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Original Article

Paper-based Microfluidic Device for Bisphenol A Based Chemical Reaction and Image Analysis

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Abstract Bisphenol A (BPA) is a representative xenoestrogenic endocrine disruptor that is widely used in consumer products and remains in wastewater. A simple detection tool is urgently needed that can be used with the naked eye for environmental monitoring in situ. Therefore we studied modification methods of phenol and ferric reagents on a paper-based microfluidic device. The reaction between BPA and ferric reagent mixtures was examined using two types of ferric reagent mixtures (ferric chloride/ferricyanide and ferric nitrate/ferricyanide) at various ferric reagent mixture ratios (1 : 9, 3 : 7, 5 : 5, 7 : 3 and 9 : 1) and concentrations (BPA 100, 300, 500 and 1000 μg/mL and 1-5% ferric reagent mixtures). Verification of this paper-based microfluidic device was analyzed with a UV spectrophotometer.

In addition, the changing color of the BPA reaction was demonstrated using histograms of the image statistics including the hue, saturation and value (HSV) analysis. Of the total BPA reactions, the optimal condition was identified as 1% ferric reagent mixture (5 : 5 ratio) and 5 μL of BPA loaded onto the paper-based microfluidic device. Moreover, the BPA detection abilities of ferric chloride/ferricyanide and ferric nitrate/ferricyanide were similar to the changing images on the paper-based microfluidic device. The BPA paper-based microfluidic device is expected to be applied in situ and in factories as a low-cost, portable, simple and rapid detection system.

Keywords: Paper-based microfluidic device, Bisphenol A, Ferricyanide, Ferric chloride, Ferric nitrate

Introduction

Plastic materials containing bisphenol A (BPA) are widely used in products such as food containers, bottles (i.e., epoxy resins and polycarbonate plastics), toys, thermal paper receipts, safety equipment, medical devices and electronic equipment[1-3]. Alternatively, the toxicity of BPA is lower than that of many other chemicals and remains controversial regarding its impact on human safety and the environment. The most important problem with BPA is its role as an endocrine disruptor due to its xenoestrogen (e.g., estrogen-17-β-estradiol) status, acting as an estrogen mimicker in the human body[1].

Some studies have recently reported that BPA induces adult diseases (e.g., obesity, diabetes and heart diseases)[4-7] and several toxicities (e.g., carcinogenicity, teratogenicity, immunotoxicity, mutagenicity hepatotoxicity and neurotoxicity)[8-13]. In addition, the ecological concerns of BPA are rooted in it major endocrine disruptive effects (e.g., abnormalities, metabolism, physiological function and bioaccumulation)[14-18] on aquatic organisms. Similarly, the US Environmental Protection Agency (US EPA) noted that BPA exposure has adverse effects on humans and the environment (e.g., sensitive aquatic organisms)[19].

In particular, BPA in applicable manufacturing factories results in worker exposure levels higher than

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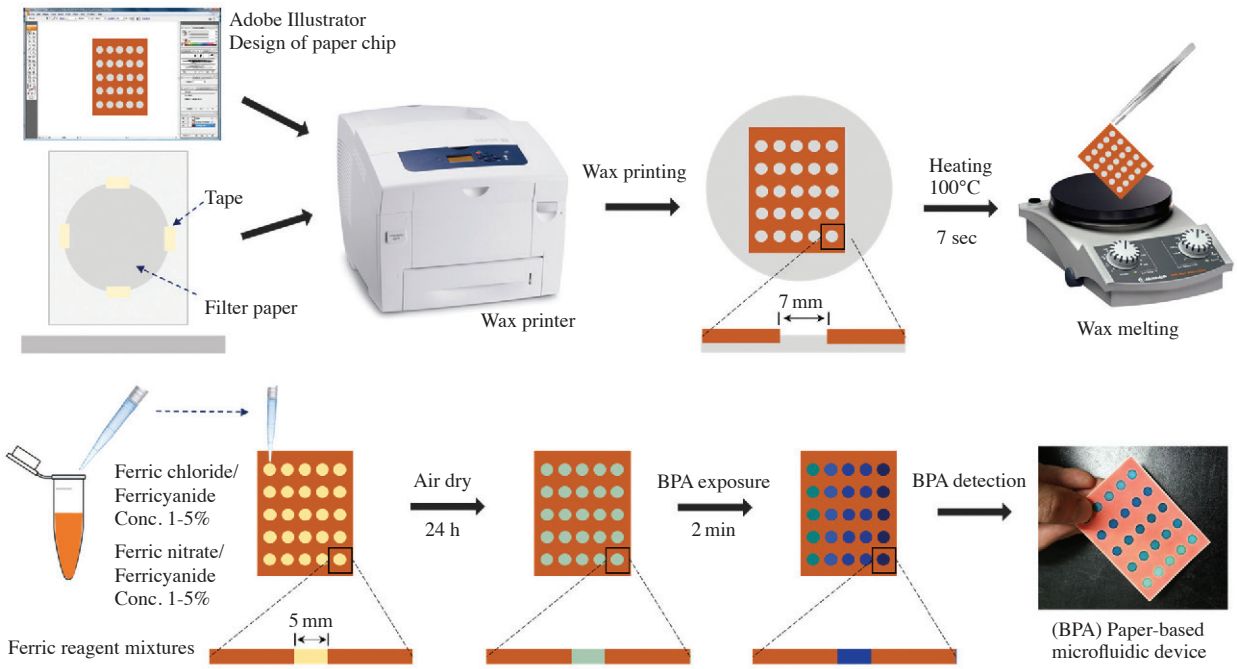

Figure 1. Schematic process of the paper-based microfluidic device for BPA detection.

those in the non-occupational population and also has an adverse effect on occupational diseases[19-22]. To this end, factory emissions of bisphenol analogues from wastewater treatment plant (WWTP) sludge in Korea and China were previously investigated[23,24].

BPA analyses often use high pressure liquid chromatography (HPLC) and gas chromatography (GC), both of which have good abilities detection, but cannot be used in situ.

A simpler method for BPA detection is based on absorbance through a ferric chloride/ferricyanide reaction using a UV-spectrophotometer, which also takes a long time and has restrictions on measurement[25].

The ferric chloride ($FeCl_3$) reaction with the phenol of BPA occurs as −OH radical catalysis bonding of the phenol, wherein the phenol appears as a dark blue color due to the condensation product of the ferric reagents[26]. The phenol of BPA could react with ferric nitrate/ferricyanide, and the color change is very useful, enabling BPA detection with the naked eye. However, these reactions have not been previously reported.

In this study, we designed a paper-based microfluidic device for BPA detection under optimal conditions using two types of ferric reagent mixtures. BPA detection was conducted via direct visualization. In addition, we compared BPA detection quality using the optimal color of ferric reagent mixtures I and II (ferric chloride/ferricyanide and ferric nitrate/ferricyanide, respectively).

Results and Discussion

Design of the Paper-based Microfluidic Device for BPA Detection

An advanced paper device can be easily formed based on hydrophilic and hydrophobic barriers using a previously described wax printing technique[27]. The paper-based microfluidic device was processed for BPA detection (Figure 1). Appropriate holes (reagent zones) present at regular intervals were designed using Adobe Illustrator CS5 software, and the filter paper for the paper device (Advantec No. 1, 150 mm in diameter) was fixed on A4-sized printer paper (210 mm in width × 297 mm in height) and then printed. The paper device holes were printed on 7-mm-diameter and then the wax melting process was conducted at 100°C for 7 second. The diameter of the paper device holes after wax melting was 5-mm, which proved to be the optimal hole size in our pretesting.

According to Mayani, S.V. *et al.*[28], the phenol in water is present as phenol analogues from various oxidative reaction pathways. The BPA reactions of oxidative catalysis are the ferric chloride ($FeCl_3 \cdot 6H_2O$) and ferric nitrate ($Fe(NO_3)_3 \cdot 9H_2O$) reactions. In order to produce strong reactions with BPA, an oxidant of po-

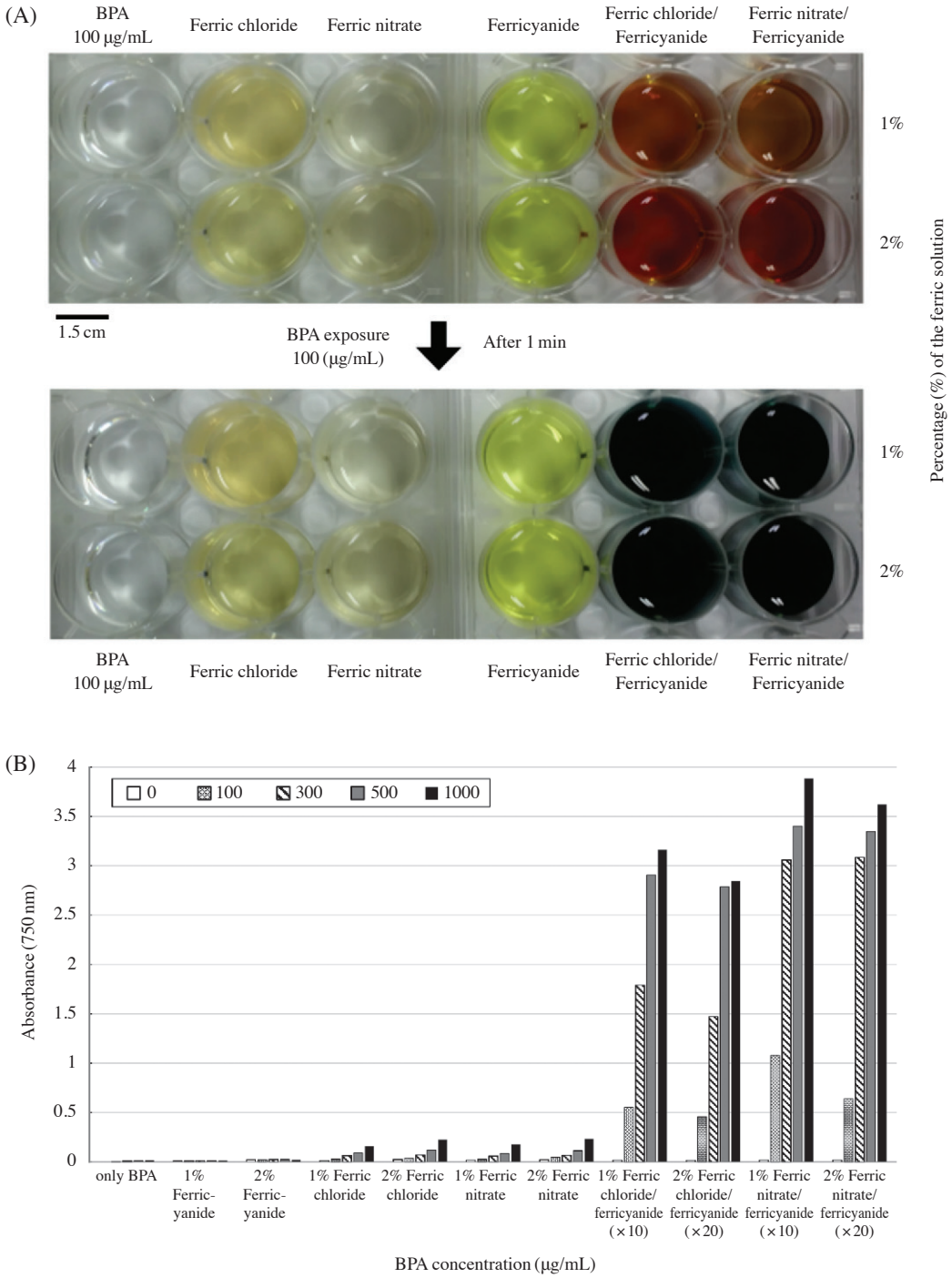

Figure 2. BPA reaction in solution: (A) color change images, and (B) UV spectrophotometry absorbance under different ferric reagents conditions. These results were obtained using dilutions (×10, ×20) of the stock solution.

tassium ferricyanide ($K_3[Fe(CN)_6]$) was added to the ferric reagents. The paper-based microfluidic device was air dried for one day with 5 μL loading volumes of ferric reagent mixture I (ferric chloride/ferricyanide) and ferric reagent mixture II (ferric nitrate/ferricyanide).

Optimal Ferric Reagent Mixture Conditions

Both ferric reagent mixtures rapidly changed from a

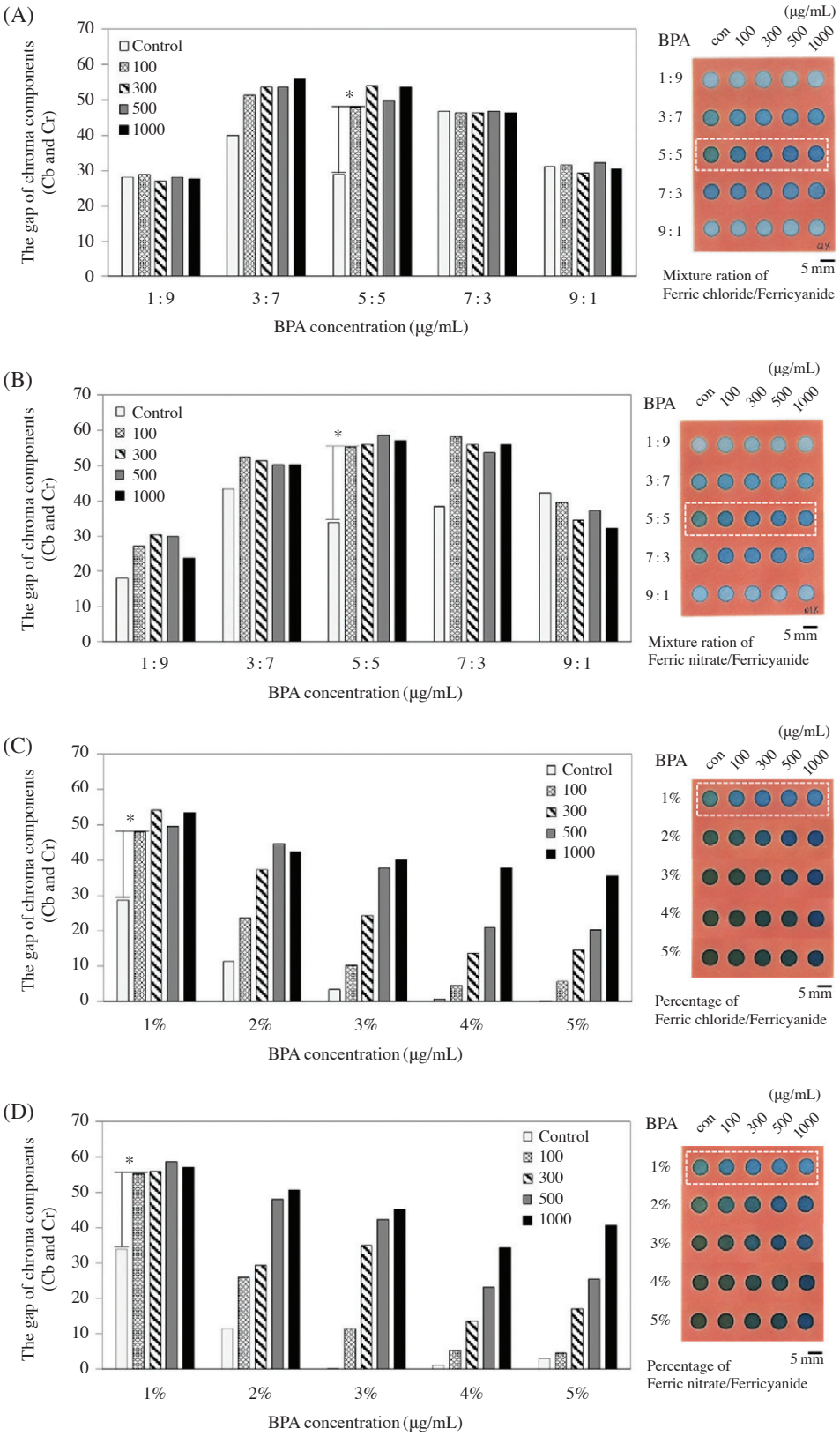

Figure 3. BPA reactions according to ferric mixture ratios (1 : 9, 3 : 7, 5 : 5, 7 : 3 and 9 : 1) and ferric reagent mixtures (1-5%) of (A, C) ferric chloride/ferricyanide and (B, D) ferric nitrate/ferricyanide.

dark orange to a blue color with exposure to 100 μg/mL BPA in solution, while unmixed ferric reagents (i.e., ferric chloride, ferric nitrate and ferricyanide) did not demonstrate such a change (Figure 2A). The color difference between ferric reagent mixture I and II upon exposure to BPA could not be distinguished with the naked eye. Moreover, there was no discernable difference in color between the 1% and 2% ferric reagent mixture solutions. In UV-Vis spectrophotometry, the absorbance of ferric reagent mixture II (ferric nitrate/ferricyanide) was higher than that of ferric reagent mixture I (ferric chloride/ferricyanide) (Figure 2B).

BPA detection via a paper device was optimized by examining various ferric reagent mixtures (1-5%) and ferric reagent mixture ratios (1 : 9, 3 : 7, 5 : 5, 7 : 3 and 9 : 1) at various BPA concentrations (100, 300, 500, and 1000 μg/mL; Figure 3A-D).

The BPA reactions in the ferric reagent mixtures could be analyzed after 2 minutes, but after 24 hours or more it was not possible to detect BPA exposure because of color changes that occurred to the paper for the control and test samples. For quantitative assessment of the ferric reagent mixtures, color distinction was observed compared to control 1% ferric reagent mixtures for both ferric reagent mixture I and II. An increase in the percentage (%) of the ferric reagent mixture produced a color change in the paper to a dark green, where the papers exposed to BPA were difficult to distinguish from the control. The papers turned various colors at the different mixture ratios (yellowish green 1 : 9; light~dark green 3 : 7, 5 : 5, 7 : 3; faded aqua 9 : 1). The color of the desired ferric reagent mixture ratio (5 : 5) was green. Although lower concentration than 100 μg/mL condition was shown the color change it couldn't distinguish control with naked eye. Therefore, the detection able the lowest BPA concentration on this paper chip device was 100 μg/mL. As a result, the optimized conditions were 1% ferric reagent mixtures and a ferric reagent mixture ratio of 5 : 5 (Figure 3).

As shown in Figure 4, the BPA exhibited a blue color with changes in the BPA concentrations (100, 300, 500, and 1000 μg/mL), but the paper colors were distinguished by loading volume (5 and 10 μL) of BPA in that the color intensity of the 10 μL by loading volume (5 and 10 μL) a color change in the paper to BPA groups (100 μg/mL) of 10 μL loading volume were difficult to distinguish from the controls in both ferric reagent mixture I and II, because BPA detection was only possible for a BPA loading volume of 5 μL.

Image Statistics and Analysis

To demonstrate the changing color of the BPA reaction with ferric reagent mixture, the hue histograms of the image statistics were analyzed. Among the three conditions, the BPA reaction of 1% ferric reagent mixture (5 : 5 ratio) and 5 μL BPA loading volume produced a green to blue color change (Figure 5). Specifically, the hue histograms of the 1% ferric reagent mixture on exposure to 100 μg/mL BPA were distinguished by a shifted range compared to those under other conditions. The higher BPA concentration caused the hue histogram distribution to shift toward the right (away from green and towards blue), suggesting that a 5 μL BPA loading volume and 1% ferric reagent mixtures (indicated by the green line) were the optimal conditions for the test. As compared in the HSV (i.e., hue, saturation and value) analysis, the optimal condition (1% ferric reagent mixture and 5 μL of BPA loading volume) was clearly illustrated based on the difference between the control and BPA exposure group compared to those of the other conditions (Figure 6). The sample loading volume of 5 μL, 1% ferric reagent mixtures I and II in the HSV range were distributed closer than others (sample volume of 10 μL, 1% ferric

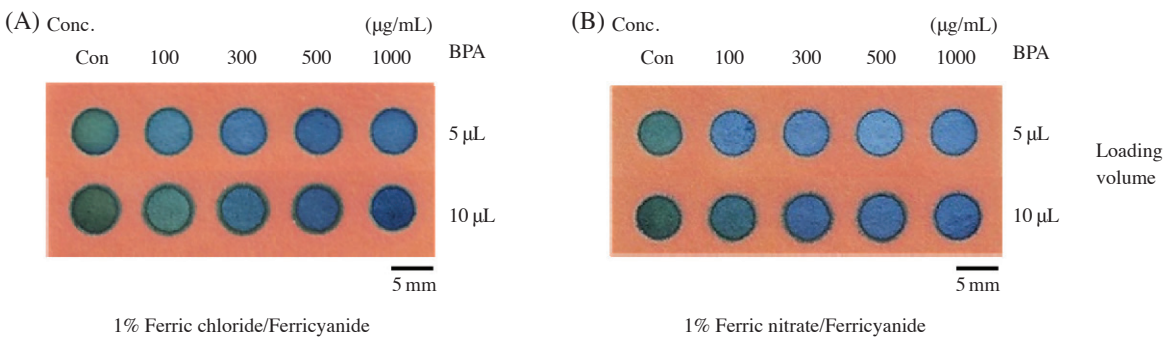

Figure 4. BPA reactions of (A) ferric chloride/ferricyanide and (B) ferric nitrate/ferricyanide by 1% ferric reagent mixture (5 and 10 μL of BPA loading volume).

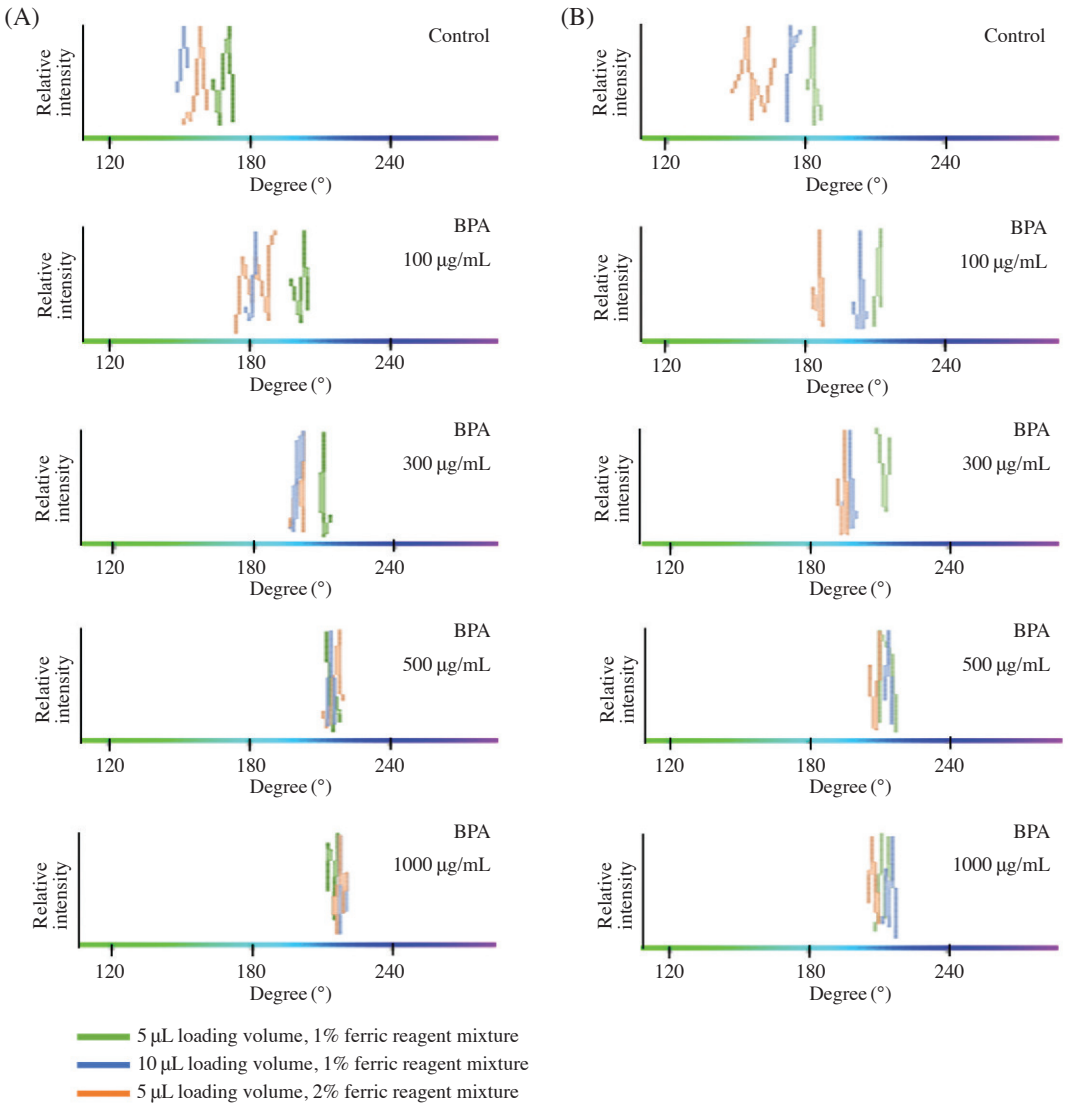

Figure 5. Hue histograms of image statistics according to the color change caused by BPA reactions of (A) ferric chloride/ferricyanide and (B) ferric nitrate/ferricyanide. The green line represent a 5 μL of BPA loading volume and 1% ferric reagent mixtures, the blue line is a 10 μL of BPA loading volume and 1% ferric reagent mixtures and the orange line is a 5 μL of BPA loading volume and 2% ferric reagent mixtures.

reagent mixtures I and II; sample volume 5 μL, 2% ferric reagent mixtures I and II). The color difference was clear, suggesting the usefulness of this method for direct visualization BPA detection using the paper device.

Conclusions

A paper-based microfluidic device was used for BPA detection. Optimal conditions were evaluated using two types of ferric reagent mixtures (i.e., ferric chloride/ferricyanide and ferric nitrate/ ferricyanide) under various mixture ratios and concentrations. As a result, BPA detection was clearly observed in the 1% ferric reagent mixture (5 : 5 ratio) at a 5 μL BPA loading volume. In addition, the colors of the BPA reactions were similar for both ferric reagent mixture I (ferric chloride/ferricyanide) and ferric reagent mixture II (ferric nitrate/ferricyanide) with naked eye. However, ferric reagent mixture II is more preferable than ferric reagent mixture I on the gap of chroma components by the YCbCr model.

The proposed paper-based microfluidic device for

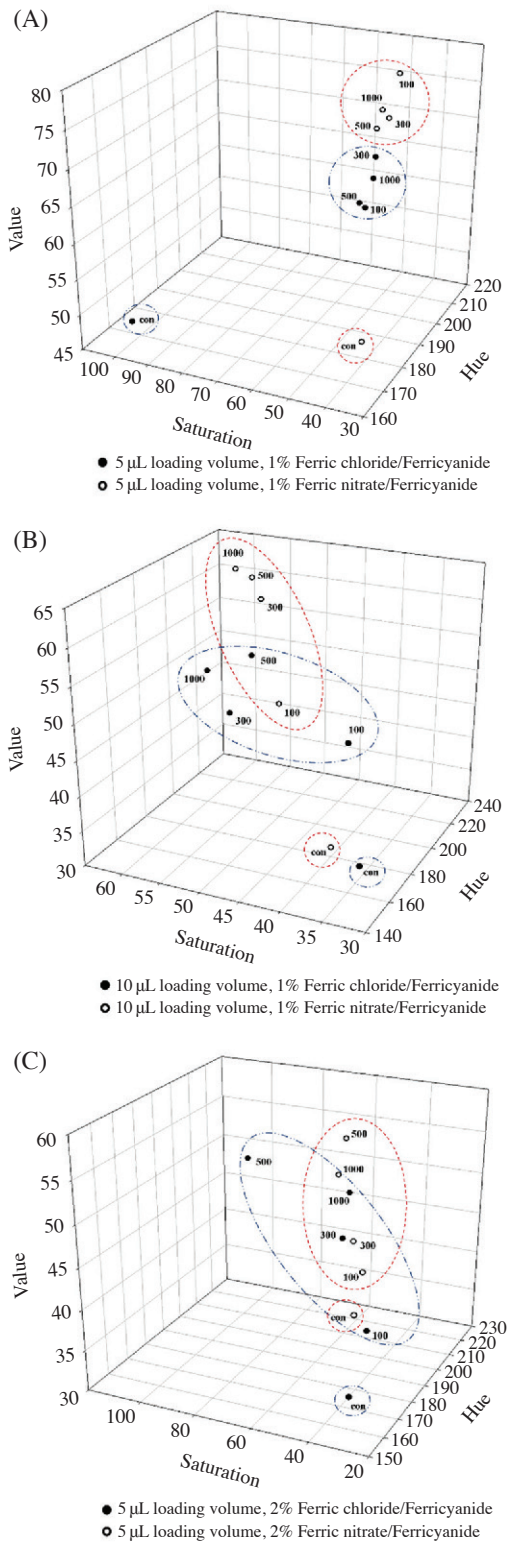

Figure 6. Comparative analysis of BPA loading volume and percentage (%) of ferric reagent mixtures by (A) 5 μL BPA loading volume and 1% ferric reagent mixtures, (B) 10 μL BPA loading volume and 1% ferric reagent mixtures and (C) 5 μL BPA loading volume and 2% ferric reagent mixtures.

BPA detection is a low-cost, portable, simple and rapid system that can be applied *in situ*.

Materials and Methods

Design of the Paper-based Microfluidic Device

Hydrophilic reagent zones and hydrophobic barriers were made on paper using a wax printing technique on qualitative filter paper (150-mm diameter, No. 1, circle type, Advantec Co., Japan)[28].

The paper-based microfluidic device was designed using Adobe Illustrator CS5 software and a wax printer (Fuji Xerox ColorQube 8570, Fuji Xerox Co., Japan). The holes in the reagent zones of the paper were designed with a 7 mm diameter. After wax printing, the paper underwent a wax melting process at 100°C for 7 seconds using hot plate stirrers (Heidolph MR Hei-Standard Magnetic Stirrer, Heidolph Co., Germany), which resulted in a slight decrease in diameter of the reagent zones to 5 mm.

Preparation of Reagents

BPA was purchased from Sigma Aldrich (bisphenol A ≥99%, St. Louis, MO, USA). Phenol was purchased from DAEJUNG (99%, Korea) as a comparison material. The reagent mixtures for BPA and phenol were purchased as ferric chloride (iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) 98%, DAEJUNG Co., Korea), ferric nitrate (iron (III) nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$) 98%, DAEJUNG Co., Korea) and ferricyanide (potassium ferricyanide $K_3[Fe(CN)_6]$ 98%, DAEJUNG Co., Korea).

Optimal Conditions for BPA Detection of Ferric Chloride, Ferric Nitrate and Ferricyanide

The reagent mixtures were applied to the materials in two combinations: (i) ferric reagent mixture I (ferric chloride/ferricyanide) and (ii) ferric reagent mixture II (ferric nitrate/ferricyanide). Optimal conditions of the ferric reagent mixtures were selected by evaluating 1-5 %, combinations of ferric chloride/ferricyanide ($FeCl_3 \cdot 6H_2O : K_3[Fe(CN)_6]$) and ferricnitrate/ferricyanide ($Fe(NO_3)_3 \cdot 9H_2O : K_3[Fe(CN)_6]$) and mixture ratios of 1 : 9, 3 : 7, 5 : 5, 7 : 3 and 9 : 1 (v/v). The loading mixtures in the paper reagent zone were air dried for 24 hours. The BPA was examined at loading volumes of 5 and 10 μL of BPA concentrations (100, 300, 500 and 1000 μg/mL). The BPA reactions with the ferric reagent mixtures were selected to observe the most distinguishable concentration at 2 minutes. Additionally,

the phenol reactions were compared to BPA reactions under similar conditions.

Detection and Data Analysis

The color values were calculated using the YCbCr model by color difference between luminance (Y) and chroma components (Cb and Cr) from RGB (Red, Green, Blue) as per the following color values equations (Figure 3):

$$Y = 0.299R + 0.587G + 0.114B \quad (1)$$

$$Cb = 0.564\,(B - Y) \quad (2)$$

$$Cr = 0.713\,(R - Y) \quad (3)$$

Ferric reagent mixtures and BPA reactions were converted to a blue color product. Quantitative measurements were analyzed by absorbance using a UV-spectrophotometer (HS-3300, HUMAS, Co., Korea). The absorbance was measured at 750 nm for 1 minute under various conditions of the substances (i.e., BPA, ferric chloride, ferric nitrate and ferricyanide) and ferric reagent mixtures (i.e., ferric chloride/ferricyanide and ferric nitrate/ferricyanide) for each of the 1 and 2% concentrations. BPA reactions of the reagent mixtures were strong, so the stock was diluted by 10-fold (1% reagent mixtures) and 20-fold (2% reagent mixtures). Other samples were measured as undiluted solutions.

Image analysis of the changed color was verified by descriptive color statistics using a color summarizer tool (http://mkweb.bcgsc.ca/color_summarizer/), and the results were displayed using SigmaPlot 12.0 software through the color codes of color picker tool (http://www.colorpicker.com/).

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