the paper's surface, usually in conductive inks instead of the conventional solid electrodes. The samples are mainly added to a specific hydrophilic region confined by the hydrophobic barrier, which then flows through a channel to reach the area of the three electrodes, each maintained at a particular potential to carry out a redox reaction involving the sample and modified with some reagents to increase the selectivity, thereby to produce an electronic signal readout depending on the electrochemical method applied. This technique has several advantages. For example, there is a considerable reliance on the selectivity and sensitivity depending on the material of the electrodes and the potential maintained. There is a minimum electrical power requirement during the field application due to its simplicity and portability. Compared to colorimetry, where the intensity of the images taken by the camera can be affected by lighting conditions and calibration, electrochemical methods facilitate the generation and transfer of more reliable data from the on-site to experts, something not commonly achievable using the colorimetry.

A study by Dossi et al., 2013, dealt with separating two common food colorants, ascorbic acid and sunset yellow, by performing planar thin layer chromatography by creating layers using the wax-based ink. The voltage of 0.9 V was applied, which caused a separation of the two dyes with distinct peaks due to the differences in their solubility in the running medium and their adsorption onto polar cellulose fibers of the paper substrate. Ascorbic acid and sunset yellow were detected in amounts as low as 30 μM and 90 μM, respectively, by this sensor with promising sensitivity and reproducibility (Dossi et al., 2013). More convenient methods recently developed include directly-writing of electrodes on paper using a pressure-based ball pen to form a paper-based electrochemical device (PED) to detect melamine in food samples. Phosphate buffered saline (PBS) solutions with increasing concentrations of melamine were used with uric acid as an indicator. Upon applying differential pulse voltammetry, the current decreased with a rise in the concentration of melamine due to the competition between the former and uric acid for adsorption onto the carbon surface. The sensor attained a detection limit of 1.0 μM of melamine (Li et al., 2015).

On newer fronts of fabricating paper-based devices, one of the latest

studies worked on the coated paper surface with zeolite-nanoflakes and graphene-oxide nanocrystals (Zeo-GO) and used it for detecting and quantifying ketamine in alcoholic and non-alcoholic drinks (**Fig. S1a**). On applying the drug on the circular testing spot of the Zeo-GO surface, the former underwent electro-oxidation, causing a change in the sensing signal of the sensor, which was correlated to the drug concentration added. The method reported a rapid-response time of only 2 s with a low detection limit of 0.001 nM/ml, which was economical, easy to prepare and disposable (Narang et al., 2017). Pencil-drawn electrodes have also extended their use to detect multiple analgesics and sedatives, viz., metamizole, paracetamol and midazolam maleate in whiskey samples (**Fig. S1b**). The electrochemical activity of the first two drugs was tested successfully as they yielded well-defined peaks on square wave voltammetry (SWV) and the sensor achieved LODs of 20 mg/l and 45 mg/l for metamizole and paracetamol, respectively. SWV studies were performed on midazolam (sedatives) in whiskey samples, which reported a LOD of 5 mg/l. Compared to other electroanalytical methods, this approach reported poor performance, but the benefits of being cost-effective, portable, reproducible and portable seem to support its future applicability (Dias et al., 2018). Additionally, electrochemical methods might face minor challenges when coupled with paper-based processes, as mentioned by Carvalhal et al. (2010), where the authors developed an electrochemical paper-based device for chromatographic separation and subsequent detection of uric acid and ascorbic acid. Still, the only major problem noted was a longer detection time compared to high-performance liquid chromatography (HPLC), which took only 16 min to separate the two analytes initially (Carvalhal et al., 2010).

### *2.4. Distance-based detection*

Distance-based methods have also attracted considerable attention in recent years (Table 1), wherein a sample reservoir and a hydrophilic channel are patterned with a colorimetric indicator specific to the sample. As the sample flows through the channel confined within the hydrophobic barrier, reactions between the former and the indicator give rise to the formation of a colored precipitate, thereby generating a colored band whose length is proportional to the concentration of the sample added. The resultant distance can be measured by keeping a ruler along the length of the channel, and thus, the need for using any electronic readers or timers is eliminated. This method is more advantageous than the colorimetric assays, but the intensity and quality of images captured using a camera or smartphone can vary based on the lighting conditions or camera quality. Moreover, no additional instruments are needed for measurement or quantification (Katelakha et al., 2021). Opinions on the qualitative outcomes might differ among the individuals due to color and brightness perception (Cate et al., 2015). These assays have been applied to detect food adulterants such as toxic food additives. A microfluidic paper-based device (uPAD) was also used to detect potassium iodate, a chemical widely used in bread and is known to have carcinogenic effects. A novel wax valve was created by printing the wax layer on the paper, separating the loading and detection zones. Potassium iodide (KI) and P-toluene sulfonic acid (PTSA) was applied to the loading zone separated from each other by a wax-coated line. At the same time, the starch solution was loaded onto the detection zone (Chen et al., 2019). Finally, potassium iodate ($KIO_3$) solution was loaded on the two chemicals in the loading zone, which triggered their reaction, thereby forming triiodide. Toluene was applied to the wax valve, dissolving it and thereby allowing the solution to flow through a hydrophilic channel to reach the starch-loaded zone, which upon reaction with triiodide, a blue complex was observed whose color intensity was directly proportional to the concentration of triiodide and which increased with distance from the loading zone, getting closer to the starch-zone. The sensor achieved a LOD of 0.05 mM (**Fig. S2a**).

A more recent study utilized colored complex formation between carminic acid (CA) and lead (Pb) for quantitative detection of the latter in century egg samples, where it is misused in the form of lead oxide (**Fig. S2b**) (Katelakha et al., 2021). A hydrophilic channel was modified with polyethyleneimine (PEI) to improve CA's adsorption efficiency onto the channel. The latter was applied to the loading zone, free to travel through the channel to longer distances. Upon adding the Pb samples in increasing concentrations, more CA reacted with the former, thereby lowering the amount of free CA that could travel and hence, lowering the travel length of the chemical on the channel, which yielded a LOD of 12.3 ug/ml. This device was successfully checked for its selectivity towards Pb by using other metal ions. However, some disturbance was reported for Cu, which was later neutralized using potassium cyanide (KCN) as a masking agent (Katelakha et al., 2021).

## 3. Integrated hybrid Paper-Based microfluidics approaches

In addition to the methods discussed above, paper-based analytical instruments have been explored using more unique approaches for determining the presence of food adulterants in collected samples, where two or more methods of detection have been helpful in a hybrid manner (Table1). One such a method aimed at the detection of Sudan I, a commonly added food dye using a self-assembled conjugated polymer made of carboxymethyl chitosan (CMC) grafted poly(p-dioxanone) or PPDO, where CMCs covered the PPDO from outside to form a water-soluble nano-micelles coating for protecting lipophilic material of the conjugated polymer poly (9,9-dioctylfluorene) or PFO; this was used to functionalize an indicator paper for exhibiting fluorescence. Upon specifically adding Sudan I, the electron-withdrawing capability of the azo group on the dye lowered the energy of the empty $\pi^*$ orbital, thereby making it a good electron acceptor responsible for quenching the fluorescence from PFO on the paper. The intensities before ($I_0$) and after (I) quenching were observed and the highest $I_0/I$ ratio was observed for Sudan I when compared to the negligible values for the other dyes, viz., monascorubrin and lycopene. The sensor reported high sensing constant of $1.74*10^7$/M for the dye (Chen et al., 2014).

Intending to utilize the electric and electrophoretic properties of analytes, a study by Wu et al., 2017, developed a paper-based sensor for separation and quantification of two food dyes, Amarnath and brilliant blue, where they applied a background electrolyte (BGE) on one end (anode) and the sample of dyes on the other end (cathode), both dipped in buffer solutions and connected via the paper (**Fig. S3a**). The two charged dyes started moving from the cathode to the anode due to the opposite charges and due to a reduction in the electric field, where they are stacked initially, but later, they are separated due to differences in their electrophoretic mobilities, where brilliant blue was found to have lesser mobility. The LOD values of this device were 0.31 μg/ml and 0.17 μg/ml for amaranth and brilliant blue, respectively, which were enhanced only after stacking was performed. The sensor's feasibility was also checked using two other analytes (Wu et al., 2017).

The electrophoretic properties of analytes were also utilized to separate food dyes, sunset yellow and carmine using a thread-based device (Xu et al., 2018). The authors created four terminals S, SW, B and BW for sample reservoir, sample waste reservoir, buffer reservoir and buffer waste reservoir, maintained at 200 V, 200 V, 50 V and 600 V, respectively, during the sample injection and separation. A thread connected terminals with a junction in the middle and upon injecting the dyes into the separation channel, two negatively charged dyes were moved from S to SW. They were separated on the way due to differences in their electrophoretic mobilities in just 3.5 min, displaying a rapid analysis of the samples (Xu et al., 2018).

The use of real-time PCR (polymerase chain reaction) as a rapid and accurate species identification is emerging rapidly. This approach was recently used by Abdel-Rahman & Ahmed, 2007, to differentiate between cow, sheep and goat DNA in yogurt samples to check their authenticity. DNA samples of the above three animals were collected from the respective yogurt samples and amplified using the PCR. Probes were created for hybridizing with each sample: oligonucleotide with a polydeoxyadenosine sequence of 30 bases at its 5′ end for cow and

probes with poly-A tails for sheep and cow. The amplified DNA samples conjugated to biotin were allowed to hybridize with each of these respective probes in separate setups, washing off the unbound sequences. The sensor was then dipped into a solution of streptavidin (SA)-bound AuNPs, which caused the latter to get pulled to the test spot due to the capillary action. If the DNA sample had been hybridized, the reaction between biotin and SA could produce a red spot.

A similar procedure was followed using a control sample with only biotin to check for the reaction between the two substrates. The respective setups were tested to be highly specific towards their DNA sequences with minimum cross-reactivity. The sensor acheived up to 0.01% adulteration detection with promising reproducibility (Bougadi & Kalogianni, 2020). Focus has also been directed towards the detection of β-agonists, commonly used in swine hair. The sensor developed produced normal chemiluminescence (CL) values due to a reaction between $K_3$[Fe (CN)$_6$] and luminol in the absence of β-agonists. On adding the analyte, it was oxidized by $K_3$[Fe (CN)$_6$], thereby lowering the latter's reaction with luminol, consequently quenching the CL. This decrease was proportional to the rise in the concentration of β-agonists. The minimum concentration detected by the sensor was $2*10^{-8}$ M and was reported to have displayed good reproducibility and specificity (Li et al., 2020).

As previously discussed, instant and efficient detection of the rape-drug ketamine in drink samples has been increasingly focused on in the recent years, using more robust methods. A team developed a sensor with a novel trimodal system for ketamine detection using potentiometric, colorimetric and fluorometric approaches. A conducting polymer coated with wax was connected to a USB plug for setting up the electric field (**Fig. S3b**). The samples containing negatively charged ketamine were loaded on the spot, which was driven by the conducting nature of the polymer towards two sections: one having citrate-dot-AuNP pairs, where the amino functionality of ketamine de-stabilized the pairing, thereby increasing the C-dot fluorescence intensity and the other section having cobalt thiocyanate, on reacting with which yielded a blue colour. The approach reportedly proved to be a sensitive, selective and affordable method of detecting ketamine with a LOD of $3.2*10^{-6}$ mol/L (Yehia et al., 2020).

A study reported using a portable paper-based microfluidic sensor to analyse different proportions of olive oil and sunflower oil blends in oil samples, which involved loading the samples at the inlet of the top of a column and applying a voltage; the electrical resistance was measured in MΩ. It was noted that the resistance increased linearly from 0.26 MΩ to 2.79 MΩ with the rise in olive oil proportion in the samples, showing that sunflower oil had more conductivity than olive oil and this was performed using three different types of filter paper. Each offered a difference in its performance due to different filtration rates and pore sizes (**Fig. S3c**). The lowest LOD reported was by a filter paper numbered 42 and it was 10.26 units (Radovanović et al., 2021).

## 4. Concluding remarks and future prospects

Massachusetts Institute of Technology (USA) recently developed a Velcro-like food sensor consisting of microneedles made of edible proteins found in silk cocoons, which can pierce through the plastic packaging and surfaces of many types of food products to draw fluids into specialized 'bio-inks' on the sensor, which changes color if the fluids contacted in the food samples are within a pH range specific to that of spoilt food. The sensor also used antibodies in one of their 'bio-inks' and successfully detected food samples contaminated with bacterial species of *E.coli* and Salmonella sp. This sensor was able to address the problem related to detecting pathogens embedded within otherwise inaccessible cavities in food. The data presented highlight the feasibility and potential usage of 'smart-packaging' for on-site detection even before the customer purchases it, potentially allowing the consumers to perform testing by themselves, thereby eliminating the use of complex instruments and laboratory conditions.

Substantial research efforts have occurred to understand the indicators of food contamination and spoilage by microorganisms, including changes in oxygen ($O_2$), carbon dioxide ($CO_2$) concentrations, humidity, pH, temperature and production-specific chemicals due to microbial growth. Sensors integrated with the packaging material of food products for detecting such changes have reached the proof-of-concept stage. They involve using colorimetric, potentiometric and fluorometric tests individually or in combination. Such intelligent fabrications have been proposed to have the least impact on the environment and specific detection ability against microbial contamination (Yousefi et al. 2019).

The prominent studies observed a lack of exploration of thread-based devices (Prabhu et al., 2021; Prabhu et al., 2020b) to detect food adulterants, which should stimulate more research activity in this field and perhaps, find even more efficient and convenient approaches. The only work discovered in this regard was for separating carmine and sunset yellow dyes using a cross-channel thread-based device, as mentioned previously (Xu et al., 2018). A possible way of utilizing thread-based tools could be the use of a container with a lid of mesh made up of a network of threads specific that traps a particular adulterant in a liquid or powdered food sample and produces a color when the sample is poured into/out of the container through the lid, thereby giving a rapid visual detection to the user. Shifting the focus back to 'smart-packaging', several potential fabrications can be suggested, one of them being able to develop a specially designed packaging material that can absorb a particular contaminant or adulterant from the food sample by capillary action and react with it to produce a compound with a strong odor, thereby allowing the customer to reject the item and switch to a better product.

A strategy can also be applied to design materials for wrapping solid food products that can react with adulterants on the surface of the product to produce a toxic compound, thereby degrading the product itself and making it easier to distinguish the item from non-contaminated products. As highlighted in work by The Massachusetts Institute of Technology before, it is essential to address the need to differentiate between spoilt and edible food products, especially when they are sold in a covered form. A futuristic way of handling this could be to design such a packaging material that could produce an acidic or toxic compound by reacting with the expected bacterial species when they start growing on the food substance after the expiration date has been crossed, thereby degrading the package itself and relieving the customer from the need of checking the expiration date of each product.

A study was already successful in creating a milk carton integrated with a paper-microfluidic device to detect urea, nitrite and proteins in milk samples (Fan et al., 2018). This is yet another indication that the applicability of paper-based analysis is being extended from the laboratory to the market. Using paper microfluidics normally involves creating hydrophobic barriers for confining the solutions of interest. However, not all types of samples can be confined by these barriers. Considering this, one could design a particular packaging material for liquid food samples like milk and juices with a hydrophobic barrier on the surface that can confine all types of solutions, except for those containing a particular adulterant, thereby allowing the consumer to get visual proof of the existence of that specific adulterant before consuming the product.

Similar to the Velcro-like food sensor previously discussed, micro-penetrating packages could be considered in the future for solid and semi-solid food products, where the microneedle-like structures can pierce through the surface of the food and draw selectively; those fluids that are injected into the food items for enhancing their weight and appearance, such as in fruits, vegetables and meat products. The drawn fluid can then reach the main packaging material and cause a particular chemical reaction to produce a characteristic color, alerting the customer beforehand. It could be interesting to see if one can design a packaging material that has a soluble coating on its inner surface, such that whenever a particular microbial growth starts on the product inside

the container, the material in the coating will get degraded by some enzyme secreted by the microbe and then expose a layer underneath coated with antibiotics, which can inhibit the growth of the microbe and perhaps, extend the shelf-life of the product (**Fig. S4**).

The success of the various methods mentioned in the upcoming years requires specific improvements and futuristic envisioning. For example, the study conducted by Zhu et al. (2015) for SERS-based detection of sunset yellow and lemon yellow proved that capillary driven fluid flow could be used to facilitate analyte transport and pre-concentration in a small area, thereby successfully separating the dyes from the drinks and concentrating them on the tips of PSS-coated paper substrates. Thus, such electrolyte-coated paper devices can be used for real-world sample preparation, concentration, and SERS-based measurement in the future (Zhu et al., 2015). Kumar & Santhanam (2019) studied the detection of metanil yellow and malachite green and commented on how further investigation is needed on the protocols for separating analytes and their subsequent transport to parts of the paper coated with SERS-based nanostructures for better performance. Additionally, silver-decorated filter paper by Das et al., (2019) for the SERS-based detection of rhodamine B and rhodamine 6G that the inexpensive and flexible substrate can open doors for real-time and on-site food and water safety monitoring. The authors envisaged other, more unique and futuristic applications in healthcare, wearable/flexible electronics, and nonenzymatic glucose sensing (Das et al., 2019).

In the case of electrochemical-based sensing, pending issues also exist concerning their reproducibility, long-term stability, and multiplexing properties. However, significant efforts are being planned to solve the same shortly. Problems are faced concerning the robustness and reproducibility of the electrodes, as commonly encountered with screen-printed electrodes (SPE), with the additional limitation of not being able to downsize them below specific dimensions. Such drawbacks can be minimized to some extent in the future by introducing other fabrication approaches such as material evaporation–and photolithography. It should also be understood that these devices will not have a high chance of thriving in the future markets unless they have minimum manual steps and user manipulation, low cost per analysis, and a lesser number of complex architectural features (Gutiérrez-Capitán et al., 2020).

Some of the strategies to improve the present limitations and make them more suitable for point-of-care applications (POC) in the next few years are selecting the most appropriate type of paper, employing efficient methods for fabricating electrodes, using proper materials for modifying the electrodes, such as nanoparticles and nanocomposites or by writing the electrodes on the surface of the paper. It has been stated that more innovation is needed in the designs of the devices, ink formulation, and the construction of the electrodes. More studies are required to explore biological entities and novel functional molecules in the inks. A great deal of focus is also suggested to be diverted towards multiplexed sensing as it is still in its infancy stages. One of the many other essential aspects to be addressed is amplifying signals by coalescing these methods with newer techniques such as CRISPR/CAS biosensing (Baharfar et al., 2020).

Modernized upgrading is also demanded in distance-based methods. First, devices with dried reagents along the channel can improve their reproducibility by using inkjet printing, where reagents can be printed along the channels with increased consistency and resolutions. The introduction of automation in such devices has also been encouraged so that the user (trained or untrained) will merely have to add the sample. At the same time, the installed system shall perform the other operations, including signal readout. Overall, further research is expected to expand the scope of applying distance-based assays, particularly in low-resource areas (Tian et al., 2016).

Concerning colorimetric sensing, several predictions have been proposed for the upcoming years. First, it has been suggested by Gharaghani et al., (2020) who had developed a paper-based colorimetric sensor for the detection of tartrazine and indigo carmine in food samples, that future research can be directed towards modifying the paper surface by newer chemical methods to build better specific interactions between the stationary phase and the analytes for chromatographic separation of the latter. The focus could be on developing compatible barriers with a wide range of solvents to make more studies possible in the next decade (Gharaghani et al., 2020). Similarly, Fan et al., (2018) who had designed a milk carton integrated with a paper microfluidic device for the detection of milk adulterants, envisaged newer methods to employ the same technology in the upcoming years by possibly transforming the paper-based device into a sticker or a label that could be applied on plastic or glass milk bottles or pouches in aseptic conditions (Fan et al., 2018). Another such study performed on milk samples by Salve et al., (2018) has highlighted the importance of increasing the stability and robustness of spot tests for adulterants on paper-based devices and of providing more investigation on developing paper microfluidic devices that would facilitate the detection of two or more contaminants in the same mixture in future experiments (Salve et al., 2018).

It is necessary to understand that to make the above-proposed methods feasible in the future; it is highly essential to have detailed knowledge about the food product, the types of adulterants commonly used in it and their chemical and/or biological properties (if any), and designing or discovering a material that could be used for packaging to detect that adulterant/ contaminant specifically by using a particular reaction. The widespread applicability of paper-based devices and related technologies can also be strengthened if the resulting products are integrated with various daily activities of the people. For example, in restaurants and other dining venues, paper napkins and straws can be made with specially designed customer-friendly materials that can produce distinctive color changes on coming in contact with solid and liquid food items contaminated with specific adulterants on rubbing their surface or dipping into liquid foods like drinks.

Similar approaches can also be used at home, where utensils can be designed for customers coated with paper functionalized with functional groups that specifically absorb some contaminants or adulterants from liquid and/or solid food items and produce a color for visual detection. Incorporating these potential technologies in consumers' daily lives can also help educate them more about food adulteration and how they can perform analysis on their own by using these do-it-yourself (DIY) products. Paper-based devices can also be developed further to improve the existing work on detecting metallic contaminants in food. For example, packaging food items with specially designed cellulosic material incorporated with a 'micro-electrode and power supply' system can extract targeted metallic compounds from the food samples into the packaging material by applying a voltage, thereby producing a detection signal that can be received on a smartphone app. In addition to detecting the adulterants at the consumer stage, it is of greater importance to screen for these substances at the production stage. Technologies can be developed to monitor parameters like pH, absorbance, color, etc. and produce 'smart' signals if these parameters go beyond a certain range, considering that some particular contaminant causes the change in them (**Fig. S4**).

It is essential to mention the contributions of other fields in detecting food adulterants. We have previously highlighted several studies in this review that have utilized nanoparticles (NPs) on paper-based substrates for the same purpose. In addition to these experiments, other research works have used a combination of palladium and gold core-shell nanocrystallines (Pd/Au CSNs) and polyamidoamine dendrimers-cadmium sulfide quantum dots CdSeQDs-PAMAM for the detection of popular food colorant, Sudan I by performing a competitive immunoassay between the dye and the CdSeQDs-PAMAM quantum dots for immobilized Sudan I antibodies and the detection was observed by the decrease in an electrochemiluminescence (ECL) due to rise in the concentration of the dye. A study had also reported an easily observable coloured complex formation as the visual detection signal due to an oxidative reaction between commonly used livestock adulterant dopamine with nanoceria

($CeO_2$ NPs). Later, sensors were also developed using AuNPs with p-nitroaniline receptors to detect melamine in milk (Mustafa & Andreescu, 2020). All these studies described in this review also aim to focus on integrating paper-based analysis with other, developing fields like nanotechnology prove to show better performance than any of the individual domains and hence, smooth and healthy exchange of information between interrelated areas must continue to gain momentum.

To minimize the lingering issue of food adulteration, advancements are needed in their timely detection and society in general. For example, paper-based packaging of food substances should be encouraged soon, which can be the most effective approach to apply these technologies at a large scale and obtain accurate results. This will also reduce the usage of plastic packaging and will offer an added advantage of biodegradability. Moreover, providing theoretical and practical knowledge about the use of paper-based microfluidics and associated techniques should be more prevalent even at the undergraduate level to inculcate the skills of designing simple, yet novel and efficient technologies for addressing important scientific issues. Apart from this, a lot of awareness has to be spread among all classes of the society regarding food adulteration, including the purchase of cheaper, paper-based packaging material.

For the successful application of the approaches mentioned above, it is also essential that considerable flexibility is given to the consumers to perform on-site testing using some of these 'smart' packaging instruments. As previously highlighted, cautiousness must be followed right from the production stages. The producer needs to consider the adverse effects on productivity, sales and brand value their company will have to endure in the long run due to such illicit practices and, therefore, implement robust strategies in response (**Fig. S4**).

In conclusion, food adulteration is a perennial problem that has penetrated the industry much more profoundly than expected. The possibility of its eradication in the future will exist only if comprehensive efforts are made by the scientific community, society, the food industry and even law. The work that has been conducted during the past decade in this field deserves a lot of appreciation as a lot of qualitative and quantitative data have been generated, which were previously not available. However, this is just the beginning of such studies, as more is yet to be explored. The efforts of the aforementioned research groups will be justified mainly by discovering the untapped possibilities of paper-based tools and instruments. Overall, the present critical review serves as a treasure for food technologists thriving towards technological improvements in food adulterant detection and introducing the noteworthy features of the paper-based microfluidics paradigm in the food sectors.

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## CRediT authorship contribution statement

**Rohitraj Ray:** Writing – original draft, Resources. **Anusha Prabhu:** Writing – original draft, Resources. **Dinesh Prasad:** Conceptualization, Writing – review & editing. **Vijay kumar Garlapati:** Writing – review & editing. **Tejraj M. Aminabhavi:** Conceptualization, Methodology, Writing – review & editing. **Naresh Kumar Mani:** Conceptualization, Writing – review & editing, Funding acquisition. **Jesus Simal-Gandara:** Conceptualization, Writing – review & editing, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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