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Development of the simultaneous colorimetric enzymatic detection of sucrose, fructose and glucose using a microfluidic paper-based analytical device

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ABSTRACT

Herein, we propose the first fabrication of a microfluidic paper-based analytical device (μPAD) as a simple, rapid and selective method for the simultaneous detection of sucrose, fructose and glucose for quality monitoring in the sugar industry. Multiplex detection of the three analytes was achieved by three detection zones designed on a single device. Each detection zone on the μPAD was modified with enzymes, chromogenic reagents and specific substrates. The enzymatic reactions between the specific enzymes, chromogenic reagents and their corresponding substrates yielded different colored products: brown for sucrose, blue for fructose and pink for glucose. All the reaction conditions were evaluated. The limits of detection were determined to be 0.9 mM, 0.8 mM and 0.4 mM for sucrose, fructose and glucose, respectively. The proposed μPAD successfully monitored sucrose, fructose and glucose within 11 min using a signal device and obtained a spike recovery of 100–103% for sucrose, 98–103% for fructose, and 100–102% for glucose. The μPAD was applied to detect sucrose, fructose and glucose in real samples, and the results were in good agreement with those obtained using high-performance liquid chromatography (HPLC). The proposed μPAD provides several advantages, including ease of use, fast results and low cost. Therefore, the developed μPAD is suitable for application in the sugar industry for monitoring sucrose, fructose and glucose in parallel.

1. Introduction

Sugar is produced from sugarcane, sugar beets, palm trees and maple trees [1–3]. Most sugar worldwide is produced from sugarcane [4,5]. Therefore, sugarcane is an important resource and serves as the world's primary source for industrial sugar [6]. Sugar quality depends on the sucrose content [7,8]. During the sugar manufacturing process, the conversion of sucrose to fructose and glucose decreases this quality [9,10]. Therefore, monitoring sucrose, fructose and glucose is quite important for quality control in sugar manufacturing. Many methods have been used to measure sucrose, fructose and glucose, including polarimetry [11–13], spectroscopy [14–16], high-performance liquid chromatography (HPLC) [17–21], and optical [22–25] and electrochemical enzymatic methods [26–28]. Polarimetry is generally used to detect sucrose in sugar factories, but this method requires sample preparation and lacks sensitivity. Spectroscopy is sensitive but has limitations, such as the need for expensive instrumentation and the inability to detect sucrose, fructose and glucose simultaneously. Although simultaneous detection is possible with HPLC, this method requires expensive instruments and specialized technicians, and it is time-consuming. Recently, optical methods have been used for sugar detection. This assay is based on the synthesis of gold nanoparticles in the presence of glucose as a reducing agent. However, this method is non-selective towards other reducing compounds. In addition, electrochemical enzymatic methods continue to be used for sugar analysis due to their high sensitivity and selectivity. These methods have some drawbacks, such as the need for several steps, skilled personnel and expensive instruments. In comparison with the methods mentioned above, microfluidic paper-based analytical devices (μPADs) detect multiple targets simultaneously using a single strip, which increases the detection speed and decreases the cost [29–33]. Such devices provide many advantages over traditional methods, such as faster analysis time, lower reagent and sample consumption, lower cost, simple operation and better suitability for on-site detection. However, the μPAD applications have some limitations regarding sensitivity, accuracy, and mass production [34,35]. Since the early 1990s, biosensors have been developed for applications in environmental [36–40], medical [41–43], biotechnological and industrial fields due to their sensitive, selective

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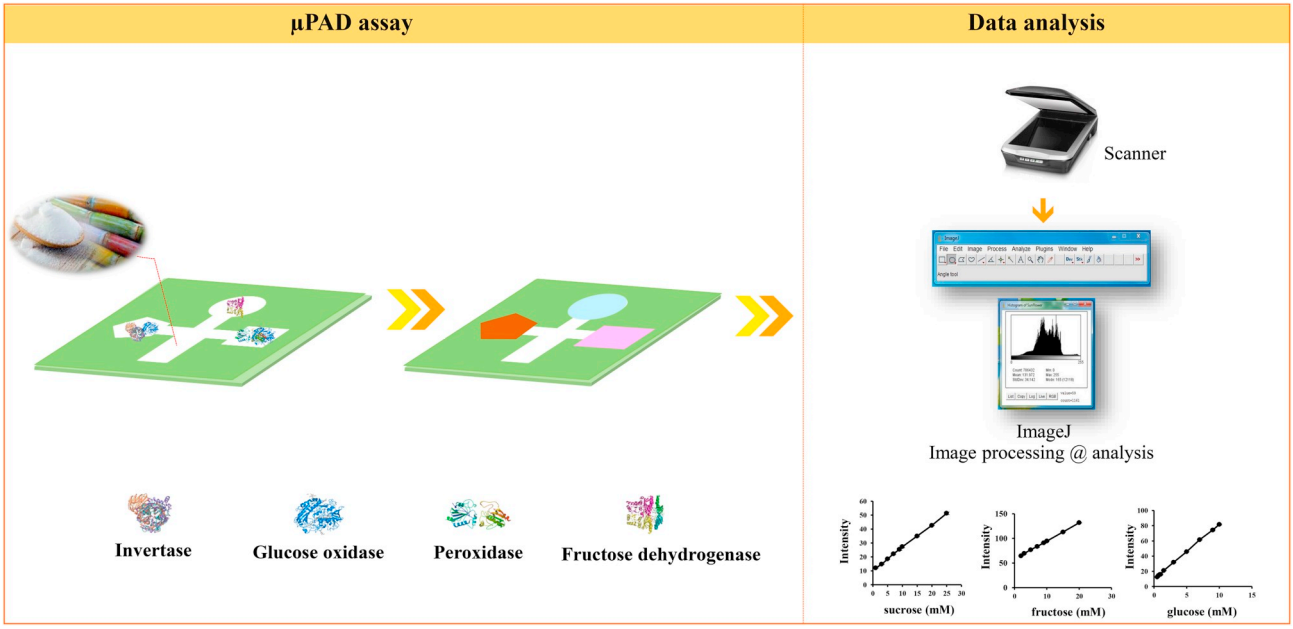

Scheme 1. Principles of the µPAD-based simultaneous analysis of sucrose, fructose and glucose detection.

and rapid methods. Now, µPADs are becoming the preferred devices for biosensors because of their many advantages.

Here, we describe the simultaneous detection of sucrose, fructose and glucose using a single paper-based device, as shown in Scheme 1. We achieved simultaneous detection by distributing samples from the sample zone (S) to three detection zones (A, B and C) for three assays (Fig. 1A). Each detection zone was modified with chemicals that caused specific enzymatic reactions, as well as their substrates, to form colored products. Then, we obtained photographs of the resultant colors using a scanner and evaluated the average color intensities of the detection zones using ImageJ software to calculate the concentration of each analyte.

To the best of our knowledge, this is the first simultaneous detection of sucrose, fructose and glucose on a µPAD based on the biosensor

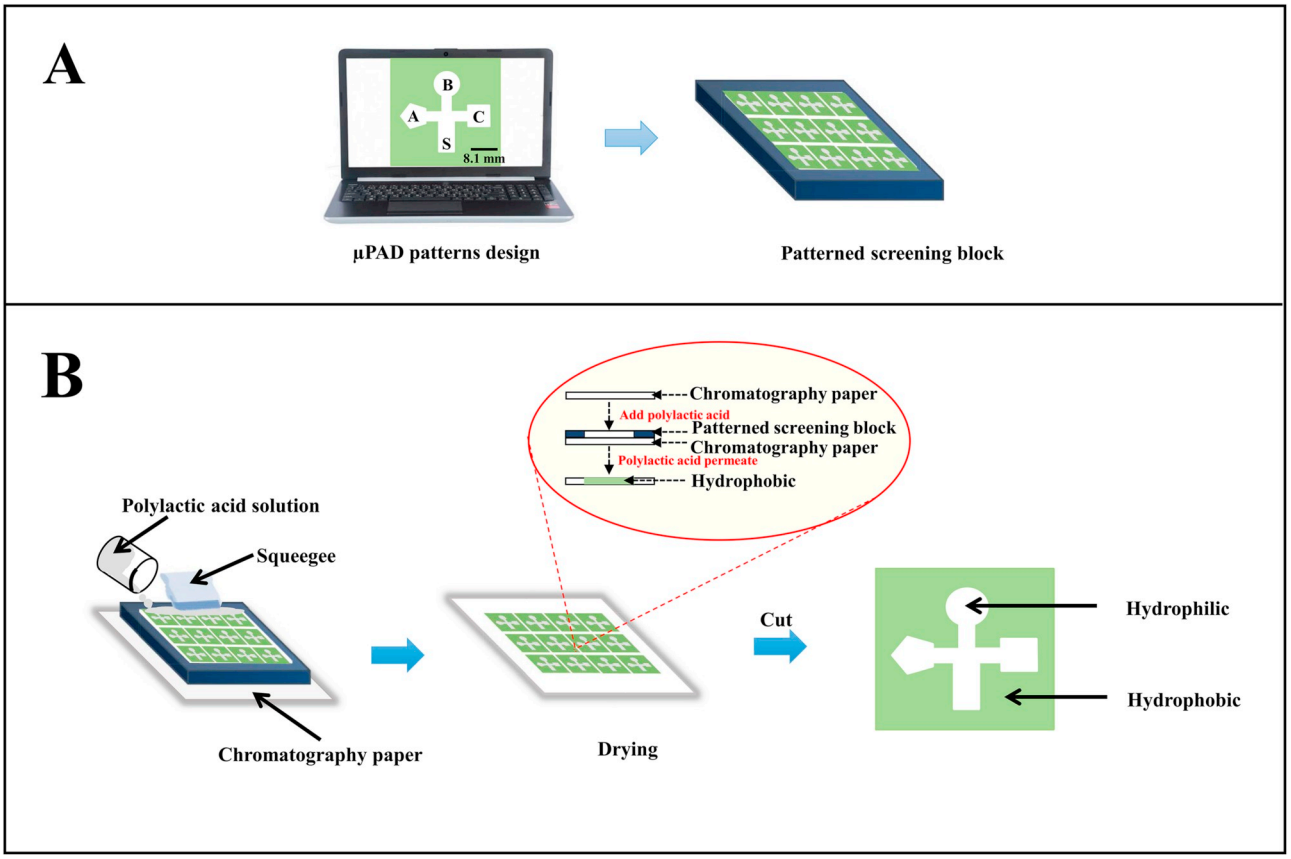

Fig. 1. Design of the µPAD for sucrose, fructose and glucose detection on one-step (A). The µPAD production process using the polymer screen-printing technique (B).

technique. The proposed analytical device detects sucrose, fructose and glucose in real samples within 11 min using a simple, portable and inexpensive device.

2. Materials and methods

2.1. Chemicals and instruments

Invertase from baker's yeast (*S. cerevisiae*) ≥300 unit/mg solid (EC 3.2.1.26), glucose oxidase from *Aspergillus niger* 163,314 unit/g (EC 1.1.3.4), peroxidase from horseradish 327 units/mg (EC 1.11.1.7), D-fructose dehydrogenase from *Gluconobacter* sp. 162 units/mg (EC 1.1.99.11), sucrose, D-fructose, *o*-dianisidine and 4-aminoantipyrine were purchased from Sigma-Aldrich (St Louis, USA). Phenol was obtained from Fisher scientific (Leicestershire, UK). D-glucose, sodium dodecyl sulfate and potassium ferricyanide were purchased from Ajax Finechem (Taren Point, Australia.). Iron (III) chloride 6-hydrate pure was purchased from PanReac AppliChem (Barcelona, Spain). Polylactic acid was purchased from Nature Works LLC. (Minnetonka, USA). All other chemicals used were analytical grade. Sugar and sugarcane juice samples were collected from Thai Sugar Industry (Rajburi, Thailand). Whatman Chromatography paper grade 4 was obtained from GE Healthcare Life Sciences (Buckinghamshire, UK). A block screen was fabricated by Chaiyaboon (Bangkok, Thailand). A scanner (CanoScan Lide 120) was acquired from Canon Marketing (Thailand) Co., Ltd. (Bangkok, Thailand). The HPLC apparatus consisted of a Waters 2690 Alliance HPLC with refractive index detector and Shodex Asahipak NH2P-50 4E column (Waters, Milford, MA, USA).

2.2. Design and fabrication of the μPAD for simultaneous assay of sucrose, fructose and glucose

The μPAD patterns were designed with Adobe Illustrator and then used to create a screen. A μPAD consists of a central sample loading zone connected by microchannels to three detection zones, as shown in Fig. 1A. Three detection zones were designed for the simultaneous assay of multiple analytes: zone A detected sucrose, zone B detected fructose and zone C detected glucose. The green part of the pattern was used to generate a hydrophobic area on the paper, and the white areas indicated the hydrophilic area.

The pattern screen was made from 100 mesh polyester with a thickness of 290 ± 10 μm on a wood frame (purchased from a local screen-printing printing shop in Bangkok, Thailand). Fig. 1B shows the μPAD fabrication process. To create a hydrophobic area on the μPAD, the patterned screen was placed on chromatography paper. A 10% w/v polylactic acid solution, prepared by dissolving polylactic acid in dichloromethane, was poured over the screen. Subsequently, the polylactic acid solution was squeezed through the mesh of the screen to create a hydrophobic area on the chromatography paper. Then, the chromatography paper was separated from the screen and was dried at room temperature. Finally, the patterned paper was cut into 3.5 cm × 3.5 cm squares to use for sucrose, fructose and glucose detection.

Sucrose was detected in zone A using a mixture of 1 μL of 60 U/mL invertase, 1 μL of 82 U/mL glucose oxidase, 1 μL of 20 U mg/mL peroxidase, and 3 μL of 3.0 mM *o*-dianisidine. To detect fructose, 4 μL of 240 U/mL fructose dehydrogenase in pH 4.50 McIlvaine buffer, 1 μL of 20.0 mM potassium ferricyanide and 1 μL of 0.01 M ferric chloride were added to zone B. For the glucose test, 6 μL of a mixture of 0.8 U/mL glucose oxidase, 2 U/mL peroxidase, 5.0 mM phenol and 2.5 mM 4-aminoantipyrine was applied to zone C. Next, a 30 μL sample or a standard solution (sucrose, fructose and glucose) was introduced to the sample zone. Then, all the enzymatic reactions on the detection zones proceeded for 10 min. After the color developed, the μPAD dried for 1 min at room temperature and was imaged using a scanner. ImageJ software (The National Institutes of Health, Bethesda, MD, USA) was used to evaluate the color intensity using the blue channel for sucrose, the red channel for fructose and the green channel for glucose. The average intensities of zones A, B and C were used to assess the concentrations of sucrose, fructose and glucose, respectively. The quantitative detections of sucrose, fructose and glucose in real samples were calculated from the regression equations of their calibration curves.

2.3. Sample preparation

For the sugar sample, a 0.1 g of sample was weighed and then dissolved in 1 mL of distilled water. For sugarcane juice A and B, 100 μL of each sample was diluted with distilled water. The sample solution was diluted with distilled water to match the detection range of the calibration curve prior to the analysis. Before the analysis, the sugar sample was diluted 30 times, and the sugarcane juice samples were diluted 19 times with distilled water. The analysis for each sample was carried out in triplicate.

2.4. The detection of sucrose, fructose and glucose using HPLC as a reference method

The quantitative determination of the sucrose, fructose and glucose in real samples was performed under the following conditions: a Shodex Asahipak NH2P-50 4E (4.6 mm ID × 250 mm) column; a column temperature of 30 °C; a mobile phase of 75% acetonitrile and 25% water; and a flow rate of 1.0 mL/mL. The detection was performed using an RID-10A refractive index detector.

3. Results and discussion

3.1. Fabrication of the μPAD

The μPAD fabrication was performed in one-step (Fig. 1B), and the polylactic acid solution was applied through the patterned screen to create hydrophobic barriers on the μPADs. The polymer solution was passed through the patterned screen into the chromatography paper. After the solvent evaporated, hydrophobic regions were formed, which provided hydrophobic barriers and hydrophilic channels on the chromatography paper. The concentrations of the polylactic acid solution used were 1, 5, 10 and 15% w/v. Fig. 2 shows the top view of the μPADs after we applied methylene blue solution to indicate the hydrophilic regions. Concentrations lower than 10% w/v provided an incomplete three-dimensional hydrophobic barrier due to the reduction in the viscosity, and the permeability of the solution on the hydrophilic portion appeared to be insufficient. Polylactic acid at higher concentrations resulted in higher viscosity and did not penetrate the cellulose fibers of the chromatography paper. When a droplet of reagent was deposited on the paper, the solution penetrated the paper's inner layer. Therefore, we used a concentration of 10% w/v to fabricate the μPADs.

3.2. Principles of the μPAD-based sucrose, fructose and glucose detection

The simultaneous detection of sucrose, fructose and glucose was achieved via the cascades of enzymatic reactions shown in equations (1)–(6). Three enzymes were used to detect sucrose, one enzyme to detect fructose, and two enzymes to detect glucose.

The analysis of sucrose was achieved by using invertase, glucose oxidase and peroxidase for the catalytic reaction. Sucrose is decomposed into gluconic acid and hydrogen peroxide (H_2O_2) by invertase and glucose oxidase, and *o*-dianisidine$_{red}$ is then oxidized to brown *o*-dianisidine$_{oxi}$ by H_2O_2 in the presence of peroxidase [44].

$$\text{Sucrose} + H_2O \xrightarrow{\text{Invertase, Glucose oxidase}} \text{Gluconic acid} + 2H_2O_2$$
$$H_2O_2 + \text{o-dianisidine}_{Red} \xrightarrow{\text{Peroxidase}} \underset{\text{(brown)}}{\text{o- dianisidine}_{Oxi}} \quad (1)$$

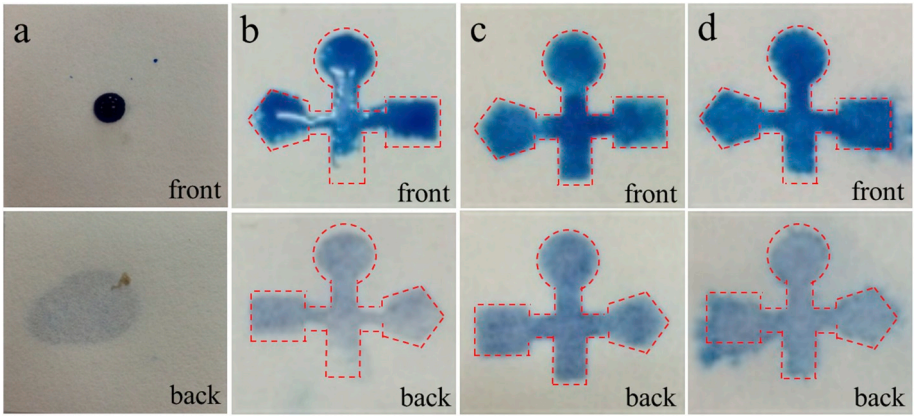

Fig. 2. Experiment polylactic acid concentrations used to create the μPAD: (a) 1% (w/v), (b) 5% (w/v),(c) 10% (w/v) and (d) 15% (w/v).

For the fructose analysis, fructose dehydrogenase uses fructose and potassium ferricyanide as substrates to produce 5-keto-D-fructose and potassium ferrocyanide. The potassium ferrocyanide then reacts with ferric chloride, resulting in Prussian blue production causing a color change to blue [45]. When this equation (2) was create to pdf, it not complete. The product of first reaction (+ Potassium ferrocyanide) and the product of second reaction (Prussian blue) are not shown. Please, revise this equation.

$$\text{Fructose+Potassium ferricyanide} \xrightarrow{\text{Fructose dehydrogenase}} 5-\text{keto}-\text{D}-\text{fructose+Potassium ferrocyanide}$$
$$\text{Potassium ferrocyanide+Ferric chloride} \rightarrow \underset{\text{(blue)}}{\text{Prussian blue}} \quad (2)$$

Finally, for the glucose assay, glucose is oxidized by glucose oxidase to form gluconic acid and H_2O_2. The H_2O_2 then reacts with 4-aminoantipyrine to produce the pink color of quinoneimine dye [46].

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$
$$H_2O_2 + 4-\text{aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \underset{\text{(pink)}}{\text{Quinoneimine dye}} + 2H_2O \quad (3)$$

To demonstrate the feasibility of the proposed μPAD, the specific enzymes and substrates were dropped onto each detection zone in the presence of sucrose, fructose and glucose in the sample zone. The colorimetric results were captured with a digital camera, as shown in Fig. 3A. The μPAD yielded multiple colors for the three analytes. Sucrose produced a brown color at zone A, while fructose and glucose produced blue and pink colors at zone B and zone C, respectively. The color was analyzed using a scanner, and the color intensity (RGB) values were determined using ImageJ software. Fig. 3B shows the RGB intensities of sucrose, fructose and glucose. The blue channel provided the highest intensity for sucrose detection, and the red and green channels provided the best signals for fructose and glucose analysis, respectively. Therefore, the blue, red and green values were selected for the quantitative analysis of sucrose, fructose and glucose, respectively. The intensity signal was calculated from $I_B - I_S$, where I_B is the control value obtained by measuring the white filter paper (blank) and I_S the measured intensity value of the sample. The concentrations of all the analytes were calculated via the calibration curve of intensity versus analyte concentration. The concentration of sucrose was calculated by subtracting the glucose signal from the sucrose signal.

3.3. Study of the reagent volume and sample volume

The reagent volume in the detection zone is an important factor that influences reactions. The reagent and sample volumes were optimized using methylene blue solution. To study of the reagent volume, a series of 1, 2, 3, 4, 5, 6 and 7 μL of methylene blue solution were applied to the detection zone. As shown in Fig. 4A, volumes ranging from 1 to 5 μL were insufficient for the color to fully cover the detection zone. However, volumes greater than 6 μL caused the reagent to spread outside the detection zone. Thus, 6 μL was selected as the optimal reagent volume. To determine the sample volume required to reach the reagent zone and develop the color, various volumes of methylene blue were dropped onto the sample zone. As shown in Fig. 4B, sample volumes of 10–20 μL did not reach the detection zone. The sample with a volume of 30 μL successfully moved to the reaction zone, whereas volumes exceeding 30 μL caused the reagent to spread over the hydrophilic area. Therefore, the sample volume was fixed at 30 μL for the subsequent experiments.

3.4. Optimization of the experimental conditions

To achieve the best performance of the μPAD for detecting sucrose, fructose and glucose, the parameter effects on the analytical signal were

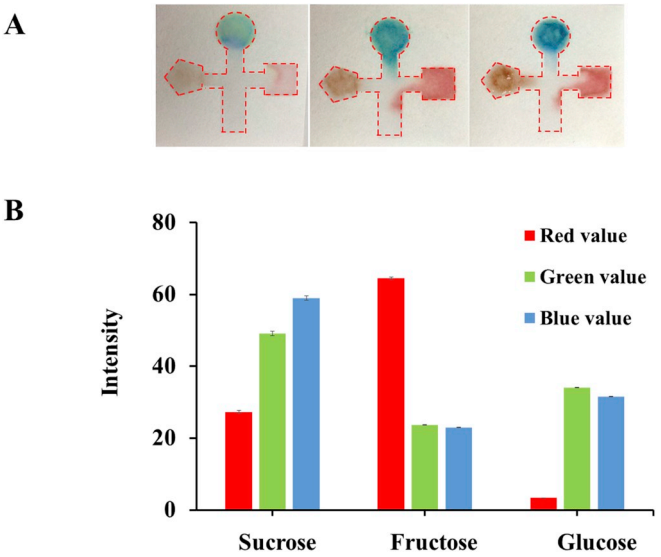

Fig. 3. Image of the μPADs used to detect sucrose (1, 10 and 25 mM), fructose (2, 10 and 10 mM), and glucose (0.5, 7 and 10 mM) (A). Quantitative RGB profiles of the three analytes (B).

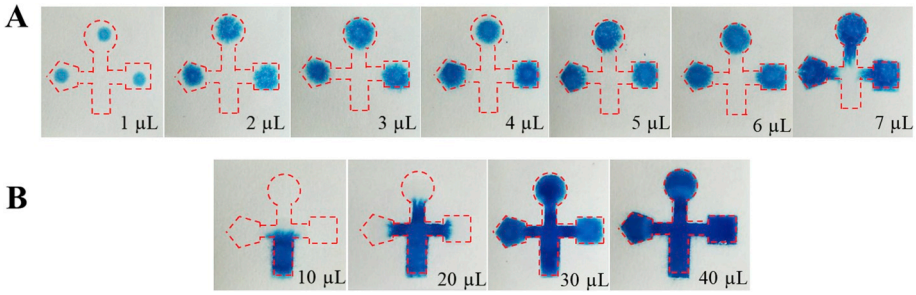

Fig. 4. Effects of reagent volume (A) and sample volume (B).

studied. All the experiments were performed in triplicate and averaged. The effects of the invertase, glucose oxidase and peroxidase concentrations played important roles. For the enzymatic reaction of sucrose, 60 U/mL invertase, 82 U/mL glucose oxidase and 20 U/mL peroxidase were chosen as the conditions for sucrose detection, because they provided the greatest color intensity (Fig. S1A, B and C). Fig. S1D shows that, as the concentration of *o*-dianisidine increased in the range in of 0.5–3.0 mM, the color intensity increased as a result of increasing o-dianisidone$_{oxi}$ products. With a further increase in the *o*-dianisidine concentration, the color intensity became saturated; a concentration of 3.0 mM was thus selected as the optimum condition. Subsequently, the pH value of each enzyme was optimized by varying the pH from 4.00 to 7.00. As shown in Fig. S1E, the highest color intensity was achieved at pH 5.50 (acetate buffer) for the invertase and glucose oxidase and pH 6.50 (phosphate buffer) for the peroxidase.

Several parameters, including the concentrations of fructose dehydrogenase, potassium ferricyanide and ferric chloride, allowed us to create a highly sensitive µPAD for the analysis of fructose. The fructose dehydrogenase concentration was optimized by testing three concentrations (80, 160 and 240 U/mL). However, concentrations higher than 240 U/mL could not be studied, because this was the original stock solution. A concentration of 240 U/mL was selected because it provided the highest intensity, as shown in Fig. S2A. Then, various concentrations of potassium ferricyanide were examined, and the results showed a complete reaction at a potassium ferricyanide concentration of 20 mM, which was considered an optimum condition (Fig. S2B). As shown in Fig. S2C, increasing the ferric chloride concentration also increased the color intensity, and there were no significant changes when the concentration of ferric chloride was increased higher than 0.01 M. Thus, a ferric chloride concentration of 0.01 M was selected.

Finally, several effects were evaluated, including the concentrations of the enzymes glucose oxidase and peroxidase and the concentrations of the substrates 4-aminoantipyrine and phenol. The concentrations of glucose oxidase and peroxidase were optimized on detection zone C. As illustrated in Figs. S3A–B, the optimized concentrations of glucose oxidase and peroxidase were 0.8 U/mL and 2 U/mL, respectively. At concentrations higher than the optimized value, the color intensity did not change significantly. The effect of the substrates 4-aminoantipyrine and phenol were studied. As shown in Fig. S3C, the color intensity increased as the concentration of 4-aminoantipyrin increased from 0.5 to 2.5 mM, reached peak intensity at 2.5 mM, and then became steady. Next, the phenol concentration, was investigated in the range of 2.0–7.5 mM (Fig. S3D). The intensity reached its maximum value at 5.0 mM. As a result, the substrate concentrations for 4-aminoantipyrine and phenol were optimized at 2.5 mM and 5.0 mM, respectively.

The reaction times for all three analytes were studied in the range of 1–30 min. Fig. S4 shows that the reactions were complete within 10 min for all three assays. Therefore, a reaction time of 10 min was selected as the reaction time for the simultaneous analysis of the three compounds.

3.5. Analytical performance

We evaluated the analytical features, including the µPAD precision, linearity, limit of detection (LOD), selectivity, accuracy and stability, under the optimum conditions. These features are summarized in Table 1.

3.5.1. Calibration and linear ranges

Fig. S5A, B and C show typical calibration curves for sucrose, fructose and glucose, respectively. The calibration curves provided linear ranges of 1–25 mM, 2–20 mM and 0.5–10 mM for sucrose, fructose and glucose, respectively.

3.5.2. LOD

The LOD was calculated using signal-to-noise ratios of 3. The LOD values were 0.9 mM for sucrose, 0.8 mM for fructose and 0.4 mM for glucose.

3.5.3. The selectivity of the µPAD

To evaluate the µPAD selectivity for sucrose, fructose and glucose detection, the possible interfering compounds, including maltose, lactose, galactose and xylose, were introduced by adding the interfering compounds or analytes on the sample zone. All the experiments used the same concentration of 10 mM. Fig. 5 shows the results. The intensities of all the interfering compounds were not significantly changed, whereas high intensities were obtained for the three analytes. This phenomenon occurred because a specific catalysis was achieved by each enzyme towards each analyte; invertase, fructose dehydrogenase and glucose oxidase catalyzed the reactions involving sucrose, fructose and glucose, respectively. Thus, our developed µPAD showed high specificity towards sucrose, fructose and glucose and is suitable for detection of all sugars in real samples.

3.5.4. Precision and accuracy of µPAD

To evaluate the precision of the developed µPADs, we studied the repeatability and reproducibility. We evaluated the repeatability of the developed assay by averaging 7 replicate analyses of 20 mM sucrose, 20 mM fructose and 10 mM glucose and determining the relative standard deviation (%RSD). The %RSD was 1.3 for sucrose, 1.9 for fructose and 1.3 for glucose. The reproducibility of the instrumental measurements, the determinations and the preparation of the devices were studied for the detection of 20 mM sucrose, 20 mM fructose and 10 mM glucose. The reproducibility of the instrumental measurements was determined using four different scanners (Canon G2000, HP DeskJet Ink Advantage 3835, HP DeskJet 2621 and Brother DCT-T310). The %RSD was 2.9 for sucrose, 1.2 for fructose and 1.1 for glucose. In addition, the reproducibility of the determinations and preparation of the devices was analyzed using 7 different µPADs, and the RSD values obtained were 3.1%, 3.4% and 1.5% for sucrose, fructose and glucose, respectively. All the results indicated that the µPADs had high precision. The µPAD accuracy was evaluated via a recovery test; three concentration levels of sucrose, fructose and glucose were spiked into a sample of sugarcane juice A. Based on Table 2, the recovery ranges were 100–103% for sucrose, 98–103% fructose and 100–102% for glucose. All the results indicated that the µPADs had high precision.

3.5.5. Stability of µPAD

The storage stability of the µPAD was determined by measuring the color intensity of sucrose, fructose and glucose. The color intensity

Table 1
Summary of μPADs' analytical performance.

Analytes	Linear range (mM)	Regression equation	r	LOD (mM)	Accuracy (%)
Sucrose	1.0–25	y = 1.6305x + 10.475	0.9996	0.9	100 ± 0.6–103 ± 1.3
Fructose	2.0–20	y = 3.6962x + 57.715	0.9997	0.8	98 ± 2.0–103 ± 1.5
Glucose	0.5–10	y = 7.2806x + 9.3507	0.9997	0.4	100 ± 0.1–102 ± 0.6

remained at 76, 82 and 84% of its initial value for the analysis of sucrose, fructose and glucose, respectively, after a storage period of 4 months at 4 °C.

3.6. Analysis of sucrose fructose and glucose in real samples

The μPAD performance was further evaluated by testing its detection ability in three real samples, one made from sugar and two made from sugarcane juice, and the analytical results were compared with those of the HPLC method. The sucrose, fructose and glucose concentrations were calculated using the calibration curve of the standard solution (Fig. 6A). Fig. 6B shows the μPAD images of real samples for simultaneous detection of sucrose, fructose and glucose. As shown in Table 3, the concentrations of sucrose, fructose and glucose obtained by the μPADs were not significantly different from those obtained with the HPLC method, based on a statistics *t*-test at 95% confidence. The proposed μPAD required less analysis time compared to that of HPLC. The average analysis time for the μPAD was 11 min, while for HPLC it was 20 min. The preparation procedure for the μPAD included only dilution, whereas the HPLC samples were filtered through a nylon membrane prior to analysis. However, the developed μPAD is limited in term of mass production for commercial use.

Table 2
The recovery of sucrose, fructose and glucose in the spiked sugarcane juice A sample using the proposed μPADs.

Analyte	Initially detected (mM)	Add (mM)	Total found (mM)	Recovery (%)
Sucrose	16.5 ± 0.0	2.9	19.4 ± 0.0	102 ± 1.2
	16.5 ± 0.0	5.0	21.5 ± 0.0	100 ± 0.6
	16.5 ± 0.0	10.0	26.8 ± 0.1	103 ± 1.3
Fructose	3.1 ± 0.1	2.6	5.8 ± 0.0	103 ± 1.5
	3.1 ± 0.1	5.0	8.0 ± 0.1	98 ± 2.0
	3.1 ± 0.1	10.0	13.1 ± 0.2	99 ± 1.8
Glucose	2.0 ± 0.0	1.2	3.2 ± 0.0	102 ± 0.6
	2.0 ± 0.0	3.0	5.0 ± 0.0	101 ± 0.6
	2.0 ± 0.0	7.0	9.0 ± 0.0	100 ± 0.1

4. Conclusions

In summary, we demonstrated that our colorimetric μPAD is the first method to facilitate the simultaneous detection of sucrose, fructose and glucose using one device. The μPAD was fabricated on chromatography paper using a polymer screening method; the materials were readily available, simple to fabricate, and the process was equipment-free and cost-effective. All the analytes were detected via enzymatic reactions with selective substrates of each analyte. The change in color on the detection zone of each assay was recorded with a scanner, and the concentrations were derived from the color intensity using ImageJ software. After the experimental conditions were optimized, the limits of detection of sucrose, fructose and glucose were 0.9, 0.4 and 0.8 mM, respectively. All the analytes were detected within 11 min without any preconcentration or clean-up steps. The total cost for the materials, including the paper devices, enzymes, reagents, chromatography paper and polylactic acid is estimated to be approximately $3/device for three analytes. Our μPAD showed excellent performance in terms of stability,

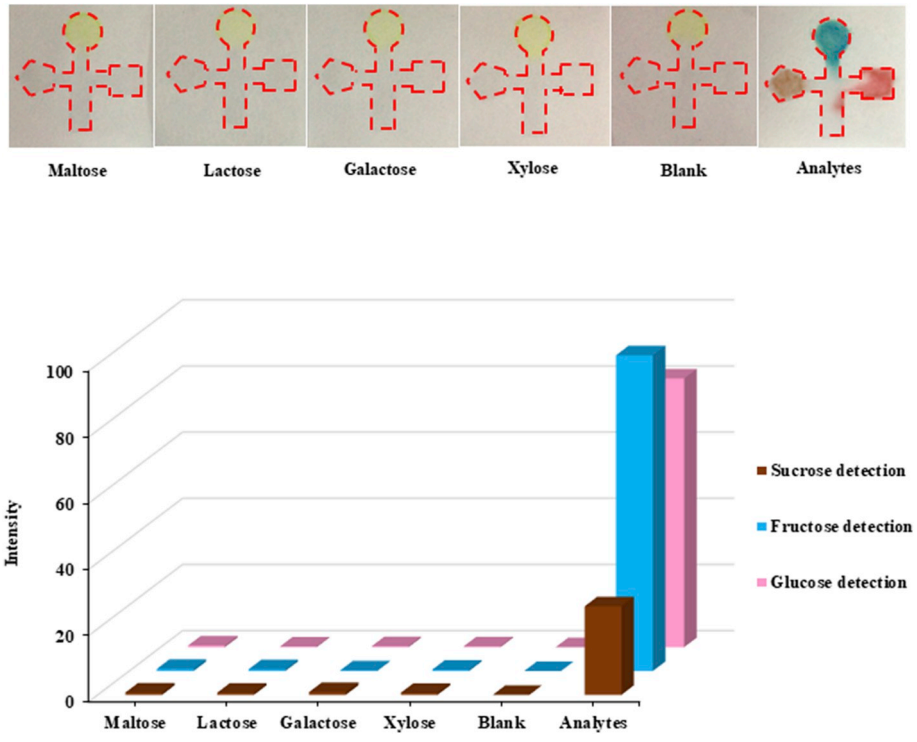

Fig. 5. Selectivity tested with maltose, lactose, galactose and xylose, which were compared with a blank μPAD and the three analytes.

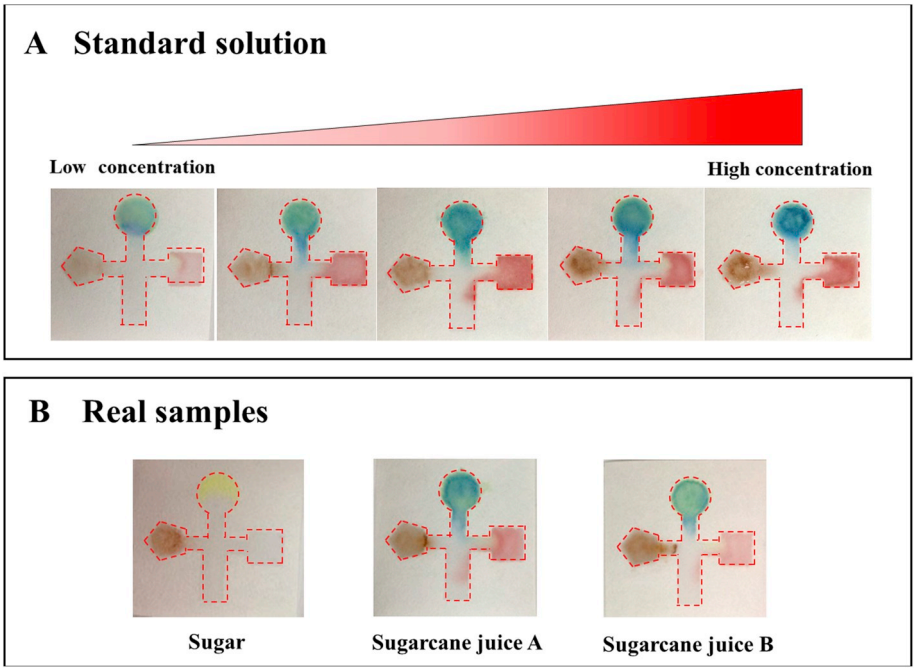

Fig. 6. Image of the μPADs tested with various concentrations of sucrose, fructose and glucose standard solution (A) and real samples (B).

Table 3
Comparison of sucrose, fructose and glucose content in real samples using μPADs and the HPLC method.

Samples	Analytes	μPADs	HPLC method	t_{cal}
		% ± SD	% ± SD	(t_{table} = 2.920)
Sugar	Sucrose	99.7 ± 0.1	99.4 ± 0.3	1.0
	Fructose	N/A	N/A	
	Glucose	N/A	N/A	
Sugarcane juice A	Sucrose	14.7 ± 0.3	14.6 ± 0.4	1.0
	Fructose	0.83 ± 0.03	0.83 ± 0.02	0.8
	Glucose	0.92 ± 0.01	1.04 ± 0.01	0.8
Sugarcane juice B	Sucrose	12.1 ± 0.3	12.9 ± 0.1	1.0
	Fructose	0.54 ± 0.02	0.50 ± 0.01	0.8
	Glucose	0.54 ± 0.02	0.62 ± 0.01	0.8

N/A: not detected.

recovery, repeatability and reproducibility for the simultaneous detection of sucrose, fructose and glucose. Additionally, we used the μPAD to analyze sucrose, fructose and glucose in real samples. This technology provides a promising alternative method for monitoring sucrose, fructose and glucose in sugar industry.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2019.120302.

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