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# A wax chalk and self-heating paper-based analytical device (SH-PAD) for the detection of bisphenol A†

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Bisphenol A (BPA) is a synthetic xenoestrogen widely present in the environment, known for its toxicity, endocrine-disrupting nature, carcinogenicity, and mutagenic effects on living organisms. The detection of BPA is essential as it infiltrates the human body through food, water, dust and dermal contact. Conventional methods currently in use are inadequate for on-the-spot detection. Consequently, there is a pressing need to build an all-in-one device that can be quickly fabricated using readily available and cost-effective off-the-shelf materials for the detection of BPA. Firstly, we have leveraged wax chalk for fabricating hydrophobic barriers on paper, which offers a hydrophilic channel resolution of  $1.64 \text{ mm} \pm 0.05 \text{ mm}$  and also the ability to confine major aqueous solvents without leakage. The fabricated device was used to detect BPA using the Folin–Ciocalteu reagent and sodium carbonate (in the presence of heat). Secondly, we have developed a self-heating paper-based analytical device (SH-PAD) using masking tape, lamination paper and Whatman filter paper. This cost-effective approach (0.017\$) is based on an exothermic reaction caused by sodium hydroxide and a small quantity of aluminium in the paper layers and can retain heat adequately for more than 5 minutes, addressing the challenge of external heat sources and enabling effective and rapid colorimetric detection of BPA using the Folin–Ciocalteu reagent and sodium carbonate. Both methods can detect up to  $2 \mu\text{g mL}^{-1}$  in spiked water samples. This developed method's user-friendliness and cost-effectiveness make it a promising candidate for point-of-care diagnostics or detection, providing testing capabilities in areas with limited resources.

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## Introduction

The contamination of food and everyday products has been a concern since the dawn of civilization, as it reduces the quality of food products and poses significant health risks.<sup>1</sup> Phenolic compounds are among the major contaminants of the environment and health sources, arising from natural or anthropogenic activities.<sup>2</sup> One of the major phenolic adulterants present is bisphenol A (BPA), a building block chemical found in epoxy resins, which can leach from these resins and hard polycarbonate plastics used in everyday items like baby bottles, toys,

water bottles, food containers, and even vehicle body primers.<sup>3</sup> Consumers are exposed to BPA through food, beverages, and water, as traces of it can migrate from packaging materials as well. The general population can be exposed to BPA through the inhalation of dust, while workers are at risk of exposure both by inhaling BPA and through dermal contact. Reportedly, higher concentrations of BPA have been detected in canned milk and infant formula.<sup>4</sup> BPA is used in large quantities in the production of synthetic polymers and thermal paper and is known for being a synthetic xenoestrogen, capable of impeding systemic endocrine activity by blocking natural hormones within the body.<sup>5</sup> Its capacity to bind to various receptors, such as estrogen, androgen, aryl hydrocarbon, and peroxisome proliferator-activated receptors, associated with hormones and other bodily systems, contributes to its endocrine-disrupting properties. Additionally, BPA demonstrates oxidative and mutagenic potential, as well as the capacity to induce hypomethylation.<sup>5</sup>

Considering the hazardous nature of bisphenol A, its detection in the environment and food products has been implemented through various analytical methods including solid-phase extraction,<sup>6</sup> mass spectrometry, surface-enhanced Raman scattering, enzyme-linked immunosorbent assay, and liquid chromatography with an electrochemical method.<sup>7</sup> Despite being

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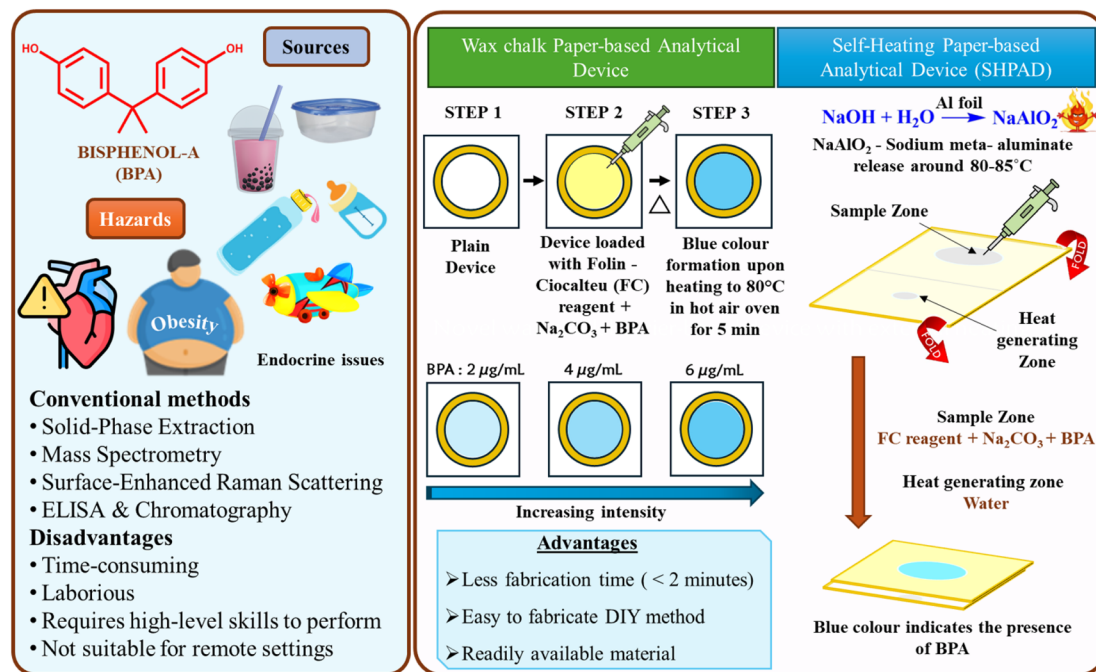


Fig. 1 Schematic illustration of BPA detection using the wax chalk and self-heating paper-based analytical device.

regarded as industry standards for detecting food adulterants due to their efficiency and accuracy, these methods have numerous limitations. They are expensive, time-consuming and laborious, requiring considerable resources and equipment, and necessitate highly skilled scientists and technicians to perform and analyze the tests. Generally, these methods are performed in centralized laboratories and can be suitable for an urban setting, limiting their applications in resource-limited areas. Consequently, there is a strong urge to develop simpler, cost-effective, and equally efficient testing methods that can be of great help to the public. Two areas gaining significant attention are paper-based microfluidics ( $\mu\text{PADs}$ )<sup>8–11</sup> and thread-based microfluidics.<sup>12–15</sup> The use of paper has largely facilitated the realization of the “lab-on-a-paper” concept.<sup>16</sup> This advancement enables experiments that once required large-scale equipment to be conducted on small, appropriately modified pieces of paper.<sup>9</sup> To convert plain paper into a paper-based device, it is essential to create hydrophobic barriers, which are generally achieved using both high throughput and low throughput techniques. High throughput methods, known for their greater reproducibility and accuracy, include photolithography,<sup>17</sup> vapor phase deposition,<sup>18</sup> PDMS printing,<sup>19</sup> inkjet printing,<sup>8</sup> wax printing,<sup>20</sup> and wax dipping.<sup>21</sup> In contrast, low throughput methods include manually drawing barriers with wax pens,<sup>22</sup> correction pens,<sup>23</sup> hydrophobic pens,<sup>24</sup> and similar tools, offering lower reproducibility and accuracy.<sup>25</sup> Wax is a common material used in both methods and is advantageous because of its availability and cost-effectiveness. However, there is a requirement for a heating step of around  $100^\circ\text{C}$  for 10–30 minutes,<sup>26</sup> which would further increase the fabrication cost.

To address the aforementioned limitation, we present a novel method by leveraging wax chalk, which results in the fabrication

of hydrophobic barriers within 30 seconds using free-hand drawing (Fig. 1). The heating time required is less than 1.5 min, which enables the melting of wax particles penetrating through the paper pores and thus forming a hydrophobic barrier. Further, the fabricated wax chalk-based paper-device was leveraged for detecting bisphenol A using the Folin–Ciocalteu reagent. To facilitate on-spot detection, we have developed a self-heating paper-based analytical device for bisphenol A detection (Fig. 1). The principle of generating heat is based on the exothermic reaction that takes place between sodium hydroxide, aluminium and water in the stacked layers of SH-PAD. The reaction between BPA, the Folin–Ciocalteu reagent and sodium carbonate changes the color from yellow to blue. The vital observable product in this reaction is the formation of a blue-colored complex. The intensity of the blue color observed is directly proportional to the BPA concentration that is present in the reaction mixture.<sup>27</sup> To the best of our knowledge, this is the first time that a self-heating paper device has been developed for onsite detection of analytes.

## Experimental

### Materials

Whatman filter paper (grade 1) was purchased from Whatman, India. Wax chalk was bought from a local stationary shop. 2 N Folin–Ciocalteu reagent and phenol were bought from Loba Chemie, India. Bisphenol A (BPA) was procured from Kanton Laboratories, India. Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and sodium hydroxide ( $\text{NaOH}$ ) were acquired from Sisco Research Laboratories (SRL), India. An aluminum foil roll and lamination pouch were acquired from a general store and a local stationary shop, respectively. Scotch masking tape and transparent tape were procured from 3 M General Purpose Masking Tape, India.



Acetone, Triton-X 100, 1 N hydrochloric acid (HCl), 1 N sodium hydroxide (NaOH), Tween-20 and dimethyl sulfoxide (DMSO) were procured from SRL, India. 1× PBS was acquired from HiMedia, India. Glycerol was procured from OOM laboratories and ethanol was bought from Hayman, India. The BPA absorbance value is determined using a spectrophotometer (Genisis 180, Thermo Scientific).

### Wax chalk barrier fabrication

To begin with, a square shape with 4 cm sides was drawn using a pencil on Whatman filter paper grade 1. Concentric circles of 15 mm and 12.5 mm were drawn using a pencil with the help of a stencil to function as a hydrophobic barrier, bearing a width of 2.5 mm shaded with yellow wax chalk twice in a uniform circular motion. The device was then heated at 100 °C for 1.5 minutes for the material to diffuse across the filter paper's barrier and reach its full depth.

### Self-heating paper-aided device fabrication

Initially, masking tape of length 10 cm was divided into 2 halves using a pencil. A 7 mm circle was cut from one part of the tape to serve as an inlet for reagents, while a 5.5 mm hole was punched on the other half to serve as an inlet for water. A piece of Whatman filter paper, measuring 3 cm by 3 cm, was adhered to the inlet with a diameter of 5.5 mm. Three pellets of sodium hydroxide (NaOH) and a 1.5 cm square piece of aluminium foil were placed on the setup. Another layer of Whatman filter paper, measuring 3 cm by 3 cm, was then stacked on top. The two layers were firmly stuck together using scotch transparent tape on all sides. A fresh piece of Whatman filter paper was taken, on which a 15 mm circle was drawn and then cut out. A lamination pouch was taken where holes of 5.5 mm were punched. The cut-out circle of 15 mm was fabricated into the lamination pouch. The laminated setup was then adhered to the masking tape setup bearing the water inlet. This setup was then folded, making it a foldable device (ESI Fig. 2†).

### Solvent compatibility

We evaluated the hydrophobic circular paper device with 20 µL of various polar and non-polar solvents, which included water, 1 N hydrochloric acid, 1 N sodium hydroxide, Tween-20, 40% ethanol, glycerol, dimethyl sulfoxide, acetone, 1× phosphate buffered saline, and 1% Triton-X 100. To facilitate observation, the solvents were dyed blue using fountain ink. Three devices were tested for each solvent to detect leakage through the barrier over a 30 minute period.

### Microscopic resolution

Straight lines with widths ranging from 3 to 4 millimeters were scribed on filter paper. To achieve this, two parallel lines of wax chalk, each 3 millimeters wide, were drawn to establish a barrier. Following the application of heat to infuse the paper with the barrier, water was added. The resulting hydrophilic channel was then examined under 40× magnification using a Euromex Oxion-Inverso microscope. The microscope's camera software captured

images of the channel, which were subsequently analyzed using Fiji software to determine the channel's resolution.

### Bulk test for BPA detection using the Folin–Ciocalteu reagent

1 N of Folin–Ciocalteu (FC) reagent, bisphenol A (BPA) and 7.5 (w/v%) of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solutions were taken in a volumetric ratio of 1 : 4 : 4. BPA solution was taken as 2, 4, 6, 8 and 10  $\mu\text{g mL}^{-1}$  in the vials, which displayed a significant color change with the intensity of the blue color increasing from 2 to 10  $\mu\text{g mL}^{-1}$  solution of BPA. These trials were conducted in triplicate at 55 °C.

### Paper-based circular device for BPA detection

Whatman filter paper grade 1 measuring 4 cm by 4 cm was taken and concentric circles of 15 mm and 12.5 mm were drawn, creating a 2.5 mm wide ring, which was then shaded with wax chalk. This setup was heated at 100 °C for 1.5 minutes. After heating, scotch transparent tape was affixed to the rear side to prevent any leakage. Varying concentrations of BPA from 2  $\mu\text{g mL}^{-1}$  to 10  $\mu\text{g mL}^{-1}$  were taken in triplicate. The setup included 50  $\mu\text{L}$  of  $\text{Na}_2\text{CO}_3$  (7.5 w/v%), 12.5  $\mu\text{L}$  of FC reagent (1 N) and 50  $\mu\text{L}$  of BPA solution made in ethanol. The circular device was placed in a moist chamber and heated at 55 °C for 5 to 7 minutes. After heating, a blue-colour complex was formed and was observable on the circular device.

### Self-heating paper-based analytical device for BPA detection

SH-PAD included 50  $\mu\text{L}$  of  $\text{Na}_2\text{CO}_3$ , 12.5  $\mu\text{L}$  of FC reagent and 50  $\mu\text{L}$  of BPA solution made in ethanol. The device was kept still for 2 minutes after each reagent was added, ensuring thorough absorption of the reagents. The device was then inverted and 900  $\mu\text{L}$  of water was added through the 5.5 mm hole present on the rear side. A blue-colored complex was formed in 15 seconds, which was observable on the front side of the device, with its intensity being directly proportional to the concentration of BPA. The heat generated was measured using an infrared gun, which amounted to 80–85 °C, resulting in a quicker and effective detection than conventional methods.

## Results and discussion

### Fabrication and characterization of the wax chalk paper-based device

Considering the hazardous nature of bisphenol A (BPA) and the drawbacks of conventional analytical tools, we have devised two

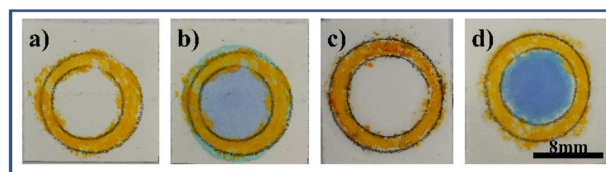


Fig. 2 Wax chalk paper device: (a) plain device before heating, (b) leakage in the device before heating, (c) plain device after heating and (d) no leakage in the device after heating.





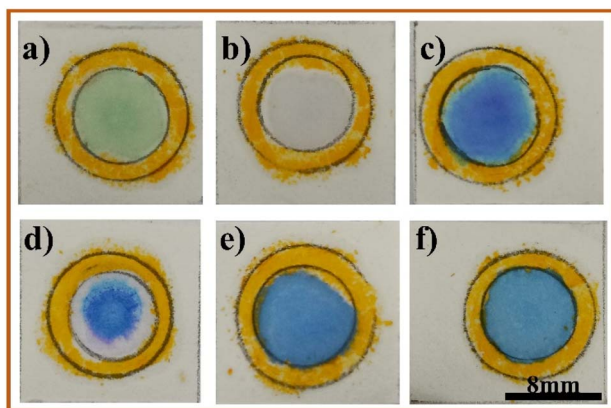


Fig. 3 Solvent compatibility studies of the wax chalk paper device with (a) 1 N HCl, (b) 1 N NaOH, (c) 1 $\times$  PBS, (d) acetone, (e) 40% ethanol, and (f) 1% Triton X-100 (all solvents coloured with ink).

innovative approaches. Firstly, wax chalk was used to create a hydrophobic barrier on filter paper. This approach is robust and cost effective and the whole fabrication process, including the application of heat, takes less than 2 minutes. Fig. 2 depicts the simple circular device fabricated using wax chalk. After heating (at 100 °C), the wax material penetrated the paper pores, resulting in sample confinement without any leakage.

Further, we have assessed the solvent compatibility of the paper device fabricated using wax chalk. Fig. 3 depicts a wide range of solvents that were successfully confined by the hydrophobic barrier. Generally, for paper-based fabrication, a wide range of approaches can be employed to create hydrophobic barriers. These include correction pens,<sup>28</sup> inkjet printing,<sup>29</sup> carbon copy<sup>30</sup> and latex.<sup>9</sup> Among all these, only latex-based hydrophobic barriers showed superior solvent compatibility.

In this study, we observed that wax chalk offers resistance against organic solvents such as acetone and 40% ethanol, and 1% Triton X 100, a surfactant. This could be because of the possible composition of the wax chalk, which is commonly available in various forms (wax-based markers, lithographic crayons, lumber crayons, and solid paint markers) with

a composition including waxes (paraffin, carnauba, and beeswax), clay, natural resins (copal and shellac), pigments, dispersants and gelling agents with enhanced strength and water resistance. These are routinely utilized for writing on surfaces of materials such as tyres, tubes, steel, plastic, plywood, brass, copper, decolun (laminated sheets) and rubber. We hypothesize that silica (present in clay) and other components may impart resistance to organic solvents and surfactants. We have also assessed the minimum hydrophilic resolution that can be achieved using this fabrication method. Using a microscope, we found that the minimum resolution that can be attained is 1.64 mm  $\pm$  0.05 (Fig. 4).

#### Detection of BPA using the Folin–Ciocalteu reagent using bulk and wax chalk paper-based devices

Further, we have used the Folin–Ciocalteu (FC) reagent for instant detection of BPA in the presence of heat.<sup>27,31</sup> The detection of bisphenol A relies on the reducing nature of the Folin–Ciocalteu (FC) reagent, which produces a blue color upon reacting with phenolic compounds.<sup>32</sup> The FC reagent, being acidic, reacts with BPA under basic conditions; therefore, sodium carbonate is used, which provides an alkaline environment, leading to an observable color change of the reaction mixture from yellow to blue. The intensity of the blue color observed is directly proportional to the BPA concentration that is present in the reaction mixture. We conducted the reaction between BPA, the FC reagent, and sodium carbonate in bulk in a ratio of 1 : 4 : 4, respectively. In the presence of 55 °C, the blue color was observed with its intensity (as well as absorbance at 760 nm) increasing with the concentration of BPA (ESI Fig. 1†). This method was applied to our wax-chalk PAD, and we observed a visible color change as depicted in the images below (Fig. 5). Additionally, we have measured the RGB intensity using FIJI software and observed an increasing trend with increasing concentration of BPA (Fig. 6).

**Detection of BPA using the self-heating PAD.** As mentioned earlier, the reaction between the Folin–Ciocalteu (FC) reagent and BPA occurs only at temperatures above 55 °C,<sup>31</sup> thus

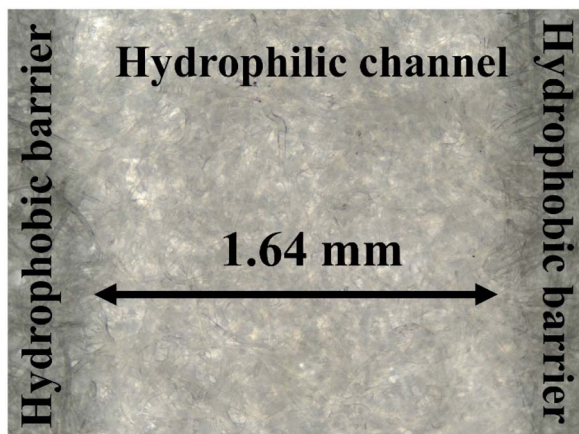


Fig. 4 Microscopic characterization of the hydrophilic channel fabricated using wax chalk.

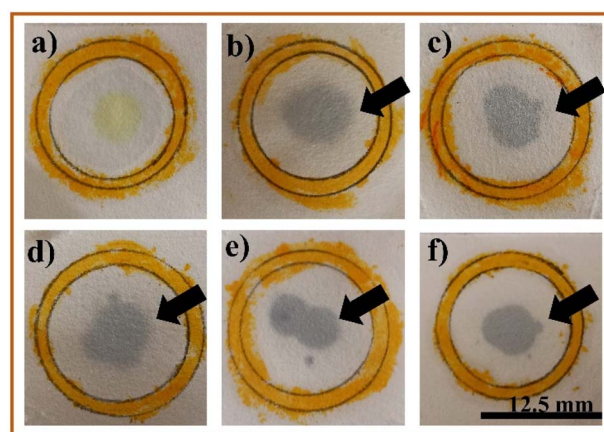


Fig. 5 Interaction between the FC reagent and BPA: (a) control, (b) 2  $\mu\text{g mL}^{-1}$ , (c) 4  $\mu\text{g mL}^{-1}$ , (d) 6  $\mu\text{g mL}^{-1}$ , (e) 8  $\mu\text{g mL}^{-1}$  and (f) 10  $\mu\text{g mL}^{-1}$ .



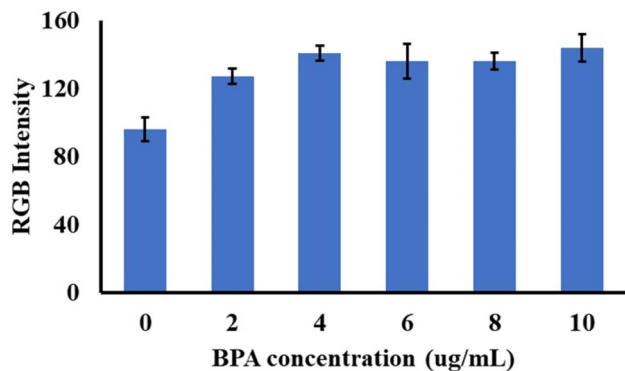


Fig. 6 RGB intensity of different concentrations of BPA in the wax chalk paper-based device.

necessitating an external heating source or equipment. This can limit the application of this approach in resource-constrained settings. Henceforth, to facilitate on-the-spot detection, there is a requirement for an all-in-one device where heat can be generated on its own without the use of any external heating source. To address this, we developed a self-heating paper origami or paper stacking device, where an exothermic reaction between sodium hydroxide pellets, water, and a very small piece of aluminium was utilized to generate the heat required. On the SH-PAD, these chemicals were properly packed and confined, ensuring no exposure to the atmosphere. We observed that the addition of water results in the generation of heat (around 80 °C). The fabricated SH-PAD was then subjected to colorimetric detection of BPA using the Folin–Ciocalteu (FC) reagent. Fig. 7 depicts the formation of a blue-colored complex with the

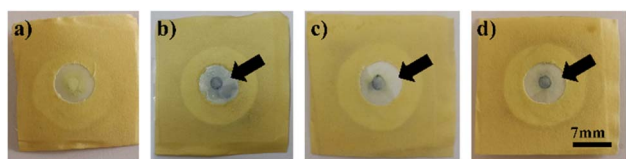


Fig. 7 SH-PAD for the detection of BPA: (a) control, (b) 2  $\mu\text{g mL}^{-1}$ , (c) 6  $\mu\text{g mL}^{-1}$ , and (d) 10  $\mu\text{g mL}^{-1}$ .

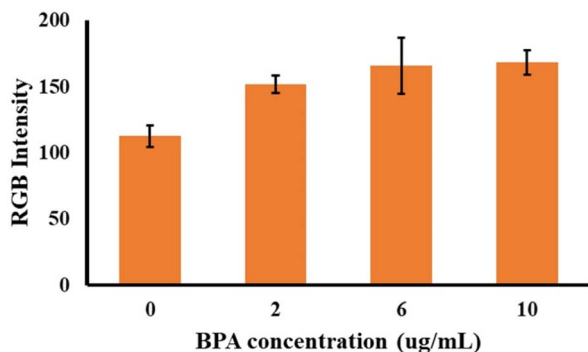


Fig. 8 RGB intensity of different concentrations of BPA in the self-heating paper-based analytical device.

intensity of the blue color increasing with the concentration. We have also observed an increased trend in the RGB intensity produced in SH-PAD (Fig. 8).

We have also assessed its shelf-life for a week, where the SH PAD was imbibed with the Folin–Ciocalteu reagent and sodium carbonate and was kept inside a refrigerator under dark conditions at 4 °C. The device was then tested by adding water and the colour change was observable in the presence of BPA (data not shown). This approach is viable and cost effective for any endothermic quantitative analysis. One major hurdle we have noticed in this approach is the overlap in standard deviation values, even though there is an increase in the intensity of color with the concentration. This could be due to non-uniform heating and device-to-device variation.

Studies have verified that significant amount of BPA is present in various environmental samples like raw leachates,<sup>33</sup> waste landfill leachate, sewage sludge, and household dust.<sup>5</sup> Workers at factories are at risk of being exposed to BPA, which may cause metabolic alterations in them.<sup>34–36</sup> The limit of detection (LOD) of BPA achieved with our developed devices is 2  $\mu\text{g mL}^{-1}$ , which is slightly above the maximum allowable levels (MALs) of 4–25  $\text{ng mL}^{-1}$ . However, the LOD achieved is comparable with the values reported in the literature.<sup>37–39</sup> Henceforth, the prospects of wax chalk and SH-PAD are highly promising, offering the potential for on-the spot detection of BPA from a wide range of samples. Moreover, further implementation of SH-PAD for detecting numerous contaminants and their integration into wearable sensors to detect biomarkers from body fluids and clinical samples will greatly help in healthcare diagnostics and personal health monitoring with the assistance of better image analysis techniques.<sup>40–42</sup> A table comparing wax chalk and SH-PAD is given in ESI Table 1.†

## Conclusions

In this work, we have demonstrated two innovative approaches to facilitate easier and quicker bisphenol A (BPA) detection. The first approach involves leveraging wax chalk, which is commonly found and inexpensive, to fabricate a hydrophobic barrier that can effectively confine the solvents without any leakage. The entire fabrication takes only 1.5 minutes. The second approach involves a self-heating paper-based analytical device (SH-PAD), which is economical (0.017\$) and possesses multiple layers of paper stacked and room for exothermic reactions using sodium hydroxide, aluminium and water. In both approaches, a blue-colored complex is formed when the Folin–Ciocalteu reagent and sodium carbonate added solution interacts with BPA. This research demonstrates the potential of straightforward fabrication methods. These methods allow established detection tests to be adapted for a significantly more portable and miniaturized medium.<sup>43,44</sup> Furthermore, our work lays the groundwork for advancements in DIY (do-it-yourself) fabrication, which offers a foundation for incorporating new functionalities, such as wearable sensors and smartphone-based detection of colorimetric signals.



## Data availability

The data supporting this article have been included as part of the ESI† as well as in the main manuscript.

## Author contributions

Conceptualization: NKM, AP, and BS; data curation: NKM, AP, and BS; formal analysis: NKM, AP, and BS; funding acquisition: NKM; investigation: NKM, AP, and BS; methodology: NKM, AP, BS, and SP; project administration: NKM, AP, and BS; resources: NKM; software: AP, NKM, and SP; supervision: NKM; validation: AP, NKM, SP, and BS; visualization: NKM; writing – original draft: NKM, AP, BS, SP, and BPQ; writing – review & editing: NKM.

## Conflicts of interest

There are no conflicts to declare.

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