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A Simple Colorimetric Detection of Bisphenol A in Exposed Drinking Bottles Using a Paper-Based Sensor

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ABSTRACT

Bisphenol-A (BPA) is a chemical widely used to produce polycarbonate plastics and epoxy resins, commonly found in bottled water containers. BPA migration into drinking water can occur due to variations in pH and temperature during storage, posing potential health risks with prolonged exposure. This study focuses on developing a rapid and accurate colorimetric paper sensor for BPA detection. The sensor employs a chromogenic reagent that selectively reacts with BPA, resulting in a visible color change from yellow to dark red. Experimental results demonstrated that the sensor exhibits high sensitivity, with a detection limit (LOD) of approximately 0.29 mmol L^{1}. Validation of water samples from plastic bottles exposed to environmental conditions confirmed the sensor's ability to identify BPA leaching effectively. Additionally, the sensor showed excellent stability, indicated by a relative standard deviation (%RSD) of 0.62%, and high accuracy, with recovery values ranging from 100.87% to 107.39%. These findings suggest that the developed colorimetric paper sensor is a promising tool for on-site monitoring of BPA contamination in drinking water, providing a simple, fast, and cost-effective alternative to conventional analytical methods.

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INTRODUCTION

Water is essential for life and helps to maintain bodily fluids and prevent dehydration. In Indonesia, 40.64% of households consume bottled or refillable gallon mineral water. Such containers are made by molding and contain polycarbonate (PC) resin produced, for example, by the polycondensation of bisphenol A (BPA) and phosgene monomer in an alkaline medium [1]. As BPA contributes to the strength and stability of plastic, it may produce health hazards during long-term use [2], [3]. BPA can leach from plastic food packaging, particularly with increasing temperature [4], [5]. BPA migration is enhanced by long-term use due to polymer hydrolysis, and over time, the plastic becomes more permeable and allows water to adhere to bottle walls [6]. As a result, the water ingested can be contaminated with BPA. The European Food Safety Authority has determined that the maximum acceptable dose of BPA for the human body is 0.05 mg/kg/day. Exposure in excess has been linked to hypertension, cancer, and disruption of reproductive hormones [7]–[9]. Therefore, it is important to control the intake of BPA to avoid its potential health risks.

Few works have quantitatively determined BPA in diverse human biofluids and plastic materials [10]. Various analytical approaches have been developed for the determination of BPA, including UV-VIS spectrophotometry [11], HPLC [12], GC-MS [13], LC-MS [14], biosensors [15], [16], and voltammetry [17]–[19]. Although such methods are sensitive and selective, the costly and complex equipment required, the need for specialized operator skills, and the time-consuming analysis process are notable drawbacks.

Colorimetric sensors are gaining much attention for their application in food safety monitoring [20]–[22]. Colorimetric responses usually involve noble metals due to their particular optical properties that exhibit strong surface plasmon resonance (SPR) effects. A colorimetric aptamer reaction of BPA with Au nanoparticles (Au NPs) has been prepared, where the colorimetric response is attributed to the aggregation of Au NPs, changing the color from red to purple in the presence of BPA in the solution [23]. Nevertheless, noble metals still suffer from various disadvantages, including their high cost, potential toxicity, and limited stability [24], [25]. A colorimetric sensor of BPA was also prepared using bifunctional $CsPbBr_3$@Cu-MOF materials [26]. MOF materials, with high specific surface area and adsorption capacity for target analytes, show great potential for colorimetric sensing [27], [28]. The sensor demonstrated a linear detection range of 1.0–80.0 nM and a limit of detection (LOD) of 0.60 nM. Although these sensors have high sensitivity, their materials must be easy to prepare and utilize, as expensive and complicated fabrication processes remain challenging.

Accordingly, this work produced a facile colorimetric sensor based on the specific reaction of BPA with a chromogenic reagent adsorbed on cellulose fibers. The colorimetric sensor was designed on the chemical reaction-induced color change between the BPA and a specific detection agent immobilized on paper. The paper-based sensor possesses a rapid and easily detectable color change to detect BPA without instruments. This sensor is also deemed to be inexpensive and appropriate for field analysis.

METHODS

1. Materials and Instruments

BPA (99% purity), 4-aminoantipyrine (4-AAP, 99%), and potassium ferricyanide ($K_3Fe(CN)_6$, 98%) were purchased from Sigma Aldrich. Getman® quantitative filter paper, ash <0.007%, Grade 42, thickness 210 μm, pore size eight μm, was obtained from Sigma Aldrich (www.sigmaaldrich.com). All the standard solutions were made using Ultrapure Water (UPW) and stored in a refrigerator (+5°C). All chemical reagents used were of analytical grade and did not require further purification.

The paper-based sensor was evaluated using ATR-IR spectra (Shimadzu instrument) from 4000 to 500 cm^1 to identify the functional groups on the paper surface. The surface topography was measured using a Scanning Electron Microscope (SEM, Tescan Vega 3) and analyzed by a 3D surface plot using ImageJ 3D Viewer. Color intensity of BPA was detected by an iPhone 14 Pro A2890 with a 1080p HD camera (48 MP and f/1.78) and quantified using ImageJ software to calculate absorbance.

2. Preparation of paper-based sensor

The filter paper used was Whatman Grade 42. This filter paper has a porosity of 2.5 µm, a thickness of 200 µm, and a diameter of 42.5 mm. The paper was cut into small disc-shaped sheets with an area of 1.0 cm². It was then immersed in 10 mL of 83.4 mM $K_3Fe(CN)_6$ solution for 10 minutes, followed by air drying at room temperature (20 °C). After this operation, the paper assumed a yellow hue (Fig. 1).

3. Real sample analysis of BPA and validation method

For a real sample application, 20 µL of water from a drinking bottle was deposited on the sensor, followed by 20 µL of 20.8 mM 4-AAP, resulting in an immediate reddish color. This indicated the adsorption of BPA. The color conversion was photographed using an iPhone 14 Pro A2890 with a 1080p HD camera (48 MP, f/1.78), and the absorbance of each BPA concentration was analyzed using ImageJ software.

The validation procedure was based on the survey of linear range, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy. The linearity between absorbance and concentration was assessed over a range of BPA standard solutions (0–80 mmol/L⁻¹). Measurements were made in duplicate. Duplicate readings: Two readings were taken for each concentration. The calibration curve method determined the linearity value based on the slope and R² value. The LOD (3SD) and LOQ (10SD) were calculated from the calibration curve.

For accurate analysis, the 7th-day sample was taken from the heated range. The resulting sample solution was replicated on the fabricated sensor 20 times (n = 20). The excursion curves were processed, and the (%)RSD was subsequently calculated. Meanwhile, the accuracy value was determined regarding percent recovery by spiking the 7th-day sample solution with 10 and 20 mmol/L⁻¹ BPA standard solutions, respectively.

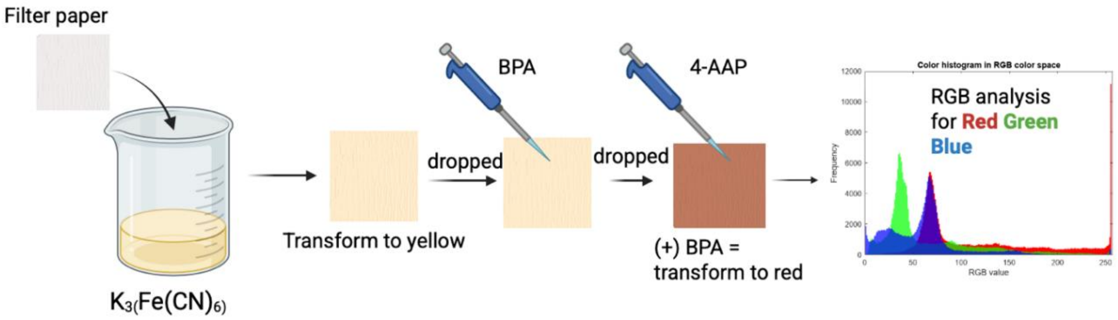
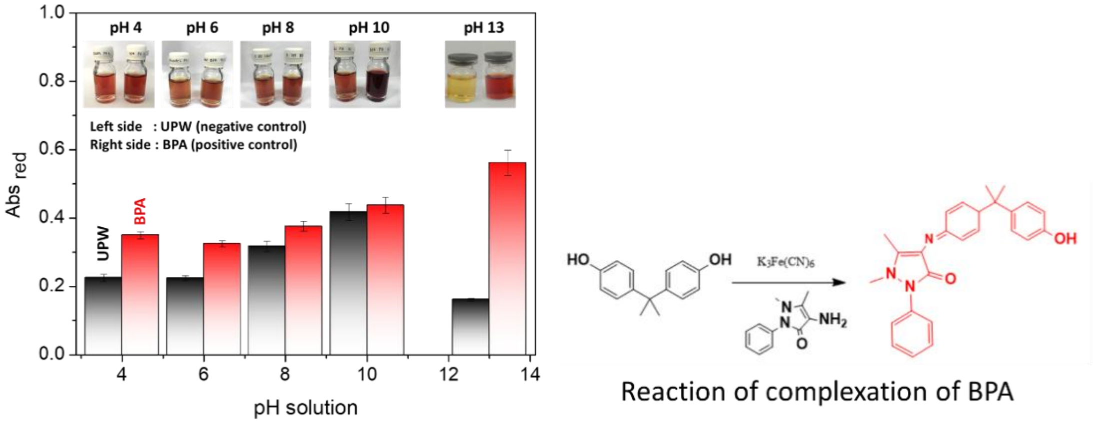


Figure 1. The illustration of sensor preparation and color quantification

RESULTS AND DISCUSSION

1. Preparation and Characterization of Paper-Based Sensor

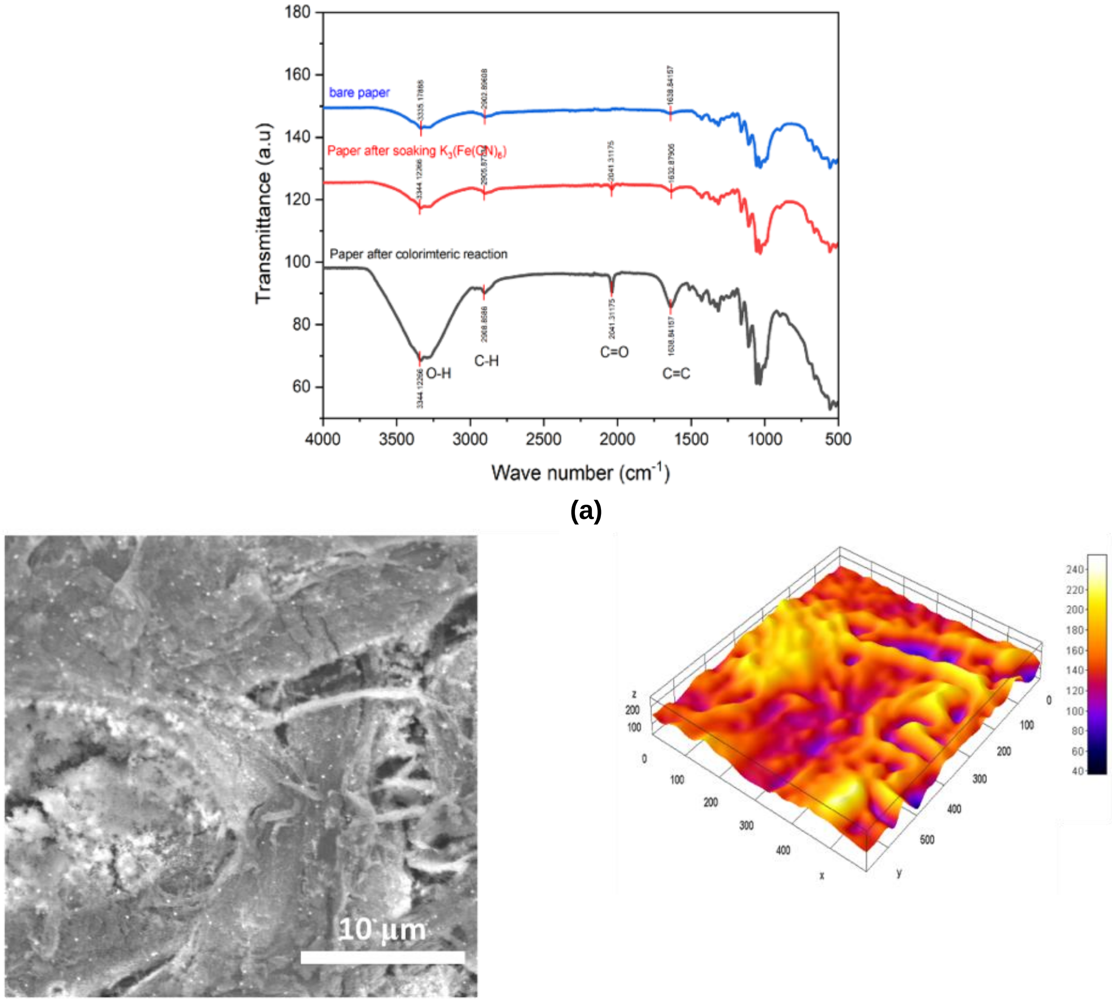
A novel rapid method of detecting BPA using a paper-based sensor has been developed in the literature. One of the key parameters in this reaction is the pH of the solution, which directly influences the stability of the complex generated [29]. It was found that the pH should be controlled in the process of BPA complexation, as demonstrated in Figure 2. A pH 13 basic solution allows for a very marked color difference between the negative control solution (without BPA) and the positive control solution (with BPA), as shown in Figure 2. In the base solution, BPA was formed, and the color changed from yellow to red since BPA reacted with 4-AAP. The color of the negative control solution without BPA remained yellow, arising from $K_3Fe(CN)_6$. Fiamegos et al. carried out a colorimetric reaction of phenolic compounds with 4-AAP [30]. The hydroxyl group of phenol is oxidized, and an azo or a conjugated quinoid can form. Because BPA is a phenolic compound possessing a hydroxyl group, and because the mechanism of the formation of complex colored substances by this method is the same as the mechanism described above, the similarity of the absorption spectrum shape and the color development trend is also confirmed by the absorption spectrum.



Reaction of complexation of BPA

Figure 2. Color and reaction complex of BPA in various pH solutions

It can be seen from Figure 2 that BPA could be detected in an alkaline solution. The paper sensor was fabricated as follows. The sensor fabrication was performed using filter paper, in this case Whatman Grade 42, to which the $K_3Fe(CN)_6$ solution was added. The chromogenic reagents, BPA and 4-AAP, can react together with $K_3Fe(CN)_6$. The 4-AAP [31], catalyzed by $K_3Fe(CN)_6$, becomes active and has a larger affinity for BPA, ultimately forming a red complex in solution. Including K $K_3Fe(CN)_6$ could improve the sensitivity and selectivity of this colorimetric approach, contributing to a clear chromatic variation responsive to BPA determination.



(a)

(b) **(c)**

Figure 3. (a) IR spectrum, (b) Morphology, and (c) 3-D surface analysis of paper-based sensor.

Prior to the detection of BPA, the paper sensor was initially characterized by IR spectroscopy to identify the functional groups present on the paper. Figure 3(a) shows the IR spectra for Whatman 42 filter paper as naked paper, $K_3Fe(CN)_6$ modifier-treated paper, and after the colorimetric reaction of BPA and 4-AAP. In addition, the intensity of the absorption peak of the hydroxyl group (O–H) in the range of 3330–3344 cm^{-1} was strengthened after the colorimetric reaction, showing that BPA could be adsorbed on the surface of the paper and that the O–H absorption intensity increased due to the adsorption of BPA. BPA has two phenolic hydroxyl groups, which can form hydrogen bonds with the hydroxyl groups on the D-glucose moiety in cellulose. The hydrogen bond contributes to the density of the –OH group appearing on the paper's surface, thus resulting in a strong absorbance intensity in the –OH band. This finding is consistent with a previously reported study, indicating that the –OH group is the principal characteristic of BPA compounds [32].

SEM observations of the paper surfaces showed a fibrous structure due to the hydrophobic cellulose fibers, which facilitate the absorption of BPA sample solutions (Figure 3(b)). The surface structure of this sensor is consistent with the morphology of cellulose fibers reported in previous references [33]. The orderly and porous structure of cellulose fibers (a loosely connected fibril network) results in a large surface area for contacting with objective substances. The 3D surface analysis also showed that color absorption was uniform and easily observed, although the surface of the paper film was not completely even (Figure 3(c)).

2. Colorimetric performance test

This sensor was applied to detect BPA. The intensity of the red color increased linearly with BPA concentration, as illustrated in Figure 4. To confirm that the colorimetric sensor is capable of responding proportionally to the change in BPA concentration, a linearity experiment was performed. An excellent linearity indicates that the sensor's response is linearly proportional to the concentration of BPA in a certain range, which provides the sensor with precision performance. As the results indicate a linear relationship with $R^2 = 0.9492$, the fabricated colorimetric sensor shows a satisfactory linear response towards the detection of BPA in the examined concentration range, and its LOD and LOQ were 0.29 mmol/L^{-1} and 0.98 mmol/L^{-1}, respectively.

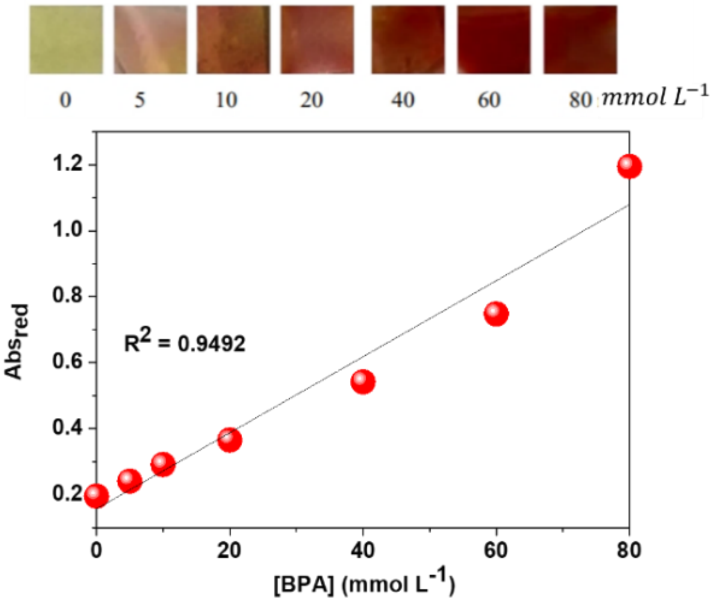


Figure 4. Linearity test of the developed paper-based sensor in various concentrations of BPA.

Validation of the fabricated paper-based sensor devices was necessary with real samples. In this work, the reported sensor was used to measure the migration of BPA from drinking bottles. The drinking bottles were pre-warmed directly under full sunlight for 0–7 days, with an average of 8 hours/day as shown in Figure 5 (stage 1).

The results of the tests indicate that during storage, BPA originating from the plastic matrix may migrate into drinking water during the heating process. The color outcomes indicate a linear correlation between the number of heating days and the browning degrees.

The precision test for sample measurements after one day of heating was analyzed to evaluate the precision of the measurement process, which was excellent since the %RSD value obtained was lower than 2% (approximately 0.62%). Accuracy was also evaluated by adding 10 and 20 mmol/L^{-1} BPA standards to the sample after 7 days of incubation. The precision was then reported as % recovery (%R). The experimental results (see Table 1) show that the accurate measurement range is 100.87–107.39%. This value is slightly beyond AOAC's limitation for accuracy (95–105%) of such methods, but still within the tolerance limit; thus, the determination can be considered accurate.

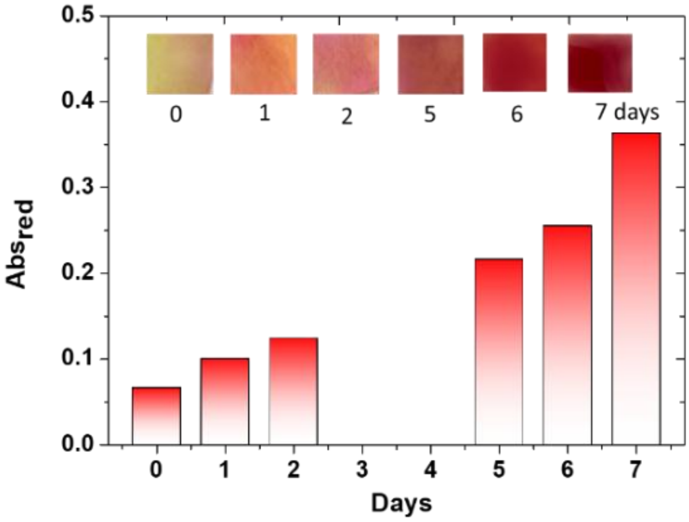


Figure 5. Measurement of BPA migration in exposed drinking bottle samples using paper-based sensors.

Although filter paper-based sensors for Bisphenol A (BPA) detection offer several advantages, such as ease of use, low cost, and visual detection capability through color change, these sensors still have some limitations that must be considered. One of the main limitations is the sensor's selectivity to BPA in the presence of other compounds with similar chemical structures, such as phenolic compounds (e.g., phenol, nonylphenol, or chlorophenol). The presence of these compounds in the sample matrix can cause signal interference, as they can also react with chromogenic reagents such as 4-AAP, producing similar color changes, thus reducing the accuracy of specific detection of BPA. In addition, sensor stability is also a consideration. Paper-based sensors generally have a limited shelf life, especially when stored in high-humidity conditions or exposed to direct light. Degradation of the active material or coloring reagents can affect the sensitivity and reproducibility of the sensor over time.

Table 1. Accuracy test of sample analysis.

Sample Days	Abs$_{ref}$	C found (mmol L^{-1})	C$_{std}$ added (mmol L^{-1})	%R
7	0.363	17.921	0	
	0.479	20.009	10	100.87
	0.610	39.400	20	107.39

CONCLUSION

Paper-based sensors for easy and rapid detection of BPA were developed and employed. The sensor fabricated in this study demonstrated excellent detection stability of BPA applied to drinking water bottles, and the sensitivity was high (LOD = 0.29 mM). The recoveries of BPA from tap water were in the range of 100.87–107.39% with the precision of %RSD < 2%, and these results indicated that BPA could still migrate from drinking water bottles due to long-term storage. The present study could be developed as a field analysis to determine BPA in samples with few low interferences. Although this sensor is promising as a fast and easy screening tool, advances are required to enhance its selectivity, stability, and suitability for different environmental/sample matrices.

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