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Paper-based colorimetric sensor for mercury ion detection using smartphone digital imaging

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Abstract

In this research, we have developed a colorimetric paper-based sensing platform for the determination of mercury ion (Hg^{2+}) using gold nanoparticles (AuNPs). To evaluate the selectivity in the response to a special target, urea is used as recognizing ligands on AuNPs for selective sensing Hg^{2+}. Colorimetric readings in the presence of various environmentally relevant metal ions, such as Na^{+}, K^{+}, Zn^{2+}, Pb^{2+}, and Hg^{2+}, were also studied. The results show that amongst all the tested metal ions, the SPR peaks are located at 525 nm except in the case of the present of Hg^{2+}, in which is red shifted to 537 nm. Furthermore, a new broad peak is observed at the wavelength of 695 nm corresponding to the color change from red to purple. The relations of the ratios of maximum absorbance at 695 nm and 537 nm, and the concentration of Hg^{2+} illustrate the linear behavior with the sensitivity of 2.0×10^{-5} nM^{-1} and the detection limit of 30 nM. The modified AuNPs with urea are applied on the paper-based device for quantitative Hg^{2+} detection with smartphone digital imaging. The intensity ratios between blue and red colors are linear-dependence relations which can be used to determine the concentration of Hg^{2+}.

1. Introduction

The heavy metal contamination in an aquatic system is increasingly becoming a major problem of environmental pollution. Among the heavy metal, mercury is considered as one of the most toxic ions that lead to a severe risk to human health [1]. Mercury (II) ion (Hg^{2+}) is the most stable form of inorganic mercury, which can be absorbed to human body through drinking water and food chain [2]. The maximum allowable level for Hg^{2+} in drinking water is 30 nM as recommended by the World Health Organization (WHO) [3]. Therefore, a routine detection and quantification of Hg^{2+} are necessary and urgent to the environmental monitoring used to assist prevention of Hg^{2+} contamination in the drinking water and the environment.

There are widely used methods for determination of Hg^{2+} in aqueous media such as inductively coupled plasma spectrometry [4], Atomic absorption spectroscopy [5] and Atomic fluorescence spectroscopy [4]. However, these methods require expensive equipment, time consumption for sample preparation, and they are not well suited for rapid on-site detection.

The development of detection techniques for real-time and long-term monitoring of Hg^{2+} contamination in environmental has become a high priority. Colorimetric sensor is one of an alternative technique that is certainly the most simple, rapid, and convenient diagnostic method in which it does not require any complex detection system. Colorimetric sensor using gold nanoparticles (AuNPs) is based on changes in the resonance wavelengths of localized surface plasmon resonance (LSPR) that exhibits different colors depending on their size and shape [6]. There are several works reported for the LSPR to detect the heavy metals such as arsenic, lead, and mercury. Arsenic in water samples was measured by the colorimetric detection using the lauryl sulphate modified gold nanoparticles as a LSPR-based sensor [7]. The valine-capped gold nanoparticles were also utilized for colorimetric and visual detection of lead ions [8]. For the mercury detection in water, citrate [2,9], DNA [10-14], dithia-diaza [15], calixarene [16] and dithioerythritol [17] functionalized with gold nanoparticles were reported. Interestingly, the nitrogen-containing molecules, like uric acid and creatinine in the urine [18] was also utilized as recognizing ligands for mercury ions. Moreover, the smartphone-based mercury contamination in water using citrate-stabilized plasmonic AuNPs. However, all these works are cuvette-based colorimetric sensing platform, the paper-based sensing platform has been demonstrated as the alternative way for the purpose of the minimum sample requirement [19].

Recently, paper-based analytical devices (PADs) have been developed for heavy metal detection in the environment [20-25] because of their instrument-free analysis technique, minimum sample requirement, and rapid analysis. Moreover, they are suitable for used as the platform for colorimetric detection that can be identified with the naked eye and quantitative analysis with the imaging devices such as digital camera and smartphones.

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Herein, we demonstrate the colorimetric sensor that is high sensitivity and selectivity for the determination of Hg^{2+} using modified AuNPs by urea. Moreover, we develop the paper-based platform in which the color changes are detected by smartphone camera and further quantitatively analyzed using free image processing software (ImageJ). The purposed platform is successfully applied for Hg^{2+} detection and that is of interest due to its disposable, low cost, and portable device.

2. Experimental

2.1 Synthesis of gold nanoparticles

Gold nanoparticles (AuNPs) were synthesized according to the published protocol [26]. Briefly, A hydrogen tetrachloroaurate ($HAuCl_4$) solution (0.5 mM, 40 mL) was heated and stirred at 80°C for 20 min. A trisodium citrate ($Na_3C_6H_5O_7$) solution (38.8 mM, 5.5 mL) was added to a boiled $HAuCl_4$ solution and further boiled for 15 min. Then the solution was cooled to room temperature while it was further stirred for 10 min.

2.2 Modified of urea-capped AuNPs

In order to study the detection of metal ions, the urea was utilized as recognizing ligands for sensing metal ions. The urea – AuNPs solutions were prepared by simply mixing urea (3.75 µL) in AuNPs solution (15 mL) for 15 min incubation and then urea – AuNPs solutions were ready for Hg^{2+} sensing [18].

2.3 Characterization method of nanoparticles

A UV-Visible spectrophotometer type-1800 (Shimadzu, Japan) matched with quartz cell was used for the absorbance measurement. AuNPs dispersion and aggregation were characterized by Transmission electron microscopy (TEM, JEOL JEM-2100).

2.4 Paper based device

The paper-based platform was designed and rendered in Fusion 360 (3D CAD/CAM design software Autodesk, USA). Subsequently, this 3D design was used as the mold master fabricated in an acrylonitrile-butadiene-styrene (ABS) filament, using a 3D printer (Flashforge Creator X), which is a low-cost technique and more convenient to make structures with more complicate shapes (see Figure 1(a)). The 3D mold was arranged on the filter paper (Whatman, 42) and glass slide, which are fixed by round magnets. After that, it was dipped in the mixing solution of paraffin and carnation oil of 75 %v/v. Then the hydrophilic areas using as detection zone were created on the filler paper below the 3D pattern, otherwise, they are the hydrophobic areas (see Figure 1(b)).

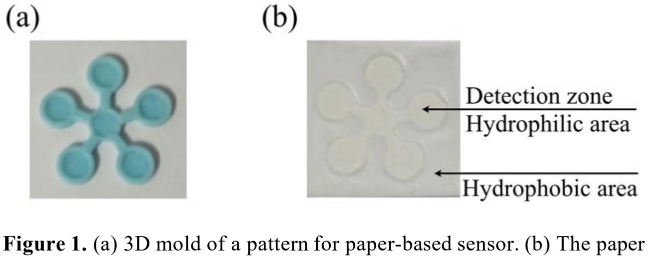

Figure 1. (a) 3D mold of a pattern for paper-based sensor. (b) The paper platform used as Hg^{2+} sensor. The detection zone is indicated in the picture.

2.5 Digital images capturing and processing

A free Android application (Open camera), installed in Samsung S6 smartphone (16 MP camera with F/1.9 aperture), was used to acquire the images of paper-based sensors with the same setting of ISO, zoom and exposure. To reduce the variation of ambient light, the dark box with the size of 20 cm × 20 cm × 20 cm was used for the capturing system with the white light source (Philip compact fluorescent lamp, 13 Watt, cool white). During the experiment, the automatic exposure (AE) and automatic focus (AF) were locked to maintain the same background. The image was stored in jpg format with intensity measurements limited to 256 levels/channel.

The acquired images were further processed using the free ImageJ software. The circle region of interest (ROI) was cropped in the detection zone from the original images. The average values of the red, green, and blue color intensity of the ROI were then determined for construct the calibration curve of color intensity and concentration of mercury ions.

3. Results and discussion

3.1 Nanoparticles analysis

The sizes of gold nanoparticles were confirmed by TEM measurement. The average sizes of AuNPs and the modified urea-AuNPs were found to be of 27.7±4.8 nm and 29.4±5.8 nm, respectively. The nitrogen components of urea are adsorbed on the AuNPs surface by electrostatic effect. The sizes of AuNPs and the capped urea-AuNPs are not significantly different which are confirmed by the little color change with no red shift in the absorption spectra. The results demonstrate that nanoparticles are almost spherical and aggregated after adding mercury ion (urea-AuNPs-Hg^{2+}) as shown in Figure 2(a-b).

3.2 Kinetic of Mercury ion detection

The UV-visible spectra of AuNPs, urea-AuNPs and urea-AuNPs-Hg^{2+} are shown in Figure 2(c). The absorption spectra of AuNPs and urea-AuNPs show the peak maximum at 525 nm. However, in the case of mercury ion, there is broad shift in LSPR peak at around 537 nm and appearance of a new peak at 695 nm, which is confirmed in TEM image result. The adding of Hg^{2+} to the solution mixture causing the interaction with the urea capped on the AuNPs surface that caused the aggregation and followed by color change (see inset of Figure 2(c)). As the increased size of AuNPs, the color of the AuNPs colloid changes from red to blue, due to the wavelength of maximum absorbance is shifted to the longer wavelengths. This relates to the red shift of the wavelength of the electron oscillations on AuNPs surface which is corresponding to the principle of localized surface plasmon resonance. Moreover, the zeta potential correlated with the surface charge and the local environment of AuNPs were measured. The zeta potential of AuNPs and the modified of urea-capped AuNPs are about -31.02 mV and -30.76 mV, respectively while that of urea-AuNPs-Hg^{2+} increases to be approximately -0.57 mV as shown in Figure 2(d). The zeta potential of colloid is more negative than 30 mV representing the well-dispersed form due to the sufficient mutual repulsion, whereas the decrease magnitude of negative charges on urea-AuNPs-Hg^{2+} indicates the significant aggregation of the nanoparticles in solution [27]. This result is consistently agreed with the UV-vis spectra and TEM images. Thus, the modification of AuNPs with urea on the surface of AuNPs, which is used as recognition moiety, can be used for colorimetric detection of mercury ion.

3.3 Selective colorimetric detection of metal ions

According to the previous studies in Hard and Soft Acids and Bases (HSAB) principle demonstrate that a soft base is strongly stabilized by forming a covalent bond with a soft acid. It has been reported previously that the colorimetric detection of Hg^{2+} is severe interference from Pb^{2+}, which may cause the problem for selective detection of Hg^{2+} [28]. Thus, the eliminating of Pb^{2+} interference from the Hg^{2+} detection is very important challenge. Moreover, other metals such as Zn^{2+}, K^{+} and Na^{+} are also reported as high interference for Hg^{2+} detection [11,18]. Hence, in this work, the selective detection of Hg^{2+} with the Pb^{2+}, Zn^{2+}, K^{+} and Na^{+} interference is also investigated. It is corresponding to the interaction of the Hg^{2+} (soft acid) with the nitrogen-containing molecules in urea (soft base), while Na^{+}, K^{+}, Zn^{2+} and Pb^{2+} are hard base, neutral, and hard acid. Thus, the urea can be utilized as recognizing ligands for sensing the mercuric ion [27]. This leads to evaluate the selectivity in the response to a special target, the colorimetric readings in the presence of various environmentally relevant metal ions, such as Na^{+}, K^{+}, Zn^{2+}, $Pb^{2+}$and Hg^{2+}, were studied and monitored by the UV-vis spectrometer. Amongst all the tested metal ions, the SPR peaks are located at 525 nm except in the case of the present of Hg^{2+}, in which is red shifted to 537 nm (see Figure 3(b)). Furthermore, a new broad peak is observed centered at 695 nm corresponding to color change from red to purple, while all other metal assay solutions retained the original color of colloidal AuNPs (see Figure 3(a)). Due to the typical change in both color and absorption spectra of treated AuNPs with Hg^{2+}, quantitative analysis of mercury ion detection is performed by comparing the absorption ratio at 695 nm / 537 nm of ($\lambda_{695} : \lambda_{537}$). This ratio is a representative of the relative quantity of aggregated and dispersed nanoparticles in the solution. Thus, the modified AuNPs with urea is specific for colorimetric mercury ion detection by the naked eye.

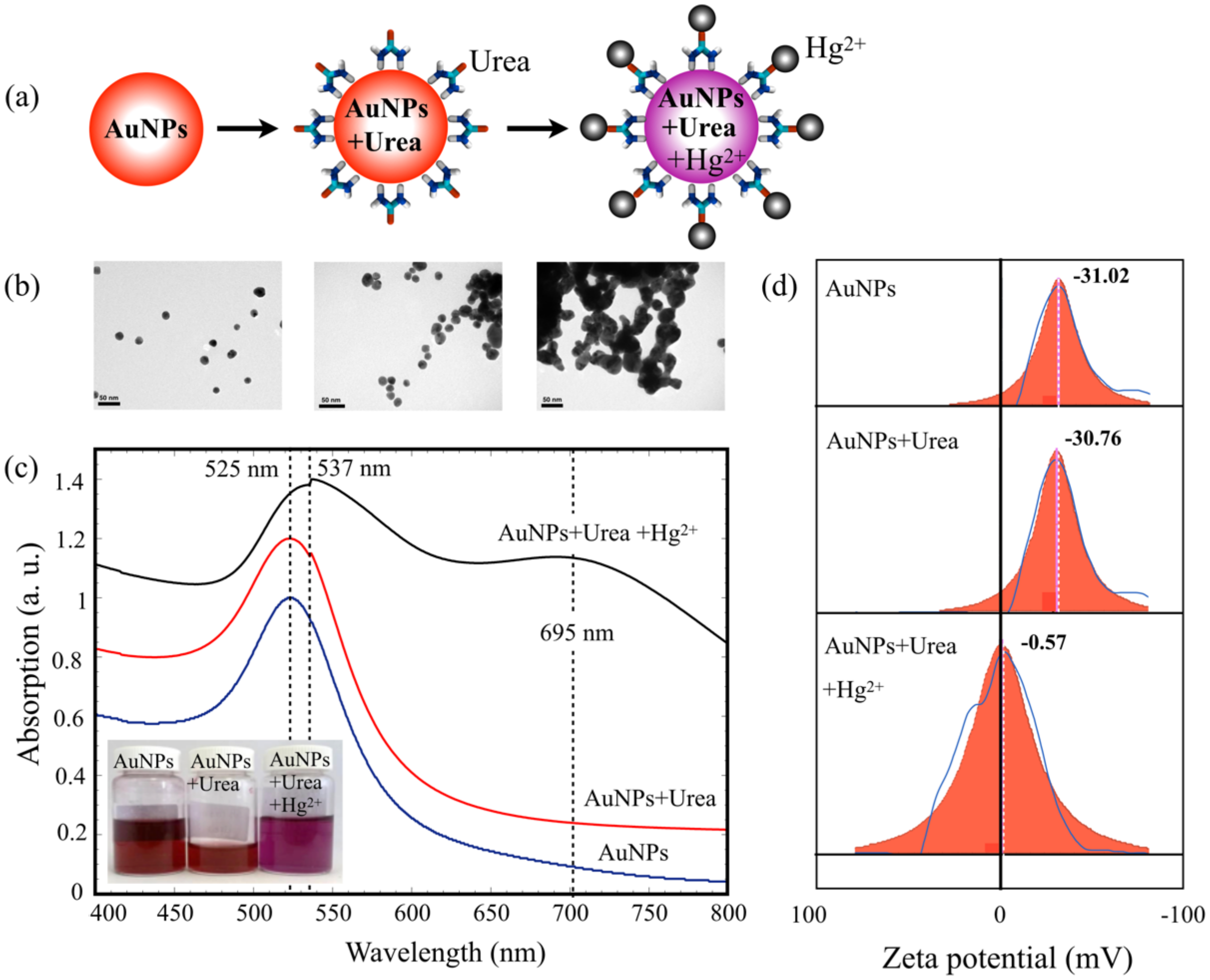

Figure 2. (a) Schematic illustration and (b) TEM images of bare AuNPs (left), urea capped nanoparticle (AuNP-urea (middle)) and after the adsorption of Hg^{2+} (AuNP-urea-Hg^{2+} (right)). (c) Absorbance spectra demonstrate the selectivity of urea-AuNPs for Hg^{2+}. Inset shows photographs of the color revolution for each reaction. (d) Zeta potential measurements of AuNPs (top), AuNP-urea (middle) and AuNP-urea-Hg^{2+} (bottom).

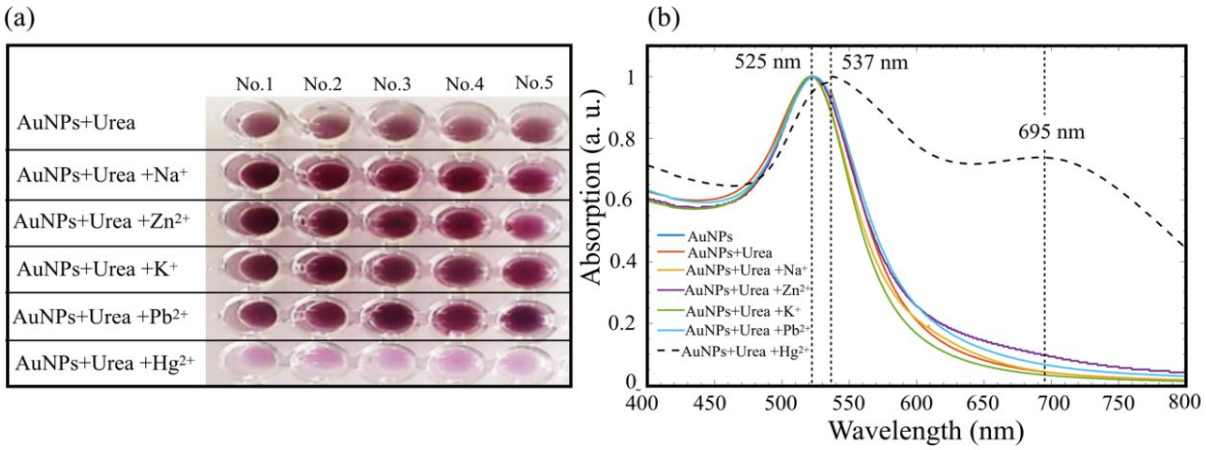

Figure 3. (a) Pictures of microwell plate containing the modified AuNPs with urea and adding of Na^+, Zn^{2+}, K^+, Pb^{2+} and Hg^{2+}, and (b) their absorption spectra illustrating the selectivity of urea-AuNPs for Hg^{2+}.

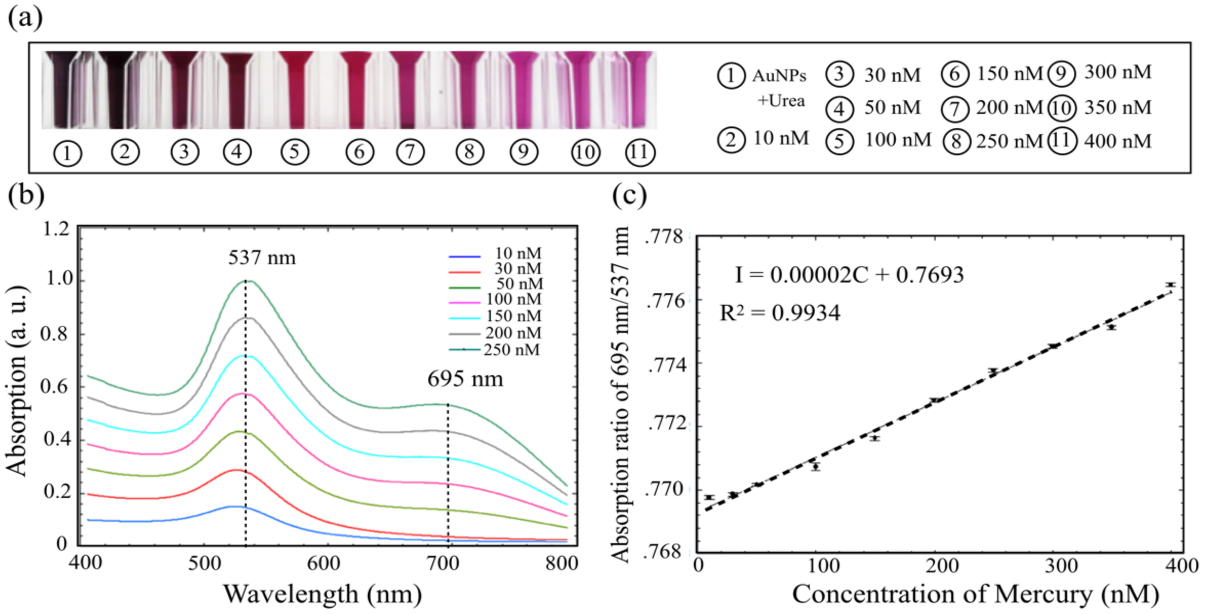

Figure 4. (a) Pictures of cuvette containing the urea-AuNPs with treating of mercury ions in different concentrations and (b) their absorbance spectra. (c) Calibration curve of mercury ions from 30 nM to 400 nM using urea-AuNPs based LSPR colorimetric sensor.

3.4 Sensitivity of gold nanoparticle toward mercury ions

To determine the sensitivity of colorimetric mercury ion detection, we monitored the color and the absorption spectra of modified AuNPs with urea and adding of mercury ion concentrations of 10 nM to 400 nM. Both colors and spectra of solutions with adding Hg^{2+} of 10 nM is invariable observations (see Figure 4(a-b)). With increasing amounts of ions Hg^{2+} from 30 nM to 400 nM, the absorption intensity of peak at 537 nm is decreased and a little red shift, while the intensity at 695 nm is simultaneously increased. Thus, the calibration curve is obtained by using the ratio of absorption intensity at 695 nm and 537 nm ($\lambda_{695} : \lambda_{537}$) as shown in Figure 4(c). There is linear relationship between the value of ($\lambda_{695} : \lambda_{537}$) (I) and concentration of Hg^{2+} (C) in the range of 30 nM to 400 nM. The level of mercury ions in sample is calculated by using the calibration curve with relation $I = 0.00002C + 0.7693$. As the results, the limit of detection is 30 nM with the sensitivity of 2×10^{-5} nM^{-1}.

3.5 Paper based mercury ion detection using smartphone digital imaging

Hydrophobic papers, containing the modified AuNPs with urea and adding of Na^+, K^+, Zn^{2+}, $Pb^{2+}$and Hg^{2+} are captured using smartphone, and shown in Figure 5(a). Pictures of the complete reactant are observed that the color changes from red to purple for mercury ion. Moreover, hydrophobic papers used as mercury ion concentration determination are observed that their colors are changed from red to purple with increasing the mercury ion concentration. Quantitative analysis of detection is performed by comparing the intensity (values range from 0-255) ratios between blue and red colors using RGB color space. The high intensity ratio is appeared for Hg^{2+} suggestion the maximum aggregation state, while all other ion metals have an intensity ratio similar to that modified urea-AuNPs (see Figure 5(b)) corresponding to the absorption ratio results. Moreover, hydrophobic

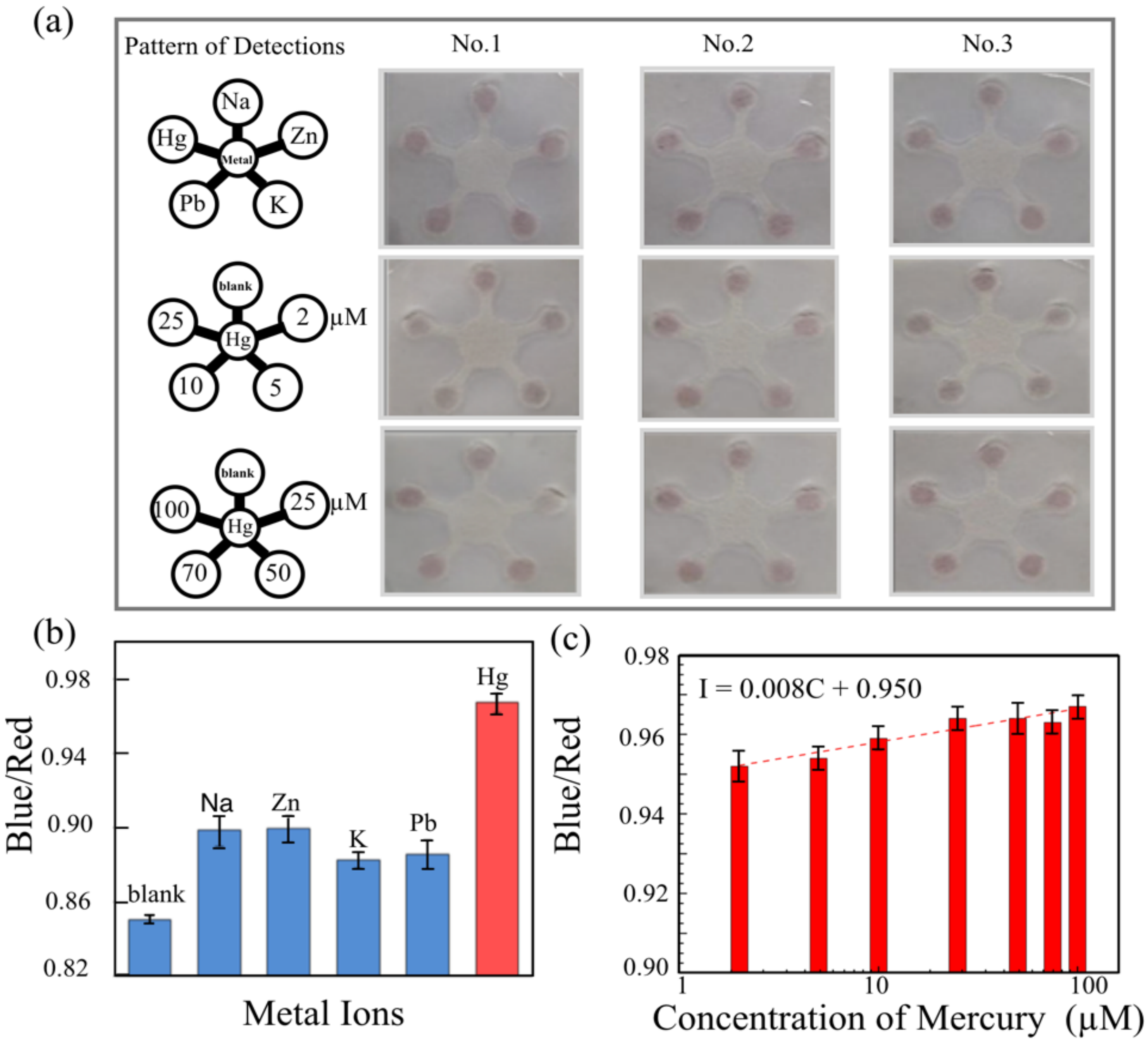

Figure 5. (a) Hydrophobic papers, containing the modified AuNPs with urea and adding of Na^+, Zn^{2+}, K^+, Pb^{2+} and Hg^{2+} and the variation of mercury ion concentrations. (b) The graph of the intensity ratios between blue and red colors using RGB color space obtained by treating urea –AuNPs with (b) metal ions and (c) variation of Hg^{2+} concentrations.

papers are also used for the detection of mercury ion concentration in which the colors are changed from red to purple with increasing the mercury ion concentration. The intensity ratios between blue and red colors are linear-dependence relations represented as $I = 0.008C + 0.950$ with I defines the averaged color intensity, and C is the concentration of Hg^{2+} ranging from 2 µM to 100 µM (see Figure 5(c)).

In order to make our sensor to be reproducibility across the various types or models of smartphone. The smartphone software must be developed to analyze the captured images to the concentration of mercury using the calibration model. The software must perform an automatically self-referencing from the background to reduce the variety of RGB values from different smartphone cameras, which can make the different the resulted concentration of mercury detection. Moreover, our sensor with the developed application can be directly determined the concentration of Hg^{2+} in real samples. However, pretreatment of real samples such as tab water and lake water sample must be performed before using the sensor by filtering with a syringe filter to remove the impurity. Hence, our system can be applied as a mercury sensor, allowing to detect the real samples at in-field locations.

4. Conclusions

The modified AuNPs with urea could be used to detect mercury ions by colorimetric detection. Since color of AuNPs colloid modified with urea is changed from red to purple while in presence of mercury ions due to the aggregation of AuNPs. The relation of the ratio of maximum absorbance at 695 nm and 537 nm and the concentration of mercury ions illustrates the linear behavior with the sensitivity of 2×10^{-5} nm^{-1} and the detection limit of 30 nM. Moreover, the modified AuNPs with urea is highly selective detection of mercury ions so that the absorption spectra are not change with the other metal ions i.e., Na^+, Zn^{2+}, K^+, and Pb^{2+}. Interestingly, the modified AuNPs with urea is applied on the hydrophobic paper device for mercury ion detection. The intensity ratios between blue and red colors are linear-dependence relations with the concentration of Hg^{2+} from 2 µM to 100 µM ($I = 0.008C + 0.950$) and then saturated. The paper-based sensor for mercury ion detection using gold nano-particles is simple, highly selective, and low cost, which is well suited to use at the site of interest.

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