Talanta 198 (2019) 542–549

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A paper-based device for simultaneous determination of antioxidant activity and total phenolic content in food samples

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ARTICLE INFO

Keywords:
Paper-based analytical devices
Antioxidant activity
Total phenolic content
Simultaneous analysis

ABSTRACT

We report the first use of a paper-based device as a simple, low-cost and rapid detection platform for simultaneous determination of antioxidant activity and total phenolic content in food samples. Two antioxidant activity assays including 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonate) radical cation (ABTS) assay and cupric reducing antioxidant capacity (CUPRAC) assay and one total phenolic content assay, Folin Ciocaltue reagent (FC) assay were simultaneously employed as a proof-of-concept. The device composed of a central sample zone connected to four pretreatment zones and consecutive detection zones to accommodate all three assays and a sample blank measurement. The analysis was achieved by dropping the samples onto the sample zone to flow to the pretreatment and detection zones containing the stored reagents for each antioxidant assay making the color change that was measured using imageJ software. Assay optimization including key reagent concentrations, reaction time, and surface modification were carried out to obtain sensitive and wide linear rage analyses. Various antioxidant standards were then evaluated to determine the analytical features of the method. The paper-based assays were successfully applied to detect antioxidant activity and total phenolic content in 10 beverage samples with similar gallic acid equivalent (GAE) values to those obtained from traditional assays at a 95% confidence interval. Moreover, the GAE values of the samples obtained from three assay analyses were well correlated to each other with relatively high Pearson's correlation coefficients. These results indicated that the assays gave accurate results and are suitable for simultaneous analysis of antioxidant activity and total phenolic content in real samples.

1. Introduction

Free radicals are known as unstable and highly reactive compounds that cause the oxidative damage to biological molecules including lipids, proteins as well as nucleic acids[1,2]. Antioxidants are substances capable of delaying, quenching or inhibiting oxidation processes generated by free radicals as well as oxygen containing compounds such as reactive oxygen species (ROS) (super oxide anion ($O_2^{\cdot-}$), hydroxyl radicals ($^{\cdot}OH$), hypochlorous acid (HOCl) and hydrogen peroxide (H_2O_2)). Therefore, they prevent food deterioration as well as several chronic illnesses such as heart disease, cancer, stroke and Alzheimer's disease [3,4]. Consuming beverages and food rich of natural antioxidants can help inhibit these diseases.

Traditionally, antioxidant activity can be measured using instrument-based methods such as gas chromatography (GC), liquid chromatography (LC), and colorimetry [5–7]. Although, GC and HPLC are effective techniques for separation and identification of antioxidants in complex samples, they are time-consuming, expensive and require trained personnel to operate. Colorimetry is a more common technique for antioxidant activity analysis because it provides lower analysis cost, is easier to perform and allows faster analysis time. These assays include total radical trapping (TRAP) [8], 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonate) radical cation ($ABTS^{\cdot+}$) [9], ferric reducing antioxidant power (FRAP) [10], oxygen radical absorbance capacity assay (ORAC) [11], cupric reducing antioxidant capacity (CUPRAC) [12,13] and the 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) [14,15] methods. Total phenolic content can be measured by the Folin-Ciocalteu reagent (FC) assay which also reflects the

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https://doi.org/10.1016/j.talanta.2019.02.048
Received 20 November 2018; Received in revised form 7 February 2019; Accepted 11 February 2019
Available online 11 February 2019
0039-9140/

antioxidant activity of the samples [16,17]. Although simpler than GC and HPLC, these methods require a large amount of sample and reagents, are time-consuming and labor-intensive manual process and hence are not suitable for fast screening of antioxidant activity. Moreover, different colorimetric assays give different antioxidant mechanisms. For example, ABTS assay provides the activity of antioxidants to scavenge $ABTS^{\bullet+}$ free radicals [9]. CUPRAC assay gives information on the antioxidant reducing capacity to Cu (II) in the Cu-neocuproine complex [12]. Folin-Ciocalteu reagent (FC) assay determine the content of phenolic compounds in the samples. To obtain all the information on the antioxidant mechanisms in the samples, it has been suggested that multiple colorimetric assays have to be performed [18]. Recently, the lab-on-a-disc method has been developed to address this problem for monitoring multiple colorimetric antioxidant assays at the same time [19]. However, the disc fabrication and operation requires complicated processes and relatively expensive instruments including milling machines, a spinning motor as well as a portable spectrophotometer.

Paper-based analytical devices (PADs) [20] are currently known as an effective and alternative method and have been applied as a detection platform in several areas including food safety [21], environmental monitoring [22] and clinical analysis [23]. PADs are attractive because they are simple, low-cost, lightweight, portable, easy to fabricate and use, disposable and provide low sample and reagent consumption. PADs have been applied for antioxidant activity analysis previously to allow for simple, high throughput and inexpensive tests. Nanoparticles have been employed to determine the reducing power of antioxidants toward the metal nanoparticles on the PADs [24–26]. The power of antioxidants to reduce Au^{3+} to form gold nanoparticles was measured [26]. Nanoceria has also been employed to determine antioxidant reducing power also on PADs, where Ce^{4+} on the nanoceria surface was reduced to Ce^{3+} by the antioxidant leading to a change in color from yellow to brown [24,25]. The DPPH paper-based assay has also been developed to allow for fast screening of the radical scavenging activity of antioxidants [27,28]. Total phenolic content in tea samples were determined using the low-cost, portable and disposable paper sensor immobilized with $NaIO_4$ and 3-methyl-2-benzothiazolinone hydrazine (MBTH) [29]. Combination of cotton thread and paper-based devices has also been developed for simple measurement of total phenolic content and antioxidant activity [30]. However, these paper-based antioxidant and total phenolic content assays provided no integration of multiple antioxidant assays as only single assay was performed on each device.

Here, we demonstrate that multiple antioxidant assays and total phenolic content assay are able to be performed on a single paper-based device. Two antioxidant activity assays, $ABTS^{\bullet+}$ and CUPRAC, and one total phenolic content, FC assay, were simultaneously performed on a single PAD as a proof-of-concept. To accommodate multiple assay analysis, the device was designed to have a central circular sample zone connected to four pre-treatment and detection zones where 3 detection zones were used for the three assays and another detection zone was used as a sample blank (Fig. 1). Antioxidant analysis can be performed by dropping a sample solution onto the sample zone and allowed to flow to the pretreatment and detection zones that are pre-deposited with the reagents corresponding to the assays evaluated. Color changes were analyzed using a scanner and imageJ software at the detection zone and were proportional to the concentration/activity of the antioxidants. To the best of our knowledge, this is the first time that multiple antioxidant assays and total phenolic content were investigated simultaneously on a paper-based device. Therefore, the developed PAD offers portability, low reagent and sample consumption, inexpensive, rapid and high throughput analysis of antioxidant activity and total phenolic content of various samples such as teas, wines and fruit juices.

2. Materials and methods

2.1. Chemicals and instruments

Unless otherwise stated, all chemicals were purchased from Sigma Aldrich (Singapore). Ammonium acetate, copper (II) chloride were purchased from Ajax Finechem (Australia). Folin-Ciocalteu reagent (FC) and hydrogen peroxide were obtained from Merck (Germany). Wine, fruit juice and tea samples were purchased from local markets in Chon Buri province, Thailand.

A Xerox ColorQube 8870-1 wax printer was purchased from Xerox (Malaysia) for device printing. A scanner (Canon, CanoScan LiDE110) was purchased from Canon (Vietnam). Whatman filter paper no. 4 obtained from Whatman™ (China) was used as a paper material to create all PADs. A UV–vis Spectrophotometer SPECORD® 210 PLUS from Analytik Jena (Germany) was used for conventional antioxidant assay measurement.

2.2. Design and fabrication of PADs

The dimensions of the devices are described in Fig. 1 where the central sample zone was connected to four pre-treatments and four consecutive detection zones. Red, purple and yellow color were used for hydrophobic barrier around the detection zones because they are complementary to green, yellow and blue which are the colorimetric signals of the ABTS, CUPRAC and FC assays, respectively. Another detection zone surrounded by the blue hydrophobic barrier was used as a sample blank. The design was created by the Adobe illustrator CC program. The device was made of Whatman no.4 filter paper and fabricated using the wax-printing method. The printed devices were placed on a hot plate with heat at 150 °C for 90 s. This allowed the wax to penetrate through the paper to form hydrophobic barriers and create the test zones. After heating, the backside of the device was covered with tape to prevent solution from leaking out underneath the paper during the analysis.

2.3. Reagent deposition and colorimetric assays on papers-based devices

2.3.1. ABTS assay

The reagent deposition was performed in the zone that is surrounded by the red hydrophobic barrier. (3-aminopropyl)triethoxysilane (APTES) solution (0.5 µL, 5% v/v) and ABTS solution (0.5 µL, 40 mM) were consecutively added into the detection zone. $K_2S_2O_8$

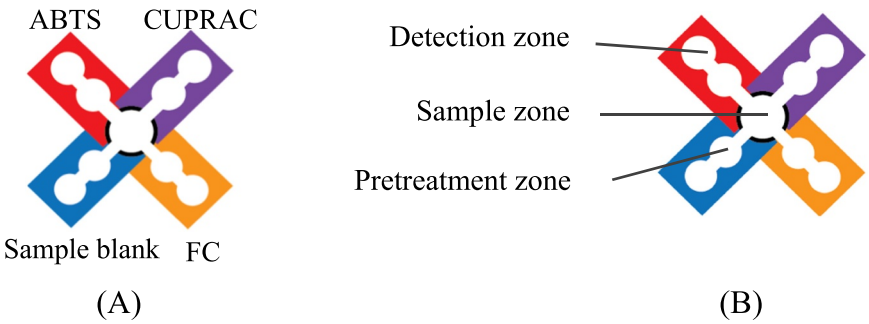

Fig. 1. Typical PADs for simultaneous measurement of antioxidant activity and total phenolic content. (A) The zones used for ABTS, CUPRAC, FC and sample blank. (B) The sample zone, pretreatment zones and detection zones on the devices. The dimension of the devices was as follows: the device was designed to have one sample reservoir (7 mm diameter), connected to 4 detection reservoirs (5 mm diameter) where 3 detection reservoirs (red, purple, yellow arms) are for 3-assay analyses and another detection reservoir (blue arm) is for the sample blank. In between the detection and the sample zones of each arm, there was a pretreatment zone (5 mm diameter) that was used to facilitate each assay reaction.

solution (0.5 μL, 70 mM) was added into the pretreatment zone. The device was allowed to dry after each reagent deposition.

2.3.2. CUPRAC assay

For CUPRAC assay, the analysis was carried out in the purple channel. $CuCl_2$ (0.5 μL, 150 mM) and ammonium acetate (0.5 μL, 10 mM, pH 7) were consecutively added into the detection zone. 0.5 μL of 600 mM neocuproine was added into the pretreatment zone. The device was allowed to dry once deposition of each reagent was done.

2.3.3. FC assay

The FC assay was performed in a yellow arm of the device. Firstly, PEG solution (0.5 μL, 40 mg/mL), FC (0.5 μL, 2 N) and Na_2CO_3 (20% w/v) were serially added into the detection zone. H_2O_2 (0.5 μL, 2 M) was added into the pretreatment zone to oxidized non-phenolic compounds that have reducing capacity such as ascorbic acid. The device was allowed to dry in between each reagent deposition.

2.3.4. Analysis of standards and samples

The antioxidant standard or sample (10 μL) was added into the sample zone followed by 20 μL of DI water to elute the residual antioxidants in the sample zone to the detection zones. The reaction of each assay on the paper-based devices was allowed to take place in the dark at ambient temperature for 5 min. After that, the device was dried using a hair-dryer. The pictures were captured using a scanner with default setting (Resolution: 300 dpi, Brightness: 0, Contrast: 0) and the color intensity was measured as mean gray intensity by an imageJ program using the procedure described in Fig. S1 (Supplementary information) [31].

2.4. Conventional assays for antioxidant activity

For method validation, the samples were also analyzed using the conventional assays for antioxidant activity (ABTS and CUPRAC assays) and total phenolic content (FC assay). Gallic acid was used as an antioxidant standard. The antioxidant activity and total phenolic content of the samples obtained from the conventional assays were expressed as gallic acid equivalent (GAE, μmol GA/g samples or μmol GA/L samples). The obtained GAE of the samples were compared to those obtained from the paper-based device analysis to determine the method accuracy.

2.4.1. ABTS assay

The ABTS assays were performed using the method previously described with some modifications [9]. Firstly, equal volumes of ABTS solution (7 mM) and potassium persulfate solution (2.45 mM) were mixed and allowed to react in the dark at ambient temperature for 16–20 h to generate $ABTS^{\bullet+}$. The $ABTS^{\bullet+}$ stock solution was then diluted by mixing 1 mL the solution with 30 mL DI water to obtain the $ABTS^{\bullet+}$ working solution. Gallic acid antioxidant standard or sample (75 μL) was mixed with 1425 μL of $ABTS^{\bullet+}$ working solution and allowed to react at ambient temperature for 10 min. The absorbance was measured at 734 nm using a spectrophotometer.

2.4.2. CUPRAC assay

The experimental procedure was carried out using the method described by Apak *et al.* with some modifications [12]. Firstly, reagents including $CuCl_2$ (5 μL, 150 mM), ammonium acetate (5 μL, 10 mM pH 7) and neocuproine (5 μL, 600 mM) were mixed. Next, gallic acid or sample (25 μL) was added into the mixture and the total volume adjusted to 3 mL with DI water. The mixture was allowed to react at ambient temperature for 30 min and the absorbance measured at 450 nm using a spectrophotometer.

2.4.3. FC assay

The FC assay was carried out according to the method previously described with some modifications [16]. Firstly, gallic acid or sample (250 μL), DI water and FC were mixed and allowed to stand at ambient temperature for 20 min. Next, 1 mL of 10% (w/v) Na_2CO_3 was added into the mixture and allowed to react for another 10 min. The absorbance was measured at 760 nm using a spectrophotometer.

2.5. Sample preparation

The samples used in this work included tea, wine and fruit juices. The tea samples were prepared by weighing 2 g of dry leaves and putting them into 200 mL of hot water (80 °C) for 5 min. The tea extract was allowed to cool to room temperature and filtered through Whatman No. 1 filter paper. The extracted solution was kept at 4 °C for up to one month. For wine samples, the solution was prepared by degasing for 15 min to remove air bubbles. For fruit juice, the samples were centrifuged at 6000 rpm for 5 min in order to separate the pulp and the clear supernatant was used for analysis.

3. Results and discussion

3.1. Assay optimization

The antioxidant assays and total phenolic content assay performed on the PADs were first optimized for the initial concentrations of the key reagents and reaction time for each assay.

The ABTS assay for determination of antioxidant activity was based on the radical scavenging activity of the antioxidants to $ABTS^{\bullet+}$ generated from ABTS and potassium persulfate (Fig. S2A, Supplementary information) [9]. The optimal concentration of ABTS was first evaluated to determine the assay sensitivity. The study was carried out without the addition of antioxidants where ABTS and potassium persulfate were deposited on the detection and pretreatment zones, respectively. Deionized water was added to the sample zone to elute the potassium persulfate to react with ABTS at the detection zone to generate green products of $ABTS^{\bullet+}$. Preliminary results showed that the observed $ABTS^{\bullet+}$ color intensity was heterogeneous throughout the detection zone and can result in variability of the color intensity measurement (Fig. 2A). This might be attributed to the high mobility of $ABTS^{\bullet+}$ flowing on the cellulose substrate. At a working pH of 7, both

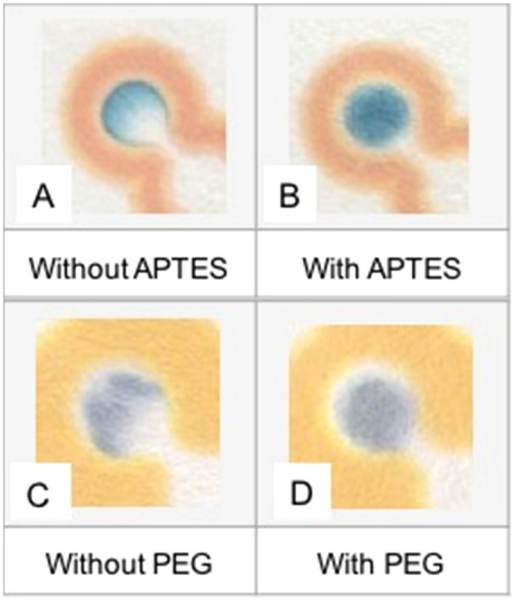

Fig. 2. Optical image showing the colorimetric assay for (A, B) ABTS assay and (C, D) FC assay on native paper without surface modifiers and with surface modifiers including APTES and PEG for ABTS and FC assays, respectively.

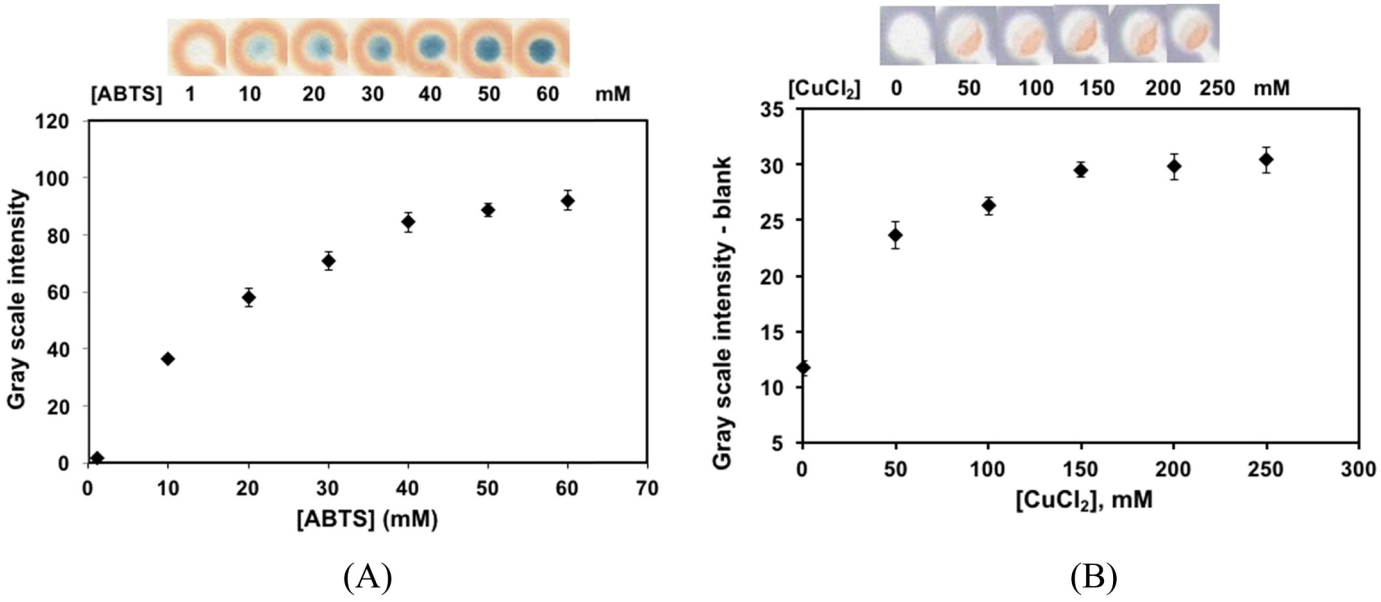

Fig. 3. Color responses versus concentration of (A) ABTS used in the ABTS assay and (B) $CuCl_2$ used for the analysis of 3 mM GA in the CUPRAC assay (n = 5).

cotton cellulose (IEP < 2.8) [32] and $ABTS^{\bullet+}$ (where its sulphonate groups (pKa < 0) [33] are deprotonated,) are negatively charged resulting in the adsorption process being limited due to electrostatic repulsion between them. To reduce this effect, APTES was employed as a surface modifier at the detection zone since its amine groups are fully protonated at pH < 9.6 giving rise to a positively charge surface [34]. Therefore, the electrostatic interaction between $ABTS^{\bullet+}$ and cotton cellulose of the paper were neutralized, resulting in higher adsorption of $ABTS^{\bullet+}$ on the surface and a uniform color distribution on the detection zone (Fig. 2B). Fig. 3A shows that the color intensity increased gradually as the ABTS concentration increased in the range of 1–40 mM as a result of increasing $ABTS^{\bullet+}$ products. At higher ABTS concentrations, the color intensity became saturated. Therefore, ABTS at a concentration of 40 mM was selected as the optimal concentration for antioxidant activity measurement to allow for a sensitive-response and wide linear range of antioxidant analysis. The results also demonstrated that it is possible to measure antioxidant activity that scavenge $ABTS^{\bullet+}$ in a dose-response manner. Reaction time between the potassium persulfate and ABTS to generate colored radicals of $ABTS^{\bullet+}$ was evaluated. The colored $ABTS^{\bullet+}$ was generated within 5 min indicating that fast reaction between potassium persulfate and ABTS occurred on the paper-based devices (Fig. S3A, Supplementary information).

Antioxidant activity analysis using CUPRAC assay was based on the reducing capacity of antioxidants toward the Cu(II)-neocuproin complex to form the yellow color of Cu(I)-neocuproin (Fig. S2B, Supplementary information)[12]. Therefore, concentration of copper solution used in the assay played an important role of antioxidant activity analysis and, hence, was first optimized. As shown in Fig. 3B, the intensity increased as the copper (II) concentration increased from 0 to150 mM from the analysis of 4 mM GA and became steady after that. Therefore, the concentration of 150 mM $CuCl_2$ was selected as the optimal value to allow for sensitive analysis of antioxidant activity.

The FC assay used to analyze total phenolic content of the samples was based on the reducing capacity of phenolic compounds toward Mo (VI) complex to produce blue color of the Mo(V) complex. The FC reagent (2 N) was used as obtained without any further dilution. Preliminary results showed that the color gradient was observed in the detection zone when 3 mM GA was analyzed due to the high mobility of the Mo(V) complex on the paper substrate (Fig. 2C). To reduce the flow rate, a low mobility and high molecular weight compound, polyethylene glycol (PEG, MW = 6000 g mol^{-1}), was employed as a paper surface modifier to allow for uniform color distribution all over the detection zone (Fig. 2D) which increased the sensitivity and accuracy of the analysis.

The reaction time for all three assays were investigated in the range of 5–45 min using both high- and low-antioxidant activity compounds including gallic acid and vanillic acid, respectively. The results showed that both types of antioxidant standards have fast reaction time with the reagents of all three assays on the developed paper-based devices where the reactions are completed within 5 min as shown by the near-zero slope of reaction time curve (Fig. S3, Supplementary information). Therefore, a reaction time of 5 min was chosen as the optimum value for all three assays for further analysis of all the antioxidant standards and samples used in this work. The variation of assay reaction time is not expected to cause the difference in the analysis since there was no significant difference in color intensity from the analysis with different reaction times for all three assays. Moreover, the reaction times used in the paper-based assays were lower than that used in the traditional assays by about 6 fold offering faster analysis time for the simultaneous analysis of the three assays.

3.2. Analysis of antioxidant and phenolic standards

Under the optimum conditions found above, the paper-based devices were used to analyze a series of antioxidants and phenolic compounds. Analytical features from the analyses including linearity, repeatability and limit of detection were determined and summarized in Table S1 (Supplementary information). The paper-based antioxidant assay and total phenolic content assays responded well with all of the antioxidant standards investigated. For example, the green color intensity was inversely proportional to the gallic acid (GA) concentration using the ABTS assay as shown in the top photograph of Fig. 4A. The yellow and blue color intensity increased as the GA concentration increased for CUPRAC and FC assays, respectively (Figs. 4B and 4C). Typical calibration curves from the analysis of the antioxidant standard, gallic acid, using ABTS, CUPRAC and FC assays are shown in Fig. 4A, B and C, respectively. For the ABTS assay, the differences in intensity ($\Delta i = i_{control}$-i_c; $i_{control}$ = intensity from control analysis and i_c = intensity from standard analysis at a given concentration) as a function of log analyte concentration was found to be linear over the range 3–13 mM which is wider than the range previous reported (0.029–0.117 mM GA). [35] For CUPRAC and FC assays, gray scale intensity as a function of GA

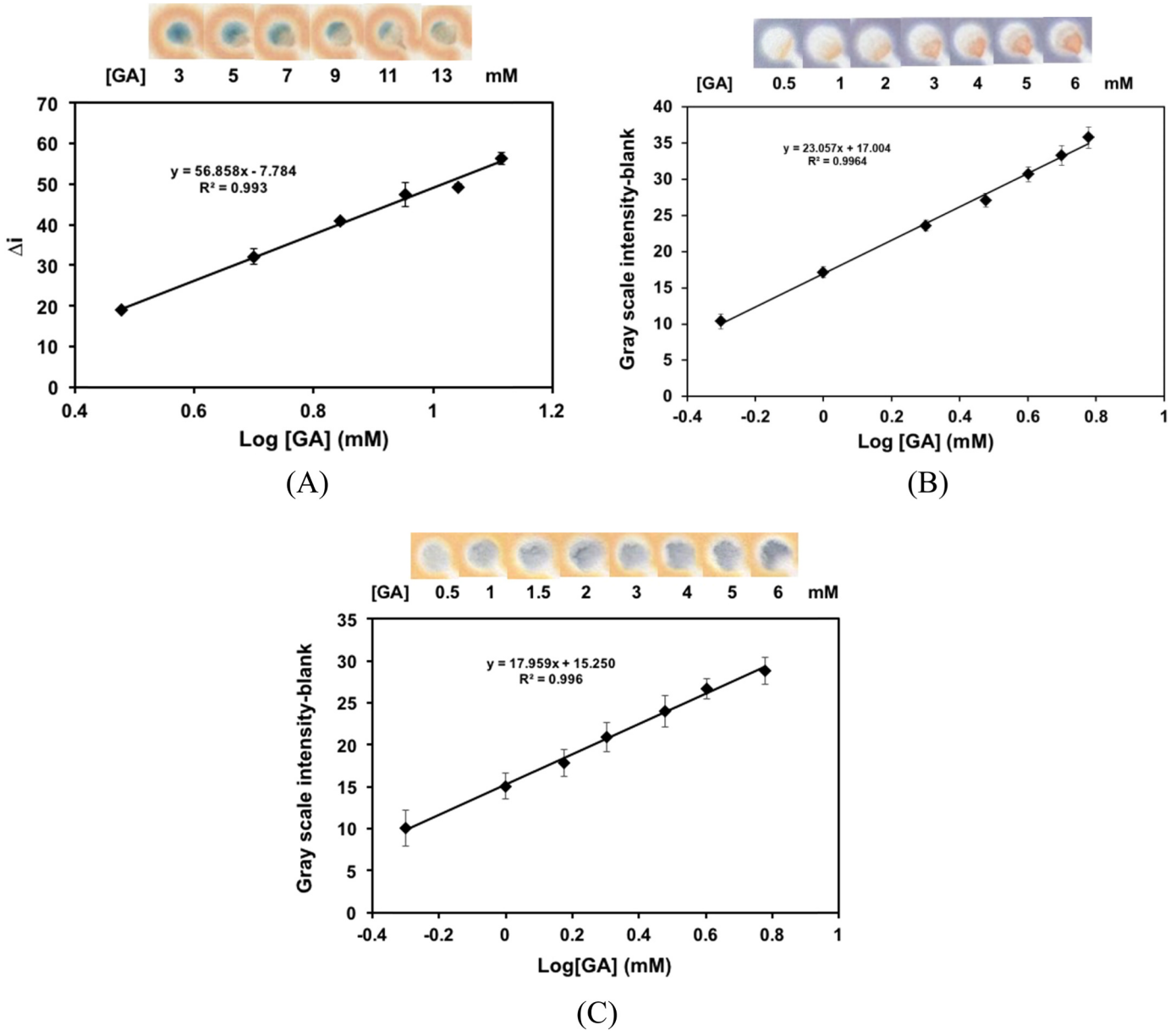

Fig. 4. Typical calibration curve for the analysis of an antioxidant standard and phenolic compound (gallic acid) on the PADs using (A) ABTS assay (B) CUPRAC assay (C) FC assay (n = 5).

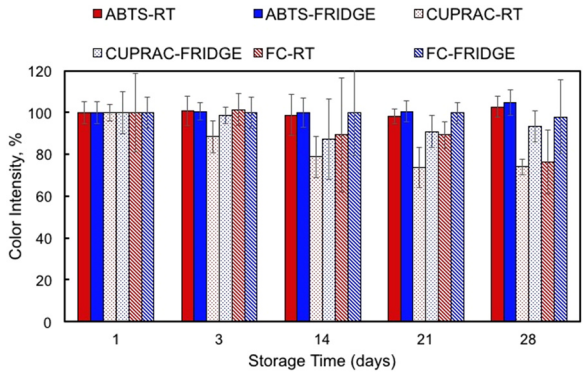

Fig. 5. Storage stability of the paper-based devices in the dark at room temperature (25–30 °C) and in the refrigerator (4 °C) demonstrated as %decrease in color with 100% being the response of freshly prepared devices for the analysis of 5 mM gallic acid (n = 6).

concentration was plotted. The wide linear ranges of 0.5–6 mM GA was obtained for FC assay which is in the similar to previous report (0.29–5.88 mM [36]). For CUPRAC assay, the linear range for GA obtained from the developed method was 0.5–6 mM. Although, this working range is at higher concentration than that obtained from the traditional CUPRAC assay (1.2–32 μM) [37], the volume of the sample/standard required for the developed method (10 μL) was about 100 times lower than that used in traditional CUPRAC assay (1.1 mL). Other antioxidant standards and phenolic compounds gave similar responses to that of GA for all three assays but different linear ranges and limits of detections (Fig. S4 and Table S1, Supplementary information). Repeatability of the assays was determined by performing 5 replicate analyses of the antioxidant standards at three different concentrations in the linear range and reported as the relative standard deviation (% RSD). High repeatability of the methods was obtained with the %RSD being in the range of 0.7–9.4% for all antioxidant standards investigated using the three assays.

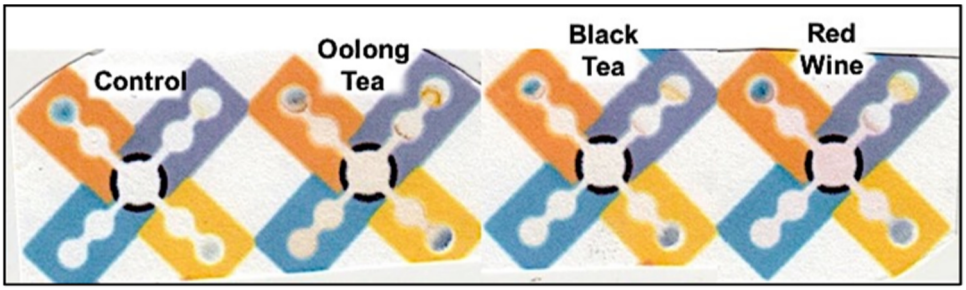

Fig. 6. Paper-based devices showing the simultaneous detection of antioxidant activity and total phenolic content of the samples including Oolong tea, black tea and red wine compared to the control.

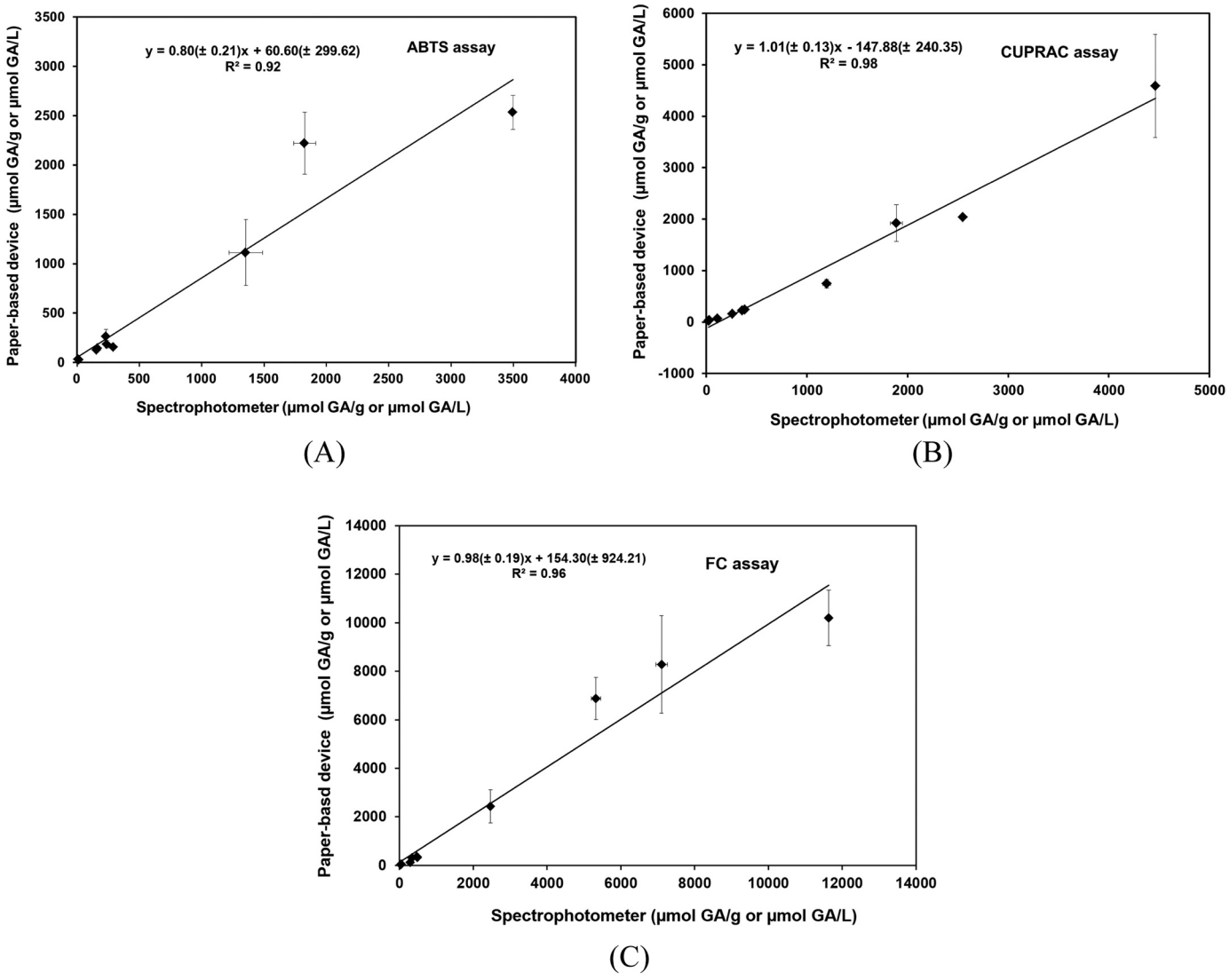

Fig. 7. Antioxidant activity and total phenolic content expressed as GAE from the analysis of 10 samples obtained from the traditional spectrophotometric assays and PAD assays. (A) ABTS, (B) CUPRAC, and (C) FC assay (n = 3).

3.3. Stability study

The stability of the developed PADs was investigated by storing the devices at room temperature (25–31 °C) and in the refrigerator (4 °C) over 28 days. The devices were pre-deposited by the reagents associated with the three assays excepted H_2O_2 for FC assay and wrapped in aluminium foil to protect from light. Gallic acid (5 mM) was used for the test and H_2O_2 (0.5 µL, 2 M) was deposited in the pretreatment zone of FC assay prior to analysis. A plot of the % color intensity, defined as % decrease with 100% being the response of the freshly prepared PADs, as a function of storage time for the analysis of 5 mM gallic acid is shown in Fig. 5. The devices that were stored in the dark at room temperature were found to be stable over a month for ABTS assay but showed a significant loss of stability over time for CUPRAC and FC assays. The devices that were stored in the refrigerator, on the other hand, were found to be relatively stable over 28 days for all three assays. The results observed here might be attributed to the neocuproine and FC reagent stability used in CUPRAC and FC assays, respectively, as

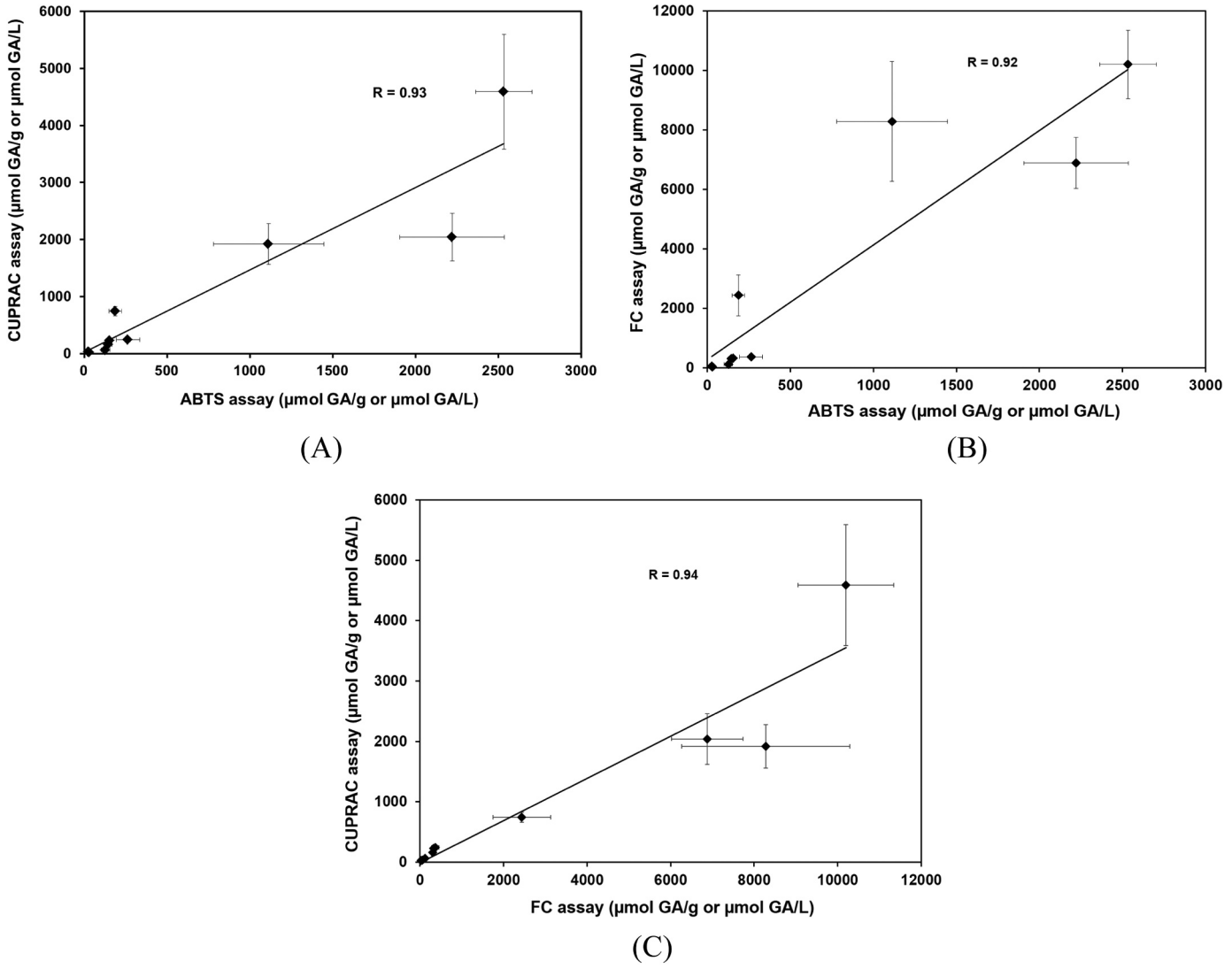

Fig. 8. Correlations of paper-based assay results expressed as GAE between (A) ABTS and CUPRAC assays, (B) ABTS and FC assays and (C) FC and CUPRAC assays (n = 3).

recommended in the safety data sheet to be stored at low temperature.

3.4. Analysis of samples

The paper-based devices were then validated against the traditional ABTS, CUPRAC and FC assays using 10 different kinds of real samples including teas and wines and fruit juices. Typical paper-based devices for the analysis of real samples are shown in Fig. 6, where all three assays where performed simultaneously. The responses from all assays were as expected from the analysis of all samples compared to the control where the color intensity decreased for the ABTS assay and increased for the CUPRAC and FC assays, respectively. The background color intensity appearing in the blue arm of the devices was subtracted from the measured signal of each assay. Moreover, some background color from pigments in the samples such as red color from red wine samples was absorbed by the filter paper at the sample zone and hence did not interfere with the assay color intensity in the detection zones.

The antioxidant activity and total phenolic content were expressed as GAE with units of μmol/g and μmol/L for dry teas and beverage samples (wine and fruit juices), respectively (Table S2, Supplementary information). Among the tea samples, the overall trend of antioxidant activity and total phenolic content can be arranged as Oolong tea > black teas > green teas which was similar to the order in previous reports [19,28]. For beverage samples, wine had higher antioxidant activity and total phenolic content than the fruit juices due to higher contents of polyphenol and phenolic compounds [38]. For most samples, the GAE values obtained from traditional assays and developed assays were similar. However, there were differences for some samples such as Mulberry green tea 1 and 2 in ABTS assays and Black tea 3 in FC assays. This might be the result of sample background color interferences in traditional spectrophotometric assays as high sample volume was required. This problem can be overcome by employing additional sample clean up step prior to analysis by traditional assays. To determine the accuracy, the GAE of all samples obtained from the paper-based devices were compared with traditional assays using the regression curve [39]. As shown in Fig. 7, the equations obtained from the comparison of all three assays with the tradition methods were presented as regression curves with the calculated confidence limits at 95% for the intercepts and slopes. For all assay comparisons, the confidence intervals included the value of 0 and 1 for the intercept and slope, respectively. These results indicated that the PAD assays had no significant differences from the traditional spectrophotometric assays at a confidence level of 95% ($p = 0.05$). With this high degree of accuracy, however, the PAD assays offer significantly reduced sample and reagent consumption, analysis time and cost.

The correlation between the antioxidant activity and total phenolic

content obtained from the three different assays on the paper-based devices was further analyzed. As shown in Fig. 8, the high values of the Pearson's correlation coefficient (R) which were 0.93 for ABTS and CUPRAC assays, 0.92 for ABTS and FC assays and 0.94 for FC and CUPRAC assays indicated that all three assays were highly correlated. These results demonstrated that the three assays can be used as an indicator to each other. For example, the measurement of CUPRAC assay to report reducing capacity can also reflect the radical scavenging activity of ABTS assay and total phenolic content obtained from FC assay and vice-versa.

4. Conclusions

A paper-based device for simultaneous analysis of antioxidant activity and total phenolic content has been developed. The analysis time for simultaneous measurement using three assays including ABTS, CUPRAC and FC assays was obtained in only 5 min. The high degree of accuracy between the developed assays on the PADs compared well to the traditional spectrophotometric assays based on the analysis of 10 samples including teas, wines and fruit juices. However, sample and reagent consumption, analysis time and cost were reduced using the developed PADs. The three assays also gave high correlation. These results indicated that the PADs can be applied for simultaneous analysis of antioxidant activity and total phenolic content with simple, cost effective, rapid analysis with low reagent and sample consumption. The application of the PADs for analysis of other sample types is currently under investigated.

Acknowledgements

This work was financially supported by (i) Thailand Research Fund (Grant no. MRG6180100) (ii) the Research Grant from Burapha University through National Research Council of Thailand (Grant no. 93/2559 and 6/2560) and for the sensor innovation research unit for development and production of test kits (iii) the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, Thailand. We would also like to thank Dr. Ron Beckett, Faculty of Science, Burapha University for comments and correction.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2019.02.048

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