Use of a Smartphone as a Colorimetric Analyzer in Paper-based Devices for Sensitive and Selective Determination of Mercury in Water Samples

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A smartphone application, called CAnal, was developed as a colorimetric analyzer in paper-based devices for sensitive and selective determination of mercury(II) in water samples. Measurement on the double layer of a microfluidic paper-based analytical device (μPAD) fabricated by alkyl ketene dimer (AKD)-inkjet printing technique with special design doped with unmodified silver nanoparticles (AgNPs) onto the detection zone was performed by monitoring the gray intensity in the blue channel of AgNPs, which disintegrated when exposed to mercury(II) on μPAD. Under the optimized conditions, the developed approach showed high sensitivity, low limit of detection (0.003 mg L⁻¹, 3SD blank/slope of the calibration curve), small sample volume uptake (two times of 2 μL), and short analysis time. The linearity range of this technique ranged from 0.01 to 10 mg L⁻¹ ($r^2 = 0.993$). Furthermore, practical analysis of various water samples was also demonstrated to have acceptable performance that was in agreement with the data from cold vapor atomic absorption spectrophotometry (CV-AAS), a conventional method. The proposed technique allows for a rapid, simple (instant report of the final mercury(II) concentration in water samples via smartphone display), sensitive, selective, and on-site analysis with high sample throughput (48 samples h⁻¹, $n = 3$) of trace mercury(II) in water samples, which is suitable for end users who are unskilled in analyzing mercury(II) in water samples.

Keywords Mercury, silver nanoparticles, smartphone, RGB system, ImageJ, microfluidic paper-based analytical device, cold vapor atomic absorption spectrophotometry, CV-AAS

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Introduction

Increasing human activities and rapid industrialization are generating a large volume of pollutants in the environment. Water contamination by heavy metals, especially mercury (Hg), is one of the most serious problems worldwide. Such contamination of natural water bodies puts human health and ecosystems at risk because only a minute amount of mercury is needed to cause health problems affecting the nervous system, urinary system, reproductive system, immune system, cardiovascular system, and endocrine system.¹² All species of mercury (Hg) are considered hazardous to human health and thus mercury was ranked third on the “Substance Priority List” by the Agency for Toxic Substances and Disease Registry (ATSDR) from 1991 - 2015.¹³ Hg²⁺ or mercuric ion is used as a substitute for inorganic mercury, which is one of the most hazardous environmental contaminants. Hg²⁺ is the most stable oxidation state because it is a soluble form and is easier to contaminate water resources.⁴⁻⁶ Maximum acceptable Hg²⁺ concentration (mg L⁻¹) levels in waters, especially in drinking water, have been established by various agencies (Table S1, Supporting Information). Therefore, an initial step of water pollution prevention involving accurate, rapid, sensitive, selective and simple methods for determination of trace Hg²⁺ in water samples are a critically important link in the nexus between monitoring and water quality management.

Conventionally, techniques for highly sensitive and selective determination of trace Hg²⁺ in water samples have been established at well-equipped laboratories using manual or automated cold vapor atomic absorption spectrophotometry (CV-AAS) and related atomic spectrophotometry techniques.⁷⁻⁸ However, these high performance techniques require high-cost instruments and special expertise of operators in sample preparation steps.⁹ For resource-limited settings, such as field sites in rural or developing regions,¹⁰ the aforementioned infrastructure is often lacking. Therefore, an alternative method for on-site water monitoring and screening that is inexpensive and can be easily operated even at home by unskilled operators is in demand. Moreover, on-site sample analysis eliminates the need for sample storage prior to analysis, minimizes losses of
the sample during collection and can provide data to instantly support management decision-making. A well-known microfluidic paper-based analytical device (µPAD), as an alternative low-cost, portable, and disposable analysis device, offers the possibility to construct new sensors for Hg²⁺ monitoring in water samples that exhibit the aforementioned desirable features.

Recently, the proof of concept for a µPAD for the determination of Hg²⁺ in water using chromatic analysis based on a simple red green blue (RGB) color model has been developed by our group. The µPAD was fabricated by alkyl ketene dimer (AKD)-inkjet printing and doped with our synthetic unmodified silver nanoparticles (AgNPs), which progressively vanished away from deep yellow to brighter yellow with increasing Hg²⁺ ion concentration in water samples, corresponding to color intensity change. The zone of images was recorded by a digital camera under an optimized control light box and the color intensity signal was further analyzed using ImageJ image processing software through the grayscale (gray intensity) in blue channel (Gb). The reaction mechanism of unmodified AgNPs with Hg²⁺ ion on the double layer µPAD can be explained as AgNPs being oxidized by Hg²⁺, resulting in desintegration of the AgNPs into smaller particles and reduction of Hg²⁺ to elementary Hg. Subsequent deposition of elementary Hg onto the surface of AgNPs also occurred, resulting in amalgam particles of mercury (Hg-Ag). Our previous prototype provided high sensitivity, a low limit of detection, short analysis time and good selectivity towards Hg²⁺ determination against other studied foreign ions in waters. Moreover, the benefits of the µPAD application compared to the same reaction in bulk solution include the advantages of simplicity in color change monitoring (by a camera), small sample volume uptake (2 μL) and minimized interference effects from suppression of charge stabilized unmodified AgNPs due to the fact that unmodified AgNPs are located inside the cellulose matrix instead of dispersed in the bulk aqueous circumstance. In addition, application of spherical AgNPs offered adjustable sensitivity depending on their particles concentration. Our previous prototype was expected to be applicable for low cost small-scale monitoring of Hg²⁺ in environmental and drinking water samples.

However, there is still room for improvement, especially regarding the detection system for µPAD in the case that intensity signal was recorded by a digital camera under an optimized control light box using image processing software. We found that the data interpretation was still unsuitable for on-site analysis with high sample throughput of trace Hg²⁺ in water samples, nor was it suitable for an end user who is unskilled in analyzing Hg²⁺ in the water samples, due to the fact that image processing needs to be performed externally with an application due to the low cost of most Android-based smartphones, the availability of open source codes and free license programs, and the large community of developers.

Using smartphones in the colorimetric determination for sensitive and selective determination of Hg²⁺ in water samples on µPAD with full integration of process (on-board application) of smartphones have not been reported elsewhere. In this work, we developed a novel use case in which a smartphone is used as a colorimetric analyzer on µPAD for sensitive and selective determination of Hg²⁺ in water samples by an unskilled user for analyzing Hg²⁺ in the water samples under a resource-limited setting with two important features: (i) development of an android application for the colorimetric determination based on a simple RGB color model for sensitive and selective determination of Hg²⁺ in water samples (instant report of the final Hg²⁺ concentration in water samples via smartphone display) under new design of µPAD combined with housing light controlling system (number of LED lights, the background color of device and flash conditions were also optimized and compared to a conventional detection system on µPAD (digital camera coupled with ImageJ software operated by computer)), and (ii) investigation of the developed Android smartphone application in different Android smartphones (with different display resolutions). Furthermore, methods for validating the developed system in practical analysis of various water samples were also performed by comparison to the data obtained from the reference method using cold vapor atomic absorption spectrophotometry (CV-AAS).

**Experimental**

**Reagents and chemicals**

All solutions were prepared in deionized water with 18 M resistance obtained from a Millipore Milli-Q purification system (Bedford, MA, USA) and all chemicals were analytical grade. Silver nitrate (AgNO₃), sodium borohydride (NaBH₄) and trisodium citrate dihydrate (C₃H₅O₃Na·2H₂O) purchased from Carlo Erba were used to prepare a high concentration (NPs mL⁻¹) of highly stable unmodified AgNPs (8.3 ± 1.4 nm) using the chemical reduction method according to our previous report. A stock solution of 50 mg L⁻¹ Hg²⁺ was freshly prepared from mercury(II) chloride (HgCl₂) obtained from Baker analyzed. Standard Reference Material (SRM) of mercury in water (1641d) with the certified value of mercury (1.557 ± 0.020 mg kg⁻¹) was purchased from the National Institute of Standards and Technology (NIST, US). All glassware were immersed in 10% (v/v) of nitric acid (HNO₃) for 24 h, thoroughly cleaned with aqua regia (volume ratio of HNO₃:hydrochloric acid (HCl), 1:3) and washed twice with deionized water prior to use. The solid form of alkyl ketene dimer (AKD wax88 konz), BASF, was dissolved in n-heptane (C₇H₁₆). Carlo Erba, and applied as hydrophobilizing agent on the µPAD surface. The µPAD was fabricated from Whatman filter paper grade 1 with a thickness of 0.180 mm by using a Canon PIXMA iP7270 inkjet printer modified with a custom-filled printer cartridge containing 3% (w/v) AKD with slight modification.

**Development of smartphone as colorimetric analyzer on double-layer µPAD for Hg²⁺ determination**

A new idea for the design of the on-demand µPAD was a hydrophilic circular area with 4 mm inner diameter, which established 10 branches for 30 detection zones (three at each branch, for triplicate analysis). Two pieces of fabricated µPAD were attached together with a glue stick, creating a piece of double-layer µPAD as demonstrated in Figs. S1a - S1c (Supporting Information). The detection zone generated on the double-layer µPAD was 3.96 ± 0.17 mm (Table S3, Supporting Information). Double-layer µPAD was coated with as-prepared...
unmodified AgNPs. Double doped 2 μL of unmodified AgNPs (drop-dried-drop) were then applied to the detection zone of the double-layer μPAD by micropipette at damp-dried state (Fig. S1d, (1), Supporting Information) and 2 μL of Hg²⁺ standards (10, 50, 500, 1000, 7000 and 10000 μg L⁻¹ for 7-point calibration or 10, 100 and 1000 μg L⁻¹ for 3-point calibration) were then applied. High throughput of samples analysis (48 sample h⁻¹, ~12 min for nine samples at maximum including blank analysis (n = 3), Fig. S2, Supporting Information) can also be accomplished with one double layer μPAD (Fig. S1d, (2-3), Supporting Information). Only gray intensity in the blue channel (Gₐ) (since the most sensitive response to the change of Hg²⁺ ions concentration were observed)²₈ on the detection zone of the μPAD were recorded by smartphone camera with the homemade software application installed (called “CAnal”) under housing light controlling system made from PVC pipe (8 × 9 cm high) with LED white light (Fig. S3, Supporting Information). The determination of Hg²⁺ by using a smartphone as a colorimetric analyzer on a double-layer μPAD is demonstrated in Scheme 1. The specifications of Android smartphones used in this study are shown in Table S4 (Supporting Information).

The smartphone camera resolution was set to 8 megapixels (MP) throughout the photographic analysis in this study. A mobile phone was fixed on the front of the housing light controlling system at the marked center position of the system in order to maintain good focus on the detection zone of the double-layer μPAD. The homemade CAnal application Ver. 4.0 was further modified from the previous version.²⁹ The homemade CAnal Ver. 4.0 software application was developed in Java platform and compiled to run on the Android operating system. Then, 1600 colored pixel values (40 × 40 pixels; measured by pixel skip pixel) located in the circular pointer, which were matched to the detection zone of the double-layer μPAD, were read and averaged for only gray intensity in blue channel (Gₐ). The intensity of Gₐ was shown to be 0 for completely dark or black image and 255 for a white image. The homemade CAnal application was developed using Eclipse IDE.²¹ The flowchart of the program is described in Fig. S4 (Supporting Information). Comparison of the current version of the CAnal application (Ver. 4.0) to the previous version (Ver. 2.0) is also illustrated in Table S5 (Supporting Information). The operation of the CAnal Ver. 4.0 application on a smartphone for establishment of calibration curve and sample analysis are explained in Fig. S5 (Supporting Information). Optimization of housing light controlling system conditions, including number of LED lights, the background color of the device and flash conditions for the colorimetric analyzer on a μPAD for Hg²⁺ determination, was also carried out.

Before sample analysis, the user needs to construct a calibration curve (at least 3-point calibration). The calibration curve is then recorded under a housing light controlling system (Figs. S4 and S5, Supporting Information). The calibration curve of three different synthesis batches of unmodified AgNPs generated by an Android smartphone camera with the homemade CAnal installed were compared to a conventional detection system on μPAD (digital camera coupled with ImageJ software operated by computer).¹⁸ For sample analysis of Hg²⁺ (Fig. S5, Supporting Information), the sample solutions were applied to the double-layer μPAD coated with the as-prepared unmodified AgNPs, and then the picture was taken by the user.

The circle pointer to the corresponding detection area of double-layer μPAD picture was placed by the user. The CAnal application processes the image information from the selected area and converts them into analyte concentrations and the report was instantly shown in ppb (μg L⁻¹) unit by using the established calibration curve on CAnal application. Due to the fact that different smartphone displays have different resolutions, the performance of the developed CAnal application in different Android smartphones was also investigated.

Real water sample application and method validation

The use of smartphones with our developed application, called CAnal, installed to be used as a colorimetric analyzer on double-layer μPAD for sensitive and selective Hg²⁺ determination was practical, according to the analyses of various water samples that were validated to the data obtained from cold vapor atomic absorption spectrophotometry (CV-AAS) used as the conventional method. Samples of commercial brand drinking water and mineral water were obtained from a convenience store in Thailand. Samples of tap water and pond water were collected from Ubon Ratchathani province in Thailand. The recovery for all of the water samples were investigated by two spiked concentrations of Hg²⁺ standard at 0.025 and 0.05 mg L⁻¹, respectively. For our developed smartphone approach, only one-step filtration using 0.45 μm PVDF syringe filter
(Simplepure) was applied prior to sample analysis. For method validation using CV-AAS, all water samples as mentioned above were prepared and analyzed according to the Method 245.1, Rev. 3.0: Determination of Mercury in Water by Cold Vapor Atomic Absorption Spectrometry (CV-AAS) of the United States Environmental Protection Agency (EPA). Then, 100 mL of water sample was contained in a 300-mL BOD bottle. Next, 5 mL of 98% (v/v) of concentrated sulfuric acid (Sigma-Aldrich) and 2.5 mL of 65% (v/v) of concentrated nitric acid (Sigma-Aldrich) were then added to the sample contained in the BOD bottle and 15 mL of 5% (w/v) of potassium permanganate (Carlo Erba) was subsequently added. The as-prepared sample in the BOD bottle was shaken and kept in ambient conditions for 15 min (at which point the persistence of purple color was observed). Then, 8 mL of 5% (w/v) potassium persulfate (Carlo Erba) was added to the as-prepared sample in the BOD bottle, and was further mixed and heated at 95°C for 2 h in a water bath. The as-prepared sample in the BOD bottle was cooled down at ambient conditions. Next, 6 mL of 12% (w/v) sodium chloride (Carlo Erba)-hydroxylammonium chloride (BDH) solution was added to the sample to remove an excess of permanganate and analyzed by CV-AAS (PinAAcle 900T-FIAS 100, Perkin Elmer, US) technique with the operation conditions listed in Table S4 (Supporting Information). Moreover, the Standard Reference Material (SRM) of mercury in water (1641d) with the certified value of mercury in water being 1.557 ± 0.020 mg kg⁻¹ (NIST; US) was also evaluated in this study.

Results and Discussion

Optimization of smartphone installed with homemade CAnal application as colorimetric analyzer

Variations of environmental illumination on the double-layer μPAD for Hg²⁺ determination by using a smartphone with the homemade CAnal application installed to be used as a colorimetric analyzer under a housing light controlling system include the number of LED lights (2 – 5 LED), the background color of the device (black and white) and flash conditions (Fig. 1 and Fig. S6, Supporting Information). Three LEDs illuminations with black background were chosen to be the optimized condition considering the highest slope and r² value of standard calibration curve of Hg²⁺ determination. The results also indicated that the flash is necessary for improving and controlling constant illumination under the housing light controlling system (Fig. S6b, Supporting Information).

The calibration curve (0.01 – 10 mg L⁻¹) plotted between the log(G₁-G₀) value and concentration of Hg²⁺, mg L⁻¹ comparing three syntheses of unmodified AgNPs at different batches generated by digital camera coupled with ImageJ software operated by computer²⁰ were shown in good agreement when compared to the results obtained by Android smartphone camera (smartphone 1) with the developed homemade CAnal application installed, at a 95% confidence level (Fig. 2 and Table S6, Supporting Information). This suggests that our developed homemade CAnal application to be used as colorimetric analyzer on double-layer μPAD for Hg²⁺ determination under a housing light controlling system (three LED illuminations with black background and flash) can overcome the shortcomings of the typical digital camera under optimized control light box conditions using image processing software with complicated data interpretation performed externally with a computer.

Analytical characteristics and real sample application

Using the optimum conditions as mentioned above, the developed approach showed high sensitivity, low limit of detection (0.0029 ± 0.0003 mg L⁻¹, 3SD blank/slope of the calibration curve), small sample volume uptake (two times of 2 μL) and short analysis time (Fig. S2, Supporting Information). The linearity range of this technique was generated by 7 (full) points and ranged from 0.01 to 10 mg L⁻¹, y = (0.2856 ± 0.0148)x + (0.7345 ± 0.0129), r² = (0.993 ± 0.007)). The results were reproducible among three batches of unmodified AgNPs synthesis. Moreover, practical analysis of various water samples was also performed. Table 1 shows the results of Hg²⁺ determination in various water samples including SRM of mercury with the certified value of mercury in water (1641d) using the conventional method (CV-AAS) compared with the results obtained from our developed smartphone application (CAnal) installed on the smartphone (smartphone 1) to be used as colorimetric analyzer on double-layer μPAD (linear equation was established in CAnal application only once at the beginning before sample analysis and used this established standard calibration was used throughout the sample analysis (Figs. S4 – S5, Supporting Information). The results obtained from 7-point (full) standard calibration were not significantly different versus the results obtained from 3-point standard calibration (y = (0.2363 ± 0.0164)x + (0.3339 ± 0.0208), r² = (0.973 ± 0.006)) (Table 1). Meanwhile, CV-AAS was performed as the reference method to evaluate the Hg²⁺ content and the results were compared with the proposed method.

Fig. 1 Performance of standard calibration curve of Hg²⁺ reported in slope ■ and r² ■ values at various conditions including background color (black and white) of housing light controlling system, and number of LED lights (2, 3, 4, and 5) using smartphone 1 installed with the developed homemade CAnal application.
Fig. 2 The calibration curve (0.01 – 10 mg L\(^{-1}\)) plotted between the \(\log(G_\text{B}-G_{\text{B}0})\) value and concentration of Hg\(^{2+}\), \(\mu g\) L\(^{-1}\) comparing three syntheses of unmodified AgNPs at different batches; operated by digital camera coupled with ImageJ software operating by computer a) and smartphone 1 installed with the developed homemade CAnal application b). Inset: the average of three calibration curves; small error bar indicates the relative standard deviation of three repeated experiments \((n = 3)\) 

| Water sample       | Conventional method (CV-AAS) | Smartphone colorimetric analyzer |                                |                                |                                |                                |
|--------------------|-----------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                    | Hg\(^{2+}\)/\mu g L\(^{-1}\) | Recovery, \(\%\)                | Established linear equation generated by 7 points standard calibration \(y = (0.2856 \pm 0.0148)x + (0.7345 \pm 0.0129)\) | Established linear equation generated by only 3 points standard calibration \(y = (0.2363 \pm 0.0164)x + (0.3339 \pm 0.0208)\) |                                |                                |
|                    | Added          | Found          | Recovery, \(\%\) | Added          | Found          | Recovery, \(\%\) | Added          | Found          | Recovery, \(\%\) |
| Drinking water     | 25            | 25.97 \pm 0.75 | 103.89           | 25            | 23.76 \pm 0.78 | 95.04           | 25            | 23.76 \pm 0.78 | 95.04           |
|                    | 50            | 50.21 \pm 2.92 | 100.42           | 50            | 51.8 \pm 3.59  | 103.76          | 50            | 51.8 \pm 3.59  | 103.76          |
| Mineral water      | 25            | 23.91 \pm 2.14 | 95.62            | 25            | 24.06 \pm 3.47 | 96.24           | 25            | 24.06 \pm 3.47 | 96.24           |
|                    | 50            | 49.26 \pm 3.10 | 98.53            | 50            | 50.66 \pm 4.66 | 101.32          | 50            | 50.66 \pm 4.66 | 101.32          |
| Tap water          | 25            | 23.17 \pm 1.28 | 92.67            | 25            | 23.20 \pm 0.94 | 92.78           | 25            | 23.20 \pm 0.94 | 92.78           |
|                    | 50            | 53.31 \pm 3.72 | 106.63           | 50            | 57.29 \pm 3.25 | 114.58          | 50            | 57.29 \pm 3.25 | 114.58          |
| Pond water         | 25            | 26.93 \pm 1.06 | 107.73           | 25            | 25.44 \pm 2.09 | 101.74          | 25            | 25.44 \pm 2.09 | 101.74          |
|                    | 50            | 52.81 \pm 3.98 | 105.62           | 50            | 49.30 \pm 0.93 | 98.59           | 50            | 49.30 \pm 0.93 | 98.59           |
| SRM\(^a\)          | —             | —              | —                | 1570.00        | —              | —                | 1537.28        | —              |

a. Standard Reference Material 1614d (Hg\(^{2+}\) in water = 1.557 \pm 0.020 mg kg\(^{-1}\)).

(Table 1). According to the evaluation of the t-test at the 95\% confidence limit level, the experimental t\(_{\text{star}}\) value was less than the t\(_{\text{critical}}\) value (Table S7, Supporting Information). This finding indicates that only 3-point calibration generated in smartphone with the homemade CAnal application installed to be used as colorimetric analyzer on double-layer \(\mu\)PAD are necessary once at the beginning (the calibration equation has been stored throughout all sample analysis process). Furthermore, the results for the precision obtained from intra-day with different users and inter-day using the same established calibration equation (linear equation was established in the CAnal application once at the beginning before sample analysis) also suggested that the established calibration equation stored in the CAnal application was needed only once in the beginning before sample analysis (Table 2). Moreover, the recovery study for all of the water samples were acceptable by spiked concentrations of Hg\(^{2+}\) standard. In addition, the performance of the developed CAnal application installed as colorimetric analyzer on double-layer \(\mu\)PAD for Hg\(^{2+}\) determination was also investigated using three different Android smartphones (with different resolution of smartphone display, shown in Table S4, Supporting Information). The results from all smartphones, as demonstrated in Fig. 3, were obtained from 3-point standard calibration operated with two spiked concentrations of Hg\(^{2+}\) standard at 25 and 50 \(\mu g\) L\(^{-1}\) in water samples including drinking water, mineral water, tap water and pond water. The results demonstrated good accuracy and were not significantly different across three different Android smartphones at a 95\% confidence limit level (Table S8, Supporting Information). This finding
Camera resolution was set up to 8 megapixels (MP) throughout the photographic analysis, which allowed for the picture resolution of μPADs to be 8 MP (the optimal condition). It was sufficient to generate the proper detection area (colored pixels) located in the circular pointer of different resolutions on smartphone displays and sizes that was matched to the detection zone of μPAD, taking into account the size of the detection area (colored pixels) and diameter of circular pointer generated on smartphone displays (Fig. S7b, Supporting Information). As shown, a μPAD picture with resolution of 2.4 MP (Fig. S7c, Supporting Information) was compared to that with resolution of 13 MP (Fig. S7d, Supporting Information). Nowadays, most smartphone cameras have a display resolution of around 1 MP for a typical smartphone to just over 3MP for the higher-end varieties (e.g. retina display),24 which corresponded to our study of display resolution of various smartphone cameras shown in Table S4 (Supporting Information). Our developments in this work suggest that the proposed colorimetric analyzer device offers great potential for the on-site determination of Hg²⁺ in water samples with reliable results and is suitable for an end user who is unskilled in analyzing Hg²⁺ in the water samples.

### Conclusions

The developed system based on the use of a smartphone as a colorimetric analyzer that is fully integrated with our homemade Canal application under a housing light controlling system (three LED illuminations with black background and flash) on a paper-based device offers great potential with obvious advantages for the on-site (such as field sites in rural or developing regions) determination of Hg²⁺ in water samples. The system provides reliable results that are comparable to those obtained using the complicated laboratory analytical instrument that requires difficult sample preparation (CV-AAS). Our developed system can facilitate a rapid, simple (instant report of the final Hg²⁺ concentration in water samples via smartphone display), sensitive (providing a low detection limit and wide dynamic range of detection: a significant reduction in the Hg²⁺ LOD (to as low as 1 μg L⁻¹)) is achieved by in situ preconcentration (multiple applications of the test zone on μPAD), selective (minimized interference effects from foreign ions suppression of charge stabilized unmodified AgNPs located inside the paper matrix) and high throughput of samples analysis (48 sample h⁻¹, ~12 min for nine samples at maximum including blank analysis (n = 3), Fig. S2, Supporting Information). These advantages allow end users who are relatively unskilled to

### Table 2: Precision study (intra-day; with different subjects and inter-day) of Hg²⁺ determination in various water samples using smartphone 1 colorimetric analyzer (results obtained from established linear equation generated by only 3-point standard calibration) (n = 3)

| Water sample | Hg²⁺/μg L⁻¹ | Recovery, % | RSD   | Hg²⁺/μg L⁻¹ | Recovery, % | RSD   |
|--------------|-------------|-------------|-------|-------------|-------------|-------|
|              | Added       | Subject     | Found |             | Added       | Subject     | Found |
| Drinking water | 50 | 1 | 51.80 ± 3.59 | 103.76 | 6.92 | 50 | 45.52 ± 1.77 | 91.04 | 3.88 |
| Mineral water | 50 | 2 | 53.75 ± 1.81 | 107.50 | 3.37 | — | — | — |
| Tap water    | 50 | 1 | 53.10 ± 1.57 | 106.20 | 2.96 | 50 | 47.37 ± 4.01 | 94.74 | 8.46 |
| Pond water   | 50 | 2 | 51.23 ± 3.36 | 102.46 | 6.56 | — | — | — |

Fig. 3 Comparison of different smartphones as colorimetric analyzer with Canal application Ver. 4.0 installed for determination of two spiked Hg²⁺ in water samples; 25 μg L⁻¹ (△, △ mobile phone 1, 2 and 3, respectively) and 50 μg L⁻¹ (●, ● mobile phone 1, 2 and 3, respectively) of Hg²⁺.

indicates that smartphone cameras operated in a sufficiently standardized manner across different resolutions of smartphone display are effective for Hg²⁺ determination on double-layer μPAD using the developed Canal application installed in Android smartphones as colorimetric analyzer. The mechanism that allows this standardization to occur can be explained as follows. Firstly, colored pixels located in the circular pointer of different resolutions of smartphone displays were matched to the detection zone of μPADs as demonstrated in Fig. S7a (Supporting Information). The circular pointer in this investigation was set to 30 pixels. Once the Canal application was installed in different Android smartphones (with different resolutions of smartphone displays and sizes), differences in diameter of the circular pointer were observed. However, the relationship between the circular pointer and detection area (colored pixels) of different smartphone displays and sizes generated on different resolutions of μPAD pictures have also been investigated (Figs. S7b - S7d, Supporting Information). This indicates that the relationships among picture resolution, resolution on smartphone and display size are a key element of this investigation. In this study, the smartphone camera resolution was set up to 8 megapixels (MP) throughout the photographic analysis, which allowed for the picture resolution of μPADs to be 8 MP (the optimal condition). It was sufficient to generate the proper detection area (colored pixels) located in the circular pointer of different resolutions on smartphone displays and sizes that was matched to the detection zone of μPAD, taking into account the size of the detection area (colored pixels) and diameter of circular pointer generated on smartphone displays (Fig. S7b, Supporting Information). As shown, a μPAD picture with resolution of 2.4 MP (Fig. S7c, Supporting Information) was compared to that with resolution of 13 MP (Fig. S7d, Supporting Information). Nowadays, most smartphone cameras have a display resolution of around 1 MP for a typical smartphone to just over 3MP for the higher-end varieties (e.g. retina display),24 which corresponded to our study of display resolution of various smartphone cameras shown in Table S4 (Supporting Information). Our developments in this work suggest that the proposed colorimetric analyzer device offers great potential for the on-site determination of Hg²⁺ in water samples with reliable results and is suitable for an end user who is unskilled in analyzing Hg²⁺ in the water samples.
effectively analyze Hg\(^{2+}\) in water samples in resource-limited settings. Moreover, our developed smartphone application to be used as a colorimetric analyzer could lead the development of other interesting analytes with broad applications, offering a convenient and cost-effective alternative to the conventional laboratory-based optical equipment.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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