

An Instrument-free Detection of Antioxidant Activity Using Paper-based Analytical Devices Coated with Nanoceria

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This work reports a portable distance-based detection paper device that has a thermometer-like shape for rapid, instrument-free determination of antioxidant activity using a nanoceria assay. The assay is based on partial reduction of cerium ion from Ce^{4+} to Ce^{3+} on nanoceria deposited along the detection channel by antioxidants present in food, giving highly reactive oxidation products. Either these products or the parent antioxidant compounds could then bind to the OH-rich ceria nanoparticles and generate charge transfer ceria-antioxidant complexes resulting in a yellow to brown color change. The distance of the brown color on the detection channel is directly proportional to antioxidant activity, and can be easily measured using an integrated ruler without the need of any external sophisticated instrument for detection. The paper sensor has been studied for the analysis of common antioxidants and its performance was validated against traditional antioxidant assays for 11 tea sample analyses. Using the Spearman rank correlation coefficient method, the antioxidant activity of tea samples obtained from the paper device correlated with the traditional assay at the 95% confidence level. The developed sensor provided a high recovery and tolerance limit and was stable for 50 days both when stored at ambient and low temperature (6 and $-20^{\circ}C$). The results demonstrated that the developed paper device is an alternative to allow for fast, simple, instrument-free, cheap, portable and high-throughput screening of antioxidant activity analysis in real samples.

Keywords Microfluidic paper-based analytical device, distance-based detection paper device, nanoceria, antioxidant activity

(Received July 20, 2017; Accepted August 31, 2017; Published January 10, 2018)

Introduction

The consumption of antioxidant-rich foods has gained significant attention for their potential to mitigate the presence of excess reactive oxygen species (ROS), reactive nitrogen species (RNS) and other free radicals in the body that caused oxidative stress.^{1,2} Common diseases associated with oxidative stress include cancer, heart disease, diabetes, atherosclerosis, Parkinson's and Alzheimer's.^{3,4} Current methods to quantify antioxidant activity can broadly be classified into two groups, including hydrogen atom transfer (HAT)-based assays and single electron transfer (ET)-based assays.^{5,6} The HAT-based assays are based on removing hydrogen atoms from the antioxidant by the free radical, resulting in the stable radical of the antioxidants.^{6,7} HAT-based assays include oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP), the Crocin bleaching assay and lipid peroxidation tests.⁸ ET-based assays determine antioxidant power using antioxidants that are oxidized by the total antioxidant capacity reagents through electron transfer.⁹ These assays include the Folin-Ciocalteu reagent (FCR), ferric ion reducing antioxidant power (FRAP), cupric ion reducing antioxidant capacity (CUPRAC), 2,2-diphenyl-1-picrylhydrazyl radical scavenging

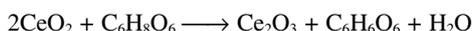
capacity (DPPH), ABTS/trolox equivalent antioxidant capacity (TEAC) or other redox metal ions such as Fe, Cu or Au.^{5,10} Although both types of assays have been widely used, they are based on UV-Vis spectrophotometry that requires large reagent and sample volumes, multiple analysis steps, are labor-intensive and time-consuming resulting in low overall throughput. Most conventional UV-Vis spectrophotometers are large, making field measurements difficult.

An improved antioxidant activity assay would require less sample and reagent, be faster, more sensitive, easier to perform, inexpensive, instrument-free and suitable for field measurements. Here, we report a microfluidic paper-based analytical device (μ PADs) for low-cost, high-throughput screening of antioxidant activity. Recently, μ PADs have gained interest for their applications in several fields such as biomedical science, genomics, immunology, chemistry, biochemistry, toxicology, environmental monitoring and food safety because they offer advantages including simplicity, rapidity, low cost, equipment-free, lightweight, portability, low reagent consumption and ease of disposal.¹¹⁻¹⁵ μ PADs can be fabricated by patterning hydrophobic materials to create hydrophilic flow channels and analysis/detection zones using various methods such as photolithography,¹⁶ wax printing¹⁷ and polymer screen-printing.^{18,19} The quantitative detection methods for μ PADs include colorimetry, fluorescence, chemiluminescence, electrochemiluminescence and electrochemistry.²⁰⁻²³ These detection methods have been widely implemented, but they can require

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external instruments for detection including scanners, potentiostats and fluorescence microscopes.^{21,24-26} Recently, the Henry group introduced a distance-based detection method that removes the need for any external equipment for detection on μ PADs.²⁴ Distance-based detection relies on flow of aqueous samples along the channels by capillary wetting to react analyte with reagents deposited on the paper, creating a colored zone.²⁴ The distance of developed color is proportional to analyte concentration and allows quantification to be read by measuring the color length with a common ruler. As a result, the analysis can be done without external equipment.

The objective of this study was to develop a μ PAD in a distance-based measurement format for analysis of antioxidant activity using nanoceria (CeO_2 nanoparticles) as the colorimetric agent. The device would allow for simple, rapid, low-cost, easy-to-use, portable and high-throughput analysis of antioxidant activity without the need for any external complicated instrument for detection. Nanoceria has recently received attention because of its applications in fuel additives, catalytic materials, solar cells, neuroprotection, anti-inflammation and cardioprotection.^{27,28} On the nanoceria surface, equal amounts of Ce^{3+} and Ce^{4+} species were observed and the nanoceria have a light yellow color. However, when the nanoceria is oxidized or reduced, the ratio of species containing Ce^{3+} and Ce^{4+} changed, resulting in a change in nanoceria color.^{1,27} Using this property, Sharpe *et al.* reported a paper-based assay for antioxidant activity using nanoceria as a colorimetric agent.¹ The assay is achieved using an ET-based method where the antioxidant compounds partially reduce cerium from Ce^{4+} to Ce^{3+} on the nanoceria surface leading to a change in color from light yellow to brown. The degree of color change is dependent on the antioxidant activity in the sample.^{1,25} A typical chemical reaction showing colorimetric detection of ascorbic acid as an antioxidant representative is shown below:



This previous reported paper-based assay was based on the color intensity measurement for quantification of antioxidant activity. Although this detection method is simple, external equipment for capturing pictures such as scanners, cameras or other optical techniques and image-processing software are required. Moreover, the influence from light during capturing pictures has to be considered to obtain accurate and quantitative analysis. To overcome these limitations, the assay developed here was performed in the distance-based detection platform where only a ruler is required for accurate quantitative analysis of antioxidant activity using nanoceria as colorimetric probes. The device was first used to analyze six antioxidant standards. Then, to test the μ PAD performance for measuring antioxidant activity, the device was validated against traditional antioxidant assays (CUPRAC, FCR and FRAP assays) and flavonoid content assay (aluminum chloride assay) using 11 tea samples as model antioxidant-rich samples. The antioxidant activity expressed as gallic acid equivalent (GAE) and epigallocatechin gallate equivalent (EGCGE) obtained from the developed paper-based method correlated with results obtained from the traditional assays compared using Spearman rank order correlation coefficients method at $P = 0.05$ confidence level. The proposed method showed high recovery from the analysis of tea samples and a high tolerance limit for potential interferences. Finally, the device was also found to be stable for almost two months when kept at room temperature, in the refrigerator or in the freezer.

Experimental

Chemicals and materials

Unless otherwise stated, all chemicals were obtained from Sigma Aldrich. Folin-Ciocalteu's reagent (FCR), aluminum chloride, iron(III) chloride anhydrous, sodium carbonate, sodium hydroxide, citric acid, DL-tartaric acid and sodium nitrite were purchased from Merck. Ammonium acetate, copper(II) chloride and sodium acetate hydrated were acquired from Ajax Finechem. Disodium L-(+)-tartrate dehydrate was purchased from TCI (Japan). Oxalic acid dehydrate was obtained from Carlo Erba Reagents. DL-Alanine was purchased from HiMedia Laboratories Pvt. Ltd. (India) and glycine was obtained from EMD Chemical Inc. (Germany).

Filter paper (No. 4) was purchased from Whatman (GE Healthcare Co., China). Xerox ColorQube 8870-13 wax printer (Flextronics Technology, Malaysia) was used to facilitate the wax-printing method for paper device fabrication.¹⁷ A desktop scanner (CanoScan LiDE 110) was used for capturing colored images of the paper device. UV-Vis spectrophotometer (Genesys 20, Thermoscientific, USA) was employed for conventional assay analysis.

Fabrication of a paper-based device

The paper-based device was fabricated using the wax-printing method.¹⁷ The details of the paper-based device's dimensions and fabrication process are described in Supporting Information and Fig. S1. To deposit reagents on the detection channel, the sample zone was detached from the detection zone by cutting to prevent wicking of reagents into the sample zone during coating. A 1% (w/v) of nanoceria dispersion was coated onto the detection zone by dipping the detection zone into the nanoceria and allowing it to dry at ambient temperature (Fig. 1A). After that, the detection zone became hydrophobic due to drying of nanoceria and preventing wetting of the detection zone. To allow for flow, 10 mg/mL of PEG was added to the detection zone (Fig. 1B).¹⁹ After drying, the detection zone and sample zone were then recombined by putting tape on the backside and the paper device was ready for antioxidant analysis (Fig. 1C).

Antioxidant activity analysis using distance-based measurement paper device

Determination of antioxidant activity was performed by applying 20 μL of antioxidant standard solutions/samples onto the sample zone, allowing the solution to flow through the detection zone *via* capillary action (Fig. 1C). Another 10 μL of deionized water was applied to the sample zone to elute the residual antioxidant. After drying, measuring of color distance was carried out using a common ruler as shown in the Fig. 1D.

Preparation of tea samples

Eleven varieties of commercial teas were purchased from local markets in Chon Buri province area, Thailand. Then, 2 g of dried teas were weighed and boiled in the 200 mL deionized water at 80°C for 5 min. Tea solutions were then filtered using Whatman No. 4 filter paper. Tea extracts were allowed to cool to ambient temperature prior to further analyses.

Conventional assays for antioxidant activity and total flavonoid content analysis

To test the performance of the developed paper devices, the results obtained from antioxidant activity analysis of 11 tea extracts using the paper-based assay were validated against those obtained from traditional assays including CUPRAC,

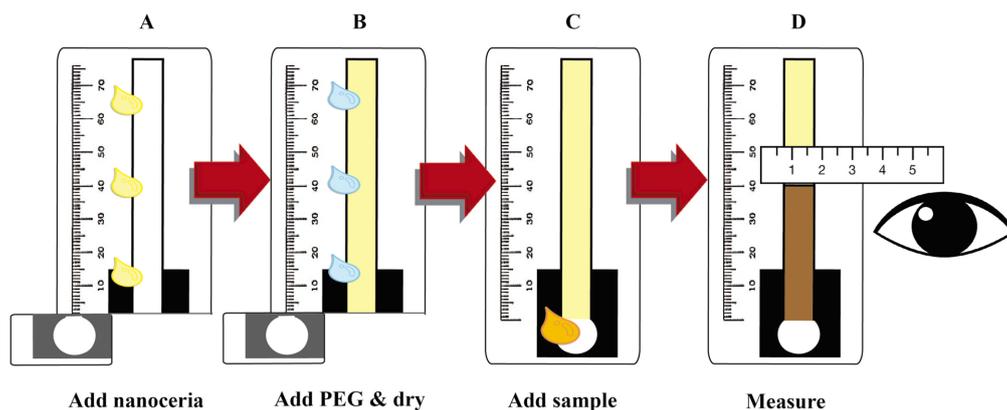


Fig. 1 Schematic representation of procedures for antioxidant activity analysis. (A) Nanoceria deposition, (B) increasing the wettability of the detection zone using PEG, (C) sample addition and (D) measurement of color distance using a common ruler.

FCR, FRAP and aluminum chloride assays. The CUPRAC, FCR and FRAP assays are used to evaluate phenolic content.^{29,30} Therefore, phenolic acids such as gallic acid were used as an antioxidant standard and the antioxidant activity of tea samples was consequently expressed as mmol gallic acid equivalent/g of tea sample (GAE). Aluminum chloride assay, on the other hand, was used to determine total flavonoid content.³¹ Therefore, we used a flavonoid compound such as EGCG as a standard and the flavonoid content of tea samples was expressed as mmol EGCG equivalent per a gram of tea sample (EGCGE). The procedure for each conventional assay is described in Supporting Information.

Results and Discussion

Optimization of the distance-based assay

Here, the antioxidant assay used nanoceria dispersion as the colorimetric redox reagent deposited along the detection channel. In the presence of an antioxidant, the nanoceria surface was partially reduced from Ce^{4+} to Ce^{3+} giving the highly reactive oxidation products and intermediates. Either product molecules or remaining parent antioxidants then bind to the nanoceria surface, generating the charge-transfer ceria-antioxidant complexes that can cause the color change from light yellow to brown in the channel.¹ The optimal nanoceria concentration and channel width were evaluated first because they affected assay sensitivity and linear range. Nanoceria dispersions in the concentration range of 1–5% (w/v) were investigated for the analysis of 10 mM GA standard. Results showed that as nanoceria concentration increased, the distance of brown color decreased (Fig. S2, Supporting Information). Lower nanoceria concentrations provided lower density of nanoceria to react with the antioxidant sample flowing from the sample zone. As a result, a longer distance is required in the detection zone to react with all of the antioxidants flowing from the sample zone. Higher nanoceria concentration, on the other hand, gave higher nanoceria density in the detection zone and hence, a shorter length of nanoceria was required to react with flowing antioxidants. Therefore, we selected 1% (w/v) of nanoceria as an optimal concentration for further experiments since the highest distance detection was obtained. We anticipated that nanoceria with the concentration lower than 1% (w/v) should give higher distance than 1% (w/v). However, based on

our preliminary experiment, it gave insufficient color intensity to observe the distance for some standards that have low antioxidant activity. Therefore, further investigation for the nanoceria with the concentration lower than 1% (w/v) has not been carried out.

We next evaluated the effect of channel width of the paper devices including 1.0, 1.5 and 2.0 mm on the detection sensitivity. A plot of measured distance as a function of GA standard concentration at each channel width is shown in Fig. S3, Supporting Information. The channel width of 1.0 mm provided high sensitivity but narrow linear range. The width of 1.5 mm can detect GA with comparable sensitivity to that of 1.0 mm but have a wider linear range. The 2.0 mm channel width showed comparable linear range to that of 1.5 mm but had lower sensitivity. These results were similar to those reported by Cate *et al.*³² as a tradeoff between linear range and sensitivity for distance-based detection paper device was observed when the channel width was evaluated. The narrower channel width provided less hydrophilic paper surface per unit distance along the flow channel and hence, longer color distance with higher sensitivity was achieved. However, at narrower channel widths, the signal became saturated at lower concentrations resulting in lower linear range. As a result, a channel width of 1.5 mm was selected as an optimal channel width as it provided high sensitivity and wide linear range and was used for further experiments.

Standard antioxidant analysis: analytical figures of merit

The detection of six standard antioxidant compounds including AA, GA, Q, CA, VA and EGCG was carried out to study the performance of the developed device for the ability to analyze several types of antioxidant compounds. For all standard analyses, the results showed that as antioxidant concentration increased, the apparent color distance on the paper device increased sharply initially and became steady at higher concentrations (Fig. S4, Supporting Information). It was also observed that different antioxidant standards gave different shades of brown color on the detection channels. This is a result of differences in the number of OH groups on the phenolic ring that can be oxidized by nanoceria and attached to nanoceria surface. The higher the number of OH groups on the phenolic ring, the darker the brown color. For example, EGCG has the largest number of hydroxyl groups (OH) on the phenolic ring (8 OH) and thus produces a darker brown color than other

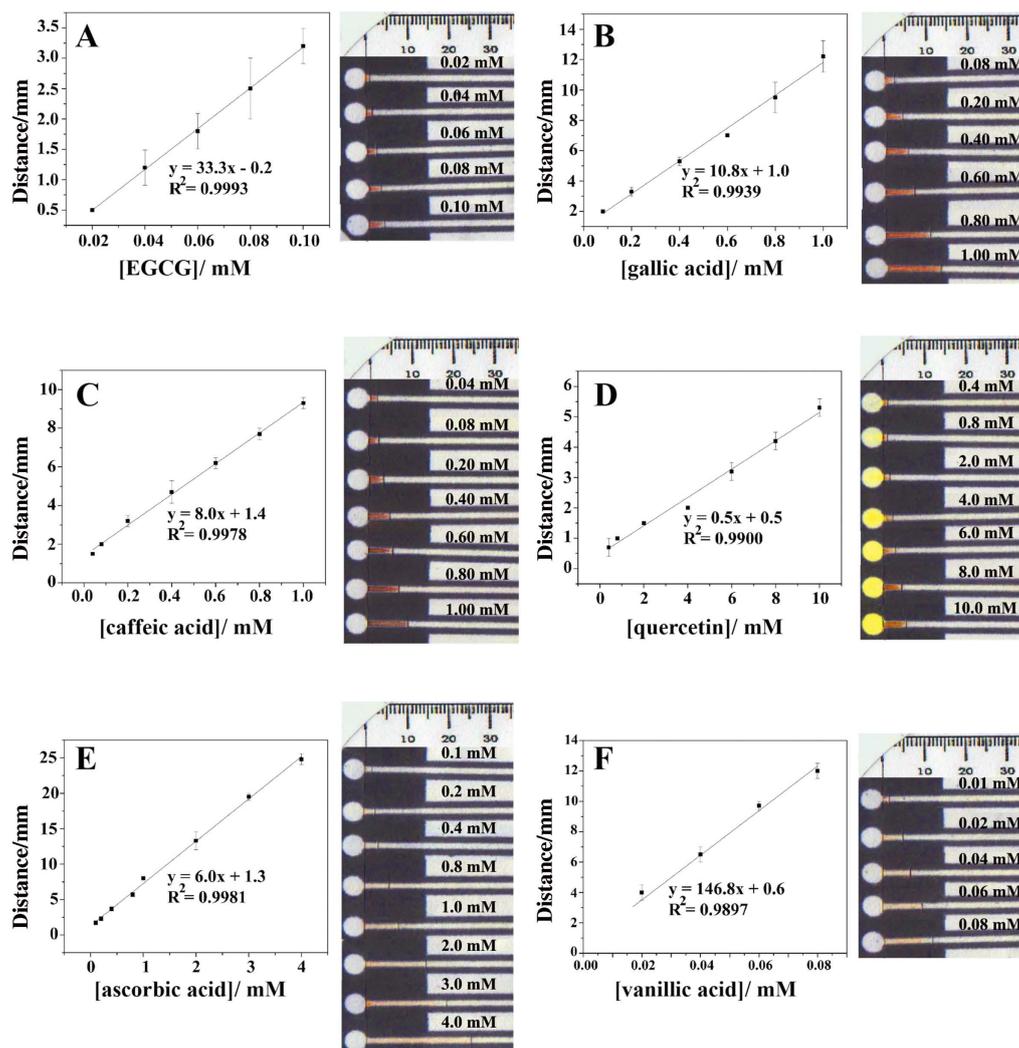


Fig. 2 Calibration curves of the distance of color development as a function of antioxidant concentration for EGCG (A) GA (B) CA (C) Q (D) AA (E) and VA (F).

antioxidants.³³ Vanilic acid, on the other hand, has the fewest OH groups on the phenolic ring (1 OH) and hence, it provided the lightest brown color. Figure 2 shows calibration curves in the linear range for all standard antioxidants studied and the corresponding obtained paper devices from the analysis at each concentration. All analytical figures of merit including linearity, reproducibility and limit of detection (LOD) were measured for all standard antioxidants and summarized in Table 1.

The reproducibility of the proposed method was quantified as the relative standard deviation (%RSD) for five replicate analyses of antioxidants at three different concentrations in the linear range using the developed devices. Good reproducibility was obtained where the %RSD was in the range of 0 - 9.5% for all evaluated standards. High inter-day reproducibility was also achieved for the analysis of 1.0 mM GA standard ($n = 5$) using the developed devices with the standard deviation of 12.7%.

The LOD for each standard antioxidant, evaluated by analyzing 10 replicates of the lowest concentration level of standard solution that gave measurable color distance,³⁴ was measured. The differences in LOD of each standard varied with the number of hydroxyl groups (OH) on the phenolic ring that can form *o*-substituted quinones, ability of each antioxidant compound for binding to the nanoceria surface and reducing

Table 1 Analytical performance characterization of the proposed method for six antioxidant compounds

Antioxidant	Analytical characterization				
	Linear range/mM	Calibration plot ^a	R^2	%RSD ($n = 5$)	LOD/ μ M
EGCG	0.02 - 0.10	$y = 33.3x - 0.2$	0.9993	1.8 - 7.5	4.0
GA	0.08 - 1.00	$y = 10.8x + 1.0$	0.9939	1.5 - 6.7	5.0
CA	0.04 - 1.00	$y = 8.0x + 1.4$	0.9978	2.0 - 9.5	6.0
Q	0.40 - 10.00	$y = 0.5x + 0.5$	0.9900	0.0 - 5.4	6.0
AA	0.10 - 4.00	$y = 6.0x + 1.3$	0.9981	0.0 - 9.4	8.0
VA	0.01 - 0.08	$y = 146.8x + 0.6$	0.9897	2.2 - 3.8	8.0

a. Unit of slope: mm/mM.

capacity from the 1,2,3-trihydroxybenzene (pyrogallol) moiety and *o*-dihydroxybenzene moiety on the compound.^{1,2,33} The higher the number of these groups, the higher the reducing activity to nanoceria on the surface, resulting in lower LOD. The antioxidants that contain pyrogallol moiety have higher reducing capacity (lower LOD) than those contain *o*-dihydroxybenzene moiety. Chemical structures and the

functional groups that play key roles in reducing capacity and antioxidant activity of evaluated standards are shown in the Supporting Information (Table S1). Dotted circles express position of pyrogallol moiety and the thick circles show the *o*-dihydroxybenzene moiety. The lowest LOD was observed for EGCG (4.0 μM) < GA (5.0 μM) < CA, Q (6.0 μM) < AA, VA (8.0 μM). EGCG has the highest activity and lowest LOD because it contains three phenolic rings (two of them are pyrogallol moieties) and the highest number of OH groups (8 OH) among all investigated antioxidants and can form *o*-substituted quinones for four positions. GA has three OH groups on the phenolic ring, which is a pyrogallol moiety, and can form *o*-substituted quinones for two positions. CA and Q have two and four OH groups on phenolic rings, respectively. Both of them contain one *o*-dihydroxybenzene moiety and can form *o*-substituted quinones for one position, resulting in a greater reactivity with nanoceria than AA and VA. The lowest activity and highest LOD were found for AA and VA. AA does not contain a phenolic acid structure but has an enol structure that is very easily oxidized to dehydroascorbate³⁵ and has two OH groups for binding to nanoceria surface while VA has only one OH group on the phenolic ring. In comparison to previous reports, our developed device provided lower LOD. For example, the μPAD provided lower LOD for AA measurement than that of the report using optical paper-based sensor assay developed by Ferreira *et al.*³⁶ in which LODs for AA was 10.5 and 82.8 μM using transmittance detection and scanner detection, respectively. The LOD for GA and CA analysis were found to be lower than those reported by Szydłowska-Czerniak *et al.*³⁷ where silver nanoparticles were used for antioxidant analysis. In that work, LODs of GA and CA were 20.0 and 9.0 μM , respectively.

Tolerance limit

The effect of potential interferences was investigated using GA as the standard antioxidant and compounds frequently found in the antioxidant-rich sample such as tea including organic acids (citric acid, tartrate, tartaric acid, oxalic acid), amino acids (glycine, alanine) and caffeine.^{2,38} Major constituents of dried green tea leaves include polyphenols, including catechins and phenolic acids (such as GA),³⁹ which are found at approximately 30 - 42% of the total dry matter. The minor components in tea are organic acids (0.5%), caffeine (3%) and amino acids (4%).³⁸ Interfering compounds were considered to affect antioxidant analysis if the measuring distances from the analysis of the mixture of 1 mM GA and interfering compound were significantly different to that of the analysis of 1 mM GA alone using pool variance *t*-test at the $P = 0.05$ confidence level. Table 2 shows that the proposed method has high tolerance to amino acids and caffeine but low tolerance to organic acids at concentrations of 2.0 - 5.0 mM. However, as has been mentioned above, the organic acids are presented in tea samples at much lower concentrations than the antioxidant compounds.

Stability of the device

The storage stability of the nanoceria-coated paper-based device was investigated under different storage conditions including ambient (desiccator), 6°C (refrigerator) and -20°C (freezer). All paper-based devices were prepared simultaneously. After preparation, all devices were sealed inside plastic zipper bags and covered with aluminum foil. A 1.0 mM GA standard solution was freshly prepared and used to test stability ($n = 3$). Figure S5 (Supporting Information) shows a plot of measured signals obtained at the three storage conditions expressed as %distance when compared with those from freshly prepared

Table 2 Effect of potential interferences for determination of GA^a

Potential interference	Tolerance limit/mM
Oxalic acid	2
Citric acid, tartrate, tartaric acid	5
Caffeine	70
Alanine	1000
Glycine	1500

a. GA concentration: 1 mM.

devices. The results demonstrate that the developed device has excellent storage stability at all three storage conditions for over 50 days. The distance decrease of the devices that were stored at room temperature was observed in the range of inter-day precision (within standard deviation of 12.7%). Therefore, the deviation or the distance fluctuation for all three storage conditions may be attributed to device variability rather than reagent degradation. This result indicated that the proposed device could be developed for a commercially ready-to-use sensor where the user need only drop the antioxidant sample on the sample zone to evaluate the antioxidant activity.

Analysis of tea samples

The performance of the proposed method was validated against the traditional assays by analyzing antioxidant activity of 11 tea samples. The proposed method was carried out by applying 20 μL aliquots of each tea sample to the sample zone without dilution and distance of developed color was measured ($n = 3$). The results obtained from the device were compared with CUPRAC, FCR and FRAP assays to evaluate the ability to determine the phenolic contents, using GA phenolic compound as the standard antioxidant and gallic acid equivalent (GAE, mmol GA/g tea) as the calculated antioxidant activity. Spearman rank correlation coefficient method was used for comparing the GAE measured by the μPAD and traditional methods. For comparison with aluminum chloride assay to investigate the performance to measure the flavonoid content of the proposed assay, the procedure was similar to the method described above excepted that EGCG was used as a flavonoid standard and the antioxidant activity of the samples was calculated in terms of EGCG equivalent (EGCGE, mmol EGCG/g tea). Table S2 (Supporting Information) summarizes the results of GAE and EGCGE for antioxidant capacity determination in tea samples obtained from the developed device and investigated traditional assays. The proposed paper devices gave antioxidant activity of the samples ranking in similar order to all conventional assays, indicating that the developed assay and the other antioxidant assays are well correlated at $P = 0.05$ level where $P < 0.002$, $P < 0.002$, $P < 0.010$ and $P < 0.002$ were obtained when the developed assay was compared with CUPRAC, FCR, FRAP and aluminum chloride assays, respectively. These results confirmed that the proposed method could be used as an alternative method for antioxidant activity analysis and could be used to determine both phenolic content as well as flavonoid content in a single measurement.

Furthermore, the accuracy of the paper-based device was evaluated through recovery experiment by spiking three different concentrations of GA (0, 1.25 and 2.50 mM) into two tea samples ($n = 3$) as shown in Table S3 (Supporting Information). Percent recovery was found in the range of 97.7 - 102.6% at 1.25 mM and 89.3 - 96.3% at 2.50 mM with precision value of 0 - 15.93%, indicating that the paper-based method gave good

accuracy for determination of antioxidants in tea samples. The applicability of the developed distance-based measurement paper assay for analysis of antioxidant activity in other samples is currently being investigated.

Conclusions

In this study, we demonstrated a distance-based detection μ PAD for determination of antioxidant activity in tea samples using nanoceria as the colorimetric probe. The antioxidant analysis is based on the partial reduction of Ce^{4+} to Ce^{3+} on the nanoceria surface in the presence of antioxidant in the sample, leading to a color change from light yellow to brown. The colorimetric response can be detected by measuring the distance of developed color on the detection zone using a common ruler. The proposed method is able to detect standard antioxidants commonly found in foods with low limit of detection and also has high tolerance to the interferences commonly found in tea samples. The developed device was also found to be stable for at least 50 days when stored at room temperature, or in a refrigerator or freezer. Moreover, the method also offers good correlation with traditional antioxidant assays. Finally, the method provided good accuracy by offering high recovery from the analysis of tea samples. These results demonstrated that the developed distance-based paper device offered accurate, rapid, low-cost, portable instrument-free and low sample and reagent analysis making it an excellent alternative assay for high throughput analysis of antioxidant activity.

Acknowledgements

This work was supported by (i) the Higher Education Research Promotion (HERP), Office of the Higher Education Commission, Ministry of Education, Thailand; (ii) DPST Research Grant 013/2557 from the Institute for the Promotion of Teaching Science and Technology, Thailand; and (iii) the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, Thailand.

Supporting Information

Detail of paper-based device fabrication, conventional assays, antioxidant chemical structures, results of optimization condition, stability and method validation is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

References

1. E. Sharpe, T. Frasco, D. Andreescu, and S. Andreescu, *Analyst*, **2013**, *138*, 249.
2. T. G. Choleva, F. A. Kappi, D. L. Giokas, and A. G. Vlessidis, *Anal. Chim. Acta*, **2015**, *860*, 61.
3. M. Koşar, F. Göger, and K. H. Can Başer, *J. Agric. Food Chem.*, **2008**, *56*, 2369.
4. J.-K. Moon and T. Shibamoto, *J. Agric. Food Chem.*, **2009**, *57*, 1655.
5. R. Apak, K. Güçlü, M. Özyürek, and S. E. Celik, *Microchim. Acta*, **2008**, *160*, 413.
6. A. Urbaniak, M. Szeląg, and M. Molski, *Comput. Theor. Chem.*, **2013**, *1012*, 33.
7. M. Leopoldini, T. Marino, N. Russo, and M. Toscano, *J. Phys. Chem. A*, **2004**, *108*, 4916.
8. L. L. Canabady-Rochelle, C. Harscoat-Schiavo, V. Kessler, A. Aymes, F. Fournier, and J.-M. Girardet, *Food Chem.*, **2015**, *183*, 129.
9. M. Özyürek, B. Bektaşoğlu, K. Güçlü, N. Güngör, and R. Apak, *Anal. Chim. Acta*, **2008**, *630*, 28.
10. D. Huang, B. Ou, and R. L. Prior, *J. Agric. Food Chem.*, **2005**, *53*, 1841.
11. D. M. Cate, J. A. Adkins, J. Mettakoonpitak, and C. S. Henry, *Anal. Chem.*, **2014**, *87*, 19.
12. L. S. A. Busa, S. Mohammadi, M. Maeki, A. Ishida, H. Tani, and M. Tokeshi, *Micromachines*, **2016**, *7*, 86.
13. L. H. Mujawar, A. A. Felemban, and M. S. El-Shahawa, *Anal. Sci.*, **2016**, *32*, 491.
14. J. Sittiwong and F. Unob, *Anal. Sci.*, **2016**, *32*, 639.
15. K. Ogawa and T. Kaneta, *Anal. Sci.*, **2016**, *32*, 31.
16. A. W. Martinez, S. T. Phillips, M. J. Butte, and G. M. Whitesides, *Angew. Chem., Int. Ed.*, **2007**, *46*, 1318.
17. E. Carrilho, A. W. Martinez, and G. M. Whitesides, *Anal. Chem.*, **2009**, *81*, 7091.
18. J. Y. Sun, C. M. Cheng, and Y. C. Liao, *Anal. Sci.*, **2015**, *31*, 145.
19. Y. Sameenoi, P. N. Nongkai, S. Nouanthavong, C. S. Henry, and D. Nacapricha, *Analyst*, **2014**, *139*, 6580.
20. H. Asano and Y. Shiraishi, *Anal. Chim. Acta*, **2015**, *883*, 55.
21. A. K. Yetisen, M. S. Akram, and C. R. Lowe, *Lab Chip*, **2013**, *13*, 2210.
22. F. Hori, Y. Harada, T. Kuretake, and S. Uno, *Anal. Sci.*, **2016**, *32*, 355.
23. K. Tominaga, S. Arimoto, K. Shimono, T. Yoshioka, F. Mizutani, and T. Yasukawa, *Anal. Sci.*, **2017**, *33*, 531.
24. D. M. Cate, W. Dungchai, J. C. Cunningham, J. Volckens, and C. S. Henry, *Lab Chip*, **2013**, *13*, 2397.
25. M. Ornatska, E. Sharpe, D. Andreescu, and S. Andreescu, *Anal. Chem.*, **2011**, *83*, 4273.
26. F. Pena-Pereira, I. Lavilla, and C. Bendicho, *Talanta*, **2016**, *147*, 390.
27. C. Xu and X. Qu, *NPG Asia Materials*, **2014**, *6*, 90.
28. Y. Zhai, Y. Zhang, F. Qin, and X. Yao, *Biosens. Bioelectron.*, **2015**, *70*, 130.
29. R. L. Prior, X. Wu, and K. Schaich, *J. Agric. Food Chem.*, **2005**, *53*, 4290.
30. R. Pulido, L. Bravo, and F. Saura-Calixto, *J. Agric. Food Chem.*, **2000**, *48*, 3396.
31. L. A. L. da Silva, B. R. Pezzini, and L. Soares, *Pharmacogn. Mag.*, **2015**, *11*, 96.
32. D. M. Cate, S. D. Noblitt, J. Volckens, and C. S. Henry, *Lab Chip*, **2015**, *15*, 2808.
33. P. A. Kilmartin and C. F. Hsu, *Food Chem.*, **2003**, *82*, 501.
34. D. A. Armbruster, M. D. Tillman, and L. M. Hubbs, *Clin. Chem.*, **1994**, *40*, 1233.
35. S. B. Nimse and D. Pal, *RSC Adv.*, **2015**, *5*, 27986.
36. D. C. M. Ferreira, G. F. Giordano, C. C. d. S. P. Soares, J. F. A. de Oliveira, R. K. Mendes, M. H. Piazzetta, A. L. Gobbi, and M. B. Cardoso, *Talanta*, **2015**, *141*, 188.
37. A. Szydłowska-Czerniak, A. Tułodziecka, and E. Szlyk, *Analyst*, **2012**, *137*, 3750.
38. Q.-Y. Shi and V. Schlegel, *Agriculture*, **2012**, *2*, 393.
39. G.-J. Du, Z. Zhang, X.-D. Wen, C. Yu, T. Calway, C.-S. Yuan, and C.-Z. Wang, *Nutrients*, **2012**, *4*, 1679.