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Simple silver nanoparticle colorimetric sensing for copper by paper-based devices

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ABSTRACT

The first investigation of silver nanoparticle (AgNP) colorimetric sensing of Cu^{2+} by paper-based analytical devices (PADs) is reported here. AgNP colorimetric sensing for the detection of Cu^{2+} was first characterized by UV–visible spectroscopy. The –SH groups on homocysteine (Hcy) and dithiothreitol (DTT) were used to modify the AgNP surface whereas the –COOH and $-NH_2$ functional groups have strong affinity to Cu^{2+} relative to other ions in solution. The plasmon resonance absorption peak intensity at 404 nm decreased and a new red-shifted band at 502 nm occurred in the presence of Cu^{2+}. Paper devices coated with the modified AgNP solution changed from yellow to orange and green-brown color after the addition of Cu^{2+} due to nanoparticle aggregation. The color intensity change as a function of Cu^{2+} concentration gave a linear response in the range of 7.8–62.8 μM ($R^2=0.992$). The limit of naked-eye detection is 7.8 nM or 0.5 μg L^{-1}. A color change observed by the naked eye with the addition of Cu^{2+} can be clearly differentiated from the other metals (As^{3+}, Cd^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, Ca^{2+}, Fe^{3+}, Na^{+}, and K^{+}) at 15.7 μM. The use of different flow directions in the PADs and μPADs for Cu^{2+} detection was also demonstrated. Levels of Cu^{2+} in real water samples were measured using the paper devices to be 2.9 ± 0.24 μM (tap water) and 3.2 ± 0.30 μM (pond water), respectively, and were within error of the values measured using an atomic absorption spectrometer (2.8 ± 0.08 μM in tap water, and 3.4 ± 0.04 μM in pond water). Thus, this work shows the successful integration of paper devices and AgNP colorimetric sensing as a simple, rapid, easy-to-use, inexpensive and portable alternative point-of-measurement monitoring.

1. Introduction

The development of simple, rapid, and inexpensive detection method for diagnostics and environmental monitoring is still needed in developing and undeveloped countries for sustainable development in public health [1]. Currently, paper devices including paper strip tests, paper-based assays, and paper-based microfluidics are attractive for portable point-of-measurement (POM) monitoring and on-site detection due to advantages which include low cost, portability, ease of use, high speed, and low reagent and sample consumption [2–4]. Furthermore, paper provides a high surface area/high optical contrast substrate for colorimetric detection. Paper devices are also suited for use by nontechnical personnel. Finally, paper devices are made from naturally abundant, biodegradable and inexpensive materials (i.e., cellulose) [5,6]. Most commercial paper devices use colorimetric detection and have been applied for qualitative and/or semi-quantitative analyte detection [1,7–11]. For colorimetric sensing, analyte flow is directed along the paper matrix by capillary action, and analyte then reacts with a label or organic dye in the test zone. The assay results are quantified by comparing the color hues and/or intensities generated by unknown analytes to known analyte concentrations. Nontechnical personnel can interpret the result by the naked eye in many cases. Paper devices therefore do not require external instrumentation for interpretation or mechanical forces to drive flow.

Gold nanoparticle (AuNP) colorimetric probes have been widely used for colorimetric assays because their extinction coefficients are high relative to common organic dyes [12–17]. AuNP colorimetric sensing has been used with two approaches. First, the result can be determined by the accumulation of an intense red color that is associated with the color of AuNPs with a diameter of 10–50 nm. The most common example of this detection mode is the commercial immunochromatographic test strip [18,19]. Alternatively, the color change of AuNPs due to aggregation or

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dispersion with the addition of analyte can be used [20,21]. For example, Lu and coworker first reported the AuNP aggregation in a dipstick assay [22]. Their devices detect analyte by immersing a lateral flow device immobilized with aggregated AuNPs into a testing solution. The blue aggregated AuNPs turned red when dispersed by the analyte in a concentration dependent manner. Inspired by Lu's work, Zhao and coworkers demonstrated the feasibility of using a AuNP-based colorimetric sensing platform on paper devices [23].

Recently, silver nanoparticles (AgNPs) have been used as an alternative colorimetric sensing system due to lower cost and higher extinction coefficients than AuNPs [24]. The high molar extinction coefficient of AgNPs leads to improved visibility based on the difference in optical brightness and increased sensitivity of UV–visible spectroscopic detection [25]. Although colorimetric applications of AgNP aggregation provide high sensitivity and selectivity for the determination of analytes such as histidine [26], homocysteine [25], cysteine [27], mercury ions [28,29], and nickel ions [30] using UV–visible spectroscopy, AgNP colorimetric sensing for copper ion by PADs has not been reported [31]. The only analytical application of AgNPs on cellulose paper that has been reported is for the determination of Rhodamine 6 G dye using Raman Spectroscopy [32]. Therefore, the aim of this study was to investigate AgNP colorimetric sensing for PADs. The utility of AgNP aggregation on paper devices was demonstrated for the detection of copper (II) ions. Copper ions play an important role in living organisms and industry but excess intake of copper ions can be toxic [33]. High copper levels in blood and liver are implicated in Alzheimer's disease and inflammatory disorders. Moreover, excessive copper in children is associated with hyperactive behavior, learning disorders such as dyslexia, and ear infections. The World Health Organization (WHO) prescribes the maximum allowable levels of copper (II) ions in drinking water at 1.3 mg L^{-1} or 20.5 μM [34]. In this work, homocysteine and dithiothreitol bind to AgNPs (~10 nm in diameter presenting yellow color) through Ag–S bonds, and then binding between Cu^{2+} and the surface-modified AgNPs results in their aggregation (Fig. 1A). The aggregation of AgNPs in the presence of copper (II) ions leads to a decrease in the surface plasmon resonance absorption peak and the formation of a new red-shifted peak. Both paper-based assay devices and paper-based microfluidic devices for Cu^{2+} detection using AgNP colorimetric sensor were studied. After the addition of Cu^{2+} solution into paper devices, the yellow color of AgNPs changed to orange or green–brown depending on the Cu^{2+} concentration. Finally, our devices were successfully applied to the detection of Cu^{2+} in real water samples.

2. Experimental

2.1. Chemicals and Materials

All chemicals used in experiment were analytical reagent (AR) grade and solutions were prepared using high pure water with a resistance of 18 MΩ cm^{-1}. Homocysteine (Hcy), dithiothreitol (DTT), cysteine (Cys), glutathione (Glu) and metal ions (As^{3+}, Ca^{2+} Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K^{+},Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) were bought from Sigma-Aldrich (St. Louis, Missouri). AgNP solution was obtained from Sensor Research Unit at Department of Chemistry, Chulalongkorn University, Thailand. Whatman No.1 filter paper was bought from Cole-Parmer (Vernon Hills, IL). All glassware was thoroughly cleaned with freshly prepared 1:1 HCl/HNO_3 and rinsed with Mill-Q 18 MΩ cm^{-1} water prior to use. All metal ion stock solutions were prepared in 0.1 M HNO_3. Working standard solutions of metal ions were prepared daily by appropriate dilution of 157 μM stock solutions with 0.1 M phosphate buffer pH 8.0.

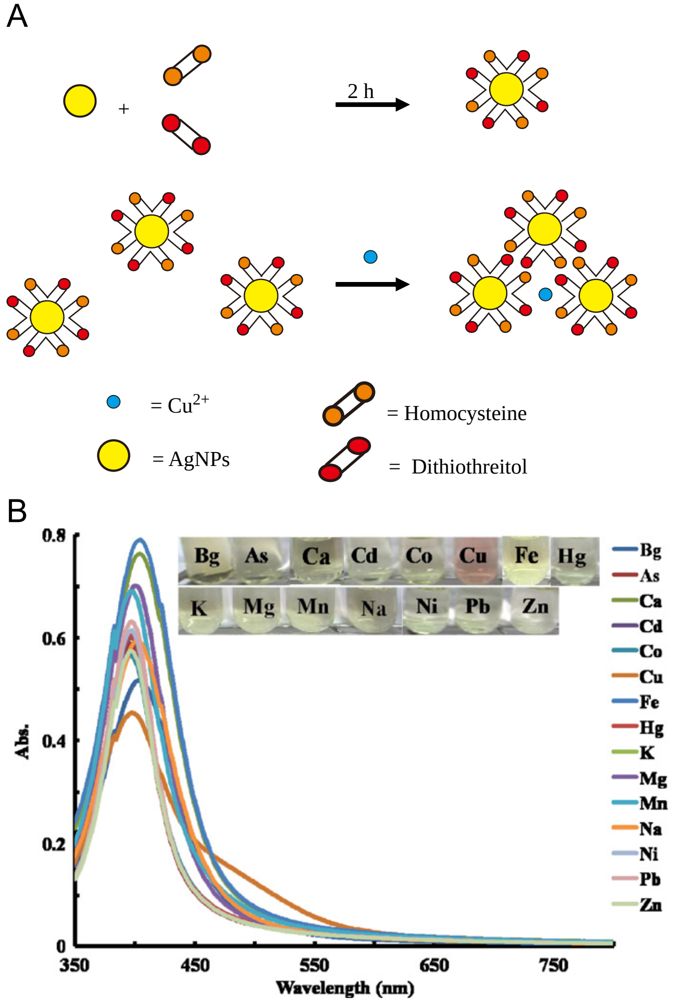

Fig. 1. (A) Schematic of aggregating process of AgNPs induced by adding Cu^{2+} and (B) UV–visible spectra and photos of Hcy-DTT-AgNPs with metal ions (7.8 nM of Cu^{2+} and 1.6 μM of other metals).

2.2. Instrumentation

UV–visible absorption spectra were recorded by a UV–visible spectrometer (Lambda 35, Perkin Elmer Instruments, USA) using quartz cuvettes (1-cm pathlength). Photographic results were recorded using a digital camera (PowerShot S95, 10.1 Megapixels, Canon). An atomic absorption spectrometer (AAS) with a hollow cathode lamp and standard air/acetylene flame (Analyst 300, Perkin Elmer Instruments, USA) was used for atomic absorbance measurements. A hollow cathode lamp was used under the following operations conditions: wavelength: 324.8 nm; slit-width: 0.70 nm; lamp current: 25 mA.

2.3. Synthesis of the modified AgNPs

AgNPs were synthesized using our previously reported methods using the chemical reduction process [25]. Sodium borohydride and methyl cellulose solution were used as the reducing agent and stabilizer, respectively. The shapes and particle size distributions of the AgNPs with nominal mean diameters of 10 nm were measured using TEM.

The Hcy-DTT-AgNP solution was prepared using self-assembly of the aminothiol on the AgNP surface. A yellow 0.1 mM AgNP solution (~10 nm in diameter) was first prepared from the dilution of 10 mM AgNPs stock solution with 18 MΩ cm^{-1} water. Then, 7 μM of 0.30 mM Hcy and 6 μL of 0.30 mM DTT were added into 6 mL of 0.1 mM AgNP solution to generate final concentrations of 3.5 μM Hcy and 3.0 μM DTT. Aminothiol compounds were self-assembled onto the surface of AgNPs by incubating the AgNPs with the aminothiol solutions for 2 h at room temperature. After this step, the AgNP aggregation was characterized using UV–visible spectrometry. 1.00 mL of modified AgNP solution was mixed with 1.00 mL of metal ion in 50 mM phosphate buffer at pH 8 (Na_2HPO_4 and NaH_2PO_4). Before analysis by the spectrometer, the mixture was incubated for 30 min at the room temperature.

2.4. Preparation of the paper devices

Whatman filter paper 1 was patterned using a cutting tool according to previously reported methods [35]. The pattern was created in CorelDraw and then transferred to the Silhouette Studio program that operated the cutter. Cutting printers (Silhouette America, Inc.) incorporate a knife in place of the traditional ink pen. The knife rotated freely on a turret, enabling precise cutting of various features, including small-radius corners, small channel or holes with 100 μm resolution. The instrument setup and actual cutting required ~60 s and ~20 s per device, respectively.

2.5. Detection of copper on the paper devices

Paper-based assay devices (PADs, holes shape with 4 mm in diameter) were modified with 0.5 μL of Hcy-DTT-AgNP solution in the test zone and allowed to dry before use. PADs were differentiated fromμPADs by the lack of a flow channel in PADs. 0.5 μL of metal ion solutions (As^{3+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) were then added to the paper devices.

Paper-based microfluidic devices (μPADs) were also prepared for Cu^{2+} detection. The test zone of μPADs was spotted with 0.2 μL of Hcy-DTT-AgNP solution (Figs. 5A and B) and allowed dry. For detection, 12 μL of 15.7 μM Cu^{2+} solution was dropped into the loading zone. An alternative pattern of μPADs is shown in Fig. 5C. In this pattern, 0.2 μL of Cu^{2+} solution at 7.8 nM, 780 nM, 7.8, 15.7, 31.4, 62.8 μM, and buffer solution was dropped into test zone number 2, 3, 4, 5, 6, 7, and 8, respectively. For detection, 12 μL of the modified AgNP solution was added to the loading zone.

2.6. Applications

To evaluate the utility of our proposed method, the Cu^{2+} in tap and pond water was quantified. Tap water samples were obtained from the chemistry building, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok, Thailand. Pond water samples were obtained from a pond in front of King Mongkut's University of Technology Thonburi, Bangkok, Thailand. Our method was validated against AAS. Prior to analysis, pre-concentration was carried out on all samples. 15 mL of sample was mixed with 10 mL of 0.1 M HNO_3 and then heated to evaporate excess water until 2.5 mL of samples remained. For AAS, samples were adjusted to a final volume of 5 mL with deionized water. For our proposed method, 50 mM phosphate buffer pH 8 was added to the sample to generate a final volume of 4 mL. The samples were then adjusted to pH 8 by the addition of 3 M sodium hydroxide and then water added to reach a final volume of 5 mL and thus a 3-fold higher concentration than the original sample.

3. Results and discussion

3.1. Characterization of AgNP aggregation

We initially investigated the AgNP aggregation by UV–visible spectrometer (Fig. 1A). As is well known, –SH group in aminothiol compounds such as Hcy, DTT, Cys, and Glu can modify the AgNP surface whereas the other functional groups have a strong affinity for metal ions [30]. In this work, four aminothiol compounds, including Hcy, DTT, Cys, and Glu, were studied. After the addition of 7.8 nM Cu^{2+} solution to the modified AgNPs solution, the solution color changed from yellow to orange color only in the mixture of Hcy, DTT and AgNPs as shown in Fig. S1. We also found a decrease in the plasmon resonance absorption peak at 404 nm and the formation of a new red-shifted band at 502 nm (Fig. 1B) suggesting that the mixture of Hcy and DTT can induce the aggregation of AgNPs in the presence of Cu^{2+}. It has been reported Cu^{2+} is an excellent catalysts for Hcy oxidation [36]. After the redox reaction between the Hcy and Cu^{2+}, Cu^{+} remained in the solution. Cu^{2+} behaved differently than other metals because of the higher stability constant between DTT and Cu^{+} relative to other metals (log $K=6.9$ (Zn^{2+}), 9.7 (Pb^{2+}), 10.8 (Cd^{2+}), 6.5 (Ni^{2+}) and 11.1 (Cu^{+})) [37]. Another possible mechanism for AgNP aggregation in the two thiols system is that the number of carbon bond (C–C) on Hcy and DTT molecule are the same. After AgNP surface was modified with the mixture of Hcy and DTT, the distance between nanoparticles at the Hcy active site is similar to the DTT active site. Therefore, the remaining Cu^{2+} and Cu^{+} can simultaneously bind with COOH– of Hcy and SH– of DTT on the Hcy-DTT-AgNPs surface. The selectivity of this method was evaluated by adding metal ions at 200 times the Cu^{2+} concentration (1.6 μM of As^{3+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) into the AgNP solution. An increase in the absorbance ratio (502 nm/404 nm) was clearly observed in the presence of Cu^{2+}, whereas no change was measured in the presence of other metals (Fig. 1B). Next, the parameters affecting the sensitivity and selectivity of Cu^{2+} detection were optimized. The concentration of Hcy and DTT, pH of phosphate buffer and incubation time were studied prior to application on paper devices. The best absorbance ratio was obtained at 50 mM phosphate buffer at pH 8, 3.5 μM of Hcy, 3.0 μM of DTT, and 30 min incubation time of the modified AgNPs and Cu^{2+}. Using the optimal conditions, the UV–Vis spectra of AgNPs with different concentrations of Cu^{2+} were investigated (Fig. 2). We expected that AgNPs could be used to quantitatively determine Cu^{2+} levels by monitoring the increase of the absorbance ratio at 502 nm/404 nm. The 502/404 absorbance ratio increased as a function of the Cu^{2+} concentration in the range of 3.2–14.2 nM. Then, the absorbance ratio stopped increasing up to 780 nM of Cu^{2+} and then decreased in the range of 780 nM −7.8 μM. The plot of absorbance ratio as a function of Cu^{2+} concentration was found to be linear within both the range of 3.2–14.2 nM ($R^2=0.997$) and 780 nM–3.9 μM ($R^2=0.998$).

3.2. Detection of copper on the paper devices

Colorimetric sensing in paper devices generally uses a higher organic indicator or nanoparticle concentration than is used in solution phase because of the low visibility of colors such as yellow and orange the paper surface. To improve visibility on paper, we investigated the concentration of AgNPs, Hcy, and DTT for nanoparticle aggregation in PADs. It was found that the 0.1 mM of AgNPs solution containing 3.5 μM Hcy and 3.0 μM DTT which provided the optimal solution detection conditions showed no significant response in the presence and absence of Cu^{2+} on the PADs by the naked eye. We therefore optimized the

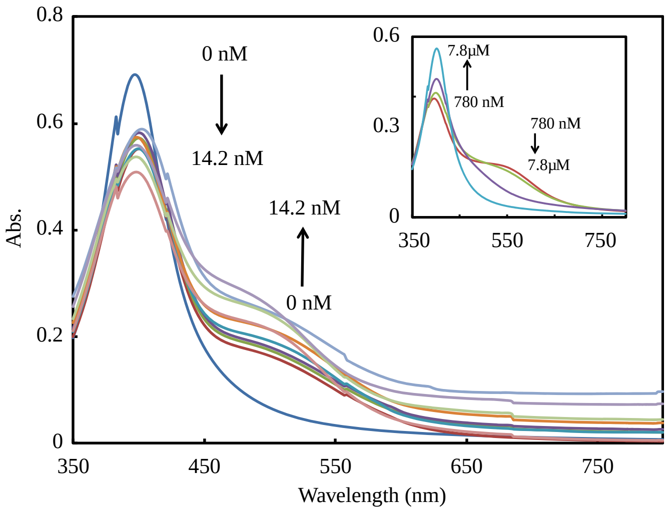

Fig. 2. UV–visible spectra of Hcy-DTT-AgNP solutions with various concentrations of Cu^{2+} in the range from 0 to 14.2 nM µg L^{-1} increasing in steps of 1.6 nM. The insert figure is various concentrations of Cu^{2+} in the range from 780 nM to 7.8 µM (780 nM, 1.6, 3.9, and 7.8 µM).

AgNP concentration in the range of 0.1–20 mM containing 7.0 µM Hcy and 6.0 µM DTT. 0.5 µL of 10 mM AgNP solution was spotted into the paper surface, followed by 0.5 µL of 7.0 µM Hcy and 6 µM DTT. An orange or green–brown color that was distant from the control experiment was observed after adding Cu^{2+} solution to the Hcy-DTT-AgNP coated paper surface, depending on Cu^{2+} concentration. A low AgNP concentration (0.1 mM) gave a light yellow color on paper devices while concentrations higher than 15 mM of AgNP concentration gave a dark yellow that made color discrimination by naked eye difficult (data not shown). As a result, we selected 10 mM of AgNP to use in the optimization of aminothiol concentration for PADs. The optimal Hcy and DTT concentration for PADs were studied next. 7.0 µM Hcy and 6 µM DTT gave the highest difference between the intensity of buffer solution and 15.7 µM Cu^{2+}, thus 7.0 µM Hcy and 6 µM DTT were chosen for the AgNP modification on PADs (Fig. S2). The optimal aminothiol concentration used in solution differed for PADs because the aggregation reactions occur in the different media surrounding. The surrounding media has a direct effect on the refractive index and the interparticles distance [24]. We also studied the linearity of color intensity with the increasing of Cu^{2+} concentration. PADs exhibited color changes as a function of Cu^{2+} concentration. Concentrations between 7.8 nM and 3.9 µM gave an orange color, while concentrations between 15.7 and 62.8 µM gave a green–brown color as shown in Fig. 3. The detection limit by the naked eye was found to be 7.8 nM or 0.5 µg L^{-1}. The green–brown color intensity as a function of the concentration of Cu^{2+} was also measured using Adobe Photoshop CS2 in gray mode. A linear calibration curve between intensity and Cu^{2+} concentration was obtained in the range of 7.8–62.8 µM ($R^2=0.992$). The World Health Organization (WHO) defines the maximum allowable levels of Cu^{2+} in drinking water at 1.3 mg L^{-1} or 20.5 µM. These results clearly show the ability to visually discriminate between the maximum allowable Cu^{2+} levels and the normal level using the modified AgNPs. Our device should therefore be useful for determining Cu^{2+} in drinking water. Although the limit of naked-eye detection in paper devices (0.5 µg L^{-1} or 7.8 nM) was higher than the previous UV–visible spectra reports (1 nM), [38] it is lower than commercial copper test strips [39,40]. Hence, our paper devices are an attractive, low cost and portable point-of-measurement (POM).

The selectivity of Cu^{2+} detection on the paper devices was then investigated at the optimal reagent concentrations (10 mM of AgNP modified with 7.0 µM Hcy, and 6.0 µM DTT). We evaluated selectivity by adding 0.5 µL each of 15.7 µM Cu^{2+} and metal ions onto paper devices coated with 0.5 µL of AgNPs. Hcy-DTT-AgNPs on the paper surfaces immediately changed colors with the addition of Cu^{2+} whereas other metal ions did not change the color (Fig. 4). Moreover, the analysis time using paper devices is only 5 min, which is 25 min faster than using UV–Vis or AAS.

Hcy-DTT modified AgNPs were next used with µPADs using the design shown in Fig. 5. The analyte solution flow direction in

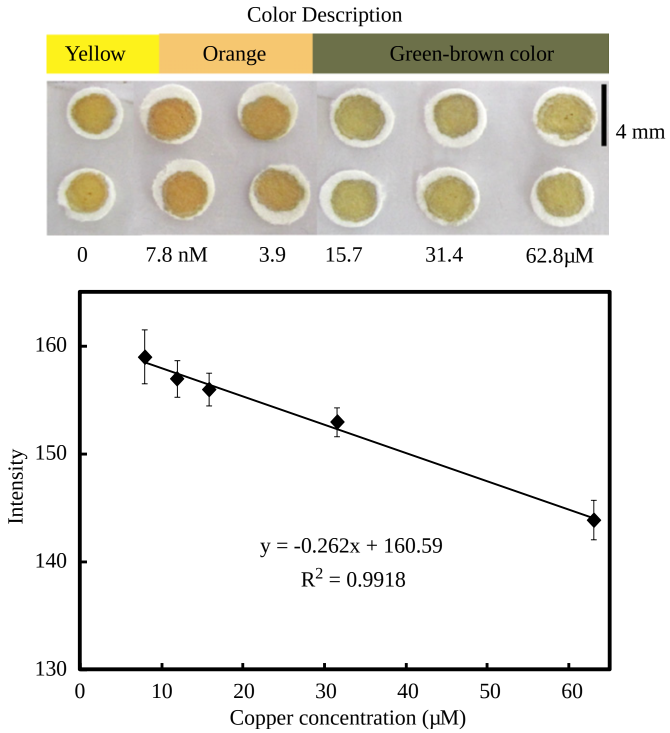

Fig. 3. Paper devices for the quantitative analysis of Cu^{2+} and the calibration plot of color intensity and the concentrations of Cu^{2+} (error bar represented the standard deviation at $n=3$). We converted one photographed digital image including all copper concentration to gray mode and selected the appropriate test zone for the device. The mean pixel values within the test zones correlate with the concentration of copper. Conditions: 10 mM of AgNP solution modified with 7.0 µM Hcy and 6.0 µM DTT. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

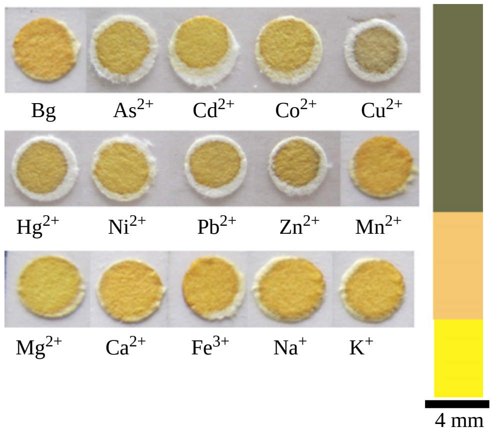

Fig. 4. Study of selectivity of the semi-quantitative analysis of Cu^{2+} toward different heavy-metal ions at 15.7 µM on paper devices. Other experimental conditions are the same as in Fig. 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

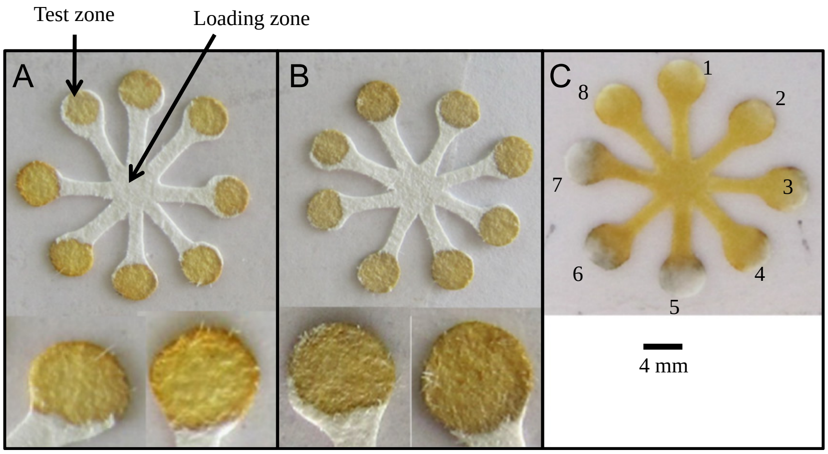

Fig. 5. μPAD for the semi-quantitative analysis of Cu^{2+} after spotting AgNPs modified with Hcy and DTT into the test zone and then (A) dropping buffer solution and (B) 15.7 μM of Cu^{2+} solution into the loading zone. (C)μ PAD for the semi-quantitative analysis of Cu^{2+} after spotting (8) 0 (2) 7.8 nM (3) 780 nM (4) 7.8 μM (5) 15.7 μM (6) 31.4 μM (7) 62.8 μM of Cu^{2+} solution and then dropping AgNPs modified with Hcy and DTT into loading zone. Other experimental conditions are the same as in Fig. 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

our PADs is the vertical with AgNP coated on paper surface whereas flow on μPADs is horizontal. More detailed information on the direction flow can be found in Fig. S3. Figs. 5A and B show the yellow color formed from buffer and the green–brown color formed from 15.7 μM Cu^{2+}, respectively. The results indicate that the nanoparticle aggregation reaction generated by the vertical flow of analyte solution (Fig. 3) was similar to the horizontal flow (Figs. 5A and B). However, the horizontal flow of modified AgNP solution to the test zone coated with Cu^{2+} (Fig. 5C) gave a different result from the horizontal flow of Cu^{2+} solution reacted with modified AgNPs at test zone (Fig. 5B). The results of the horizontal flow of modified AgNP solution into μPADs are shown in Fig. 5C. The brown–black color generated at 15.7–62.8 μM of Cu^{2+} while Cu^{2+} concentration at less than 15.7 μM did not give an orange color. The modified AgNP initially aggregated at the beginning of the test zone, blocking the horizontal flow of modified AgNP to the end of the test zone so the brown–black color in Fig. 5C obtained instead of green–brown color. Although the flow direction of AgNPs on paper devices affects the sensitivity of AgNP colorimetric sensing, the concept of our method is easy-to-use, versatile, rapid, and suitable for point of care monitoring.

3.3. Applications

To evaluate the potential application of AgNP colorimetric sensor in paper devices, the proposed method was applied for the detection of Cu^{2+} in the real water samples. Pond and tap water samples were analyzed by our devices and AAS. Levels of Cu^{2+} in tap water samples were measured using the paper devices and AAS to be 2.9 ± 0.24 and 2.8 ± 0.08 μM, respectively ($n=3$). Meanwhile, amount of Cu^{2+} in pond water samples were measured using the paper devices and AAS to be 3.2 ± 0.30, and 3.4 ± 0.04 μM, respectively ($n=3$). The paired t-test was used to validate our method versus the AAS method. The levels obtained using our approach were in good agreement with those from AAS, falling within the 95% confidence level. Thus, the analyzed values of Cu^{2+} in pond and tap water sample by our approach can be accepted.

3.4. Lifetime of the devices

For practical use in developing countries, devices must remain stable for weeks. Therefore, we studied the stability of devices after storing the prepared paper devices for 4 weeks. The modified AgNPs coated on paper devices were dried at ambient condition before storage at room temperature (~25 °C). The results were measured by the naked eye and using a digital camera. An observable signal for 15.7 μM Cu^{2+} solution dropped on the prepared paper over a period of 4 weeks (intensity in gray mode$=152.32 \pm 5.68$) generated no significant difference from the freshly prepared paper devices (intensity in gray mode$=157.47 \pm 7.36$) as shown in Fig. 6. Hence, the devices can be kept for 4 weeks without loss of activity but longer storage requires refrigeration.

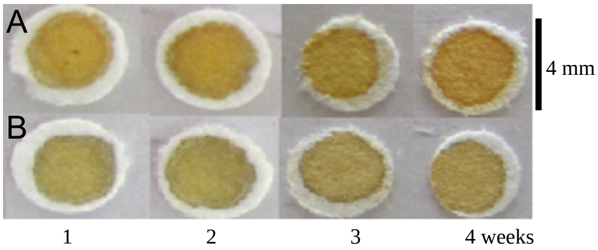

Fig. 6. Lifetime of the paper devices in the addition of (A) 0 and (B) 15.7 μM of Cu^{2+}. Other experimental conditions are the same as in Fig. 3.

4. Conclusions

This paper demonstrates for the first time the use of AgNP for colorimetric sensing of Cu^{2+} in paper devices to provide rapid, easy to use, inexpensive, and portable devices for point-of-care monitoring. The sensor is based on the aggregation of AgNPs on a paper substrate by the strong affinity between modified AgNPs and Cu^{2+}, which leads to a shift in the absorption spectrum. Furthermore, the color change of AgNPs on paper substrate can be observable by the naked eye due to the extremely high extinction coefficient of AgNPs. Cu^{2+} was clearly distinguishable color change from the other heavy metal ions under the optimum conditions at the critical level of Cu^{2+} in drinking water prescribed by WHO. Finally, our paper devices were successfully applied to the semi-quantitative analysis of Cu^{2+} in water samples. Both paper-based assay devices and paper-based microfluidic devices would be potentially modified with AgNPs for the detection of a variety of other targets.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.06.033.

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