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COMMUNICATION

A simple colorimetric device for rapid detection of Hg^{2+} in water

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A 'turn-on' fluorescent colorimetric device for Hg^{2+} sensing was built using a dual light-emitting diode system. Fluorescence generated from a rhodamine derivative (RHD), an indicator for Hg^{2+} sensing, was combined with a background red light, and the complex light was captured by a commercial charge coupled device camera or by the naked eye.

Mercury is an extremely toxic substance that is threatening to the health of human beings, which is commonly used in various products and activities. The US Environmental Protection Agency (EPA) estimates that the annual total global mercury emissions from all sources (both natural and human-generated) reach nearly 7500 tons per year. There have been many reports[1,2] on the toxicity of mercury to the nervous system, kidneys, and digestive system, consequently resulting in cell dysfunction and Minamata disease. Therefore, it is important to develop rapid, highly sensitive and selective Hg^{2+} sensors that provide real-time determination of Hg^{2+} levels in the environment, water, and food samples. Owing to their usage of sophisticated instruments, traditional quantitative approaches[3–5] for Hg^{2+} analysis are limited to their applications being in online or *in situ* detections. Although Hg^{2+} sensing systems, including electrochemical[6] and optical methods using nanoparticles,[7] polymer materials,[8] fluorophores,[9] quantum dots,[10] DNA enzymes[11,12] and gold nanoclusters,[13] could provide simple, cheap, less time-consuming as well as real-time and online detection, all of them still need complicated data processing.

Recently, the colorimetry[14–19] and fluorescence methods[20,21] for Hg^{2+} detection have attracted great attention, owing to their high sensitivity, facile operation and simplicity. To our knowledge, the human eye is able to distinguish thousands of colors, but the number of gray levels distinguished by the human eye may be much smaller.[22] Therefore, based on the mixing shade of two different colored lights, fluorescence intensity changes can be converted into different colors, which could be identified easily by the human eye. Several colorimetric sensing studies based on a dual light-emitting diode (LED) system have been reported.[23–25] LEDs have been recognized as an attractive light source due to their small size, long life and high reliability.[26] All these features make LEDs a good candidate for the device of excitation and background light sources. In this paper, we reported a turn-on fluorescence colorimetric device based on a dual LED system. This system revealed simple, fast, and 'naked-eye' discernible characteristics toward Hg^{2+}, which provided a strong colorful development compared to fluorescence intensity changes during the Hg^{2+} sensing. A schematic diagram of the proposed colorimetric device using dual LEDs for Hg^{2+} sensing was shown in Fig. 1. Considering the influence of the excitation source, two reciprocally vertical LED lamps were used in the device. In the Hg^{2+} sensing, a rhodamine derivative (RHD) was chosen as an optimal Hg^{2+} sensing indicator. An LED (λ_{max}, 520 nm) was selected as the excitation source, and a red one (λ_{max}, 660 nm) was selected to provide the background light. Simultaneously, two optical band-pass filters (530 ± 15 nm) were assembled in the device to improve the excitation optical purity and to separate the excitation light from the emission.

The structure of the RHD, the normalized excitation and emission spectra of the selected LED and RHD were shown in Fig. 2. In laboratory measurements, a constant current supplier (25 mA) was used for the LED lamps; whereas for in-field applications, a button battery set in the bottom of colorimetric device was employed.

Based on a previous report,[27] we designed a simple colorimetric device for the rapid detection of Hg^{2+} in water samples. In this device, light intensity values could be changed into a composite color with a red LED as the color background. Fig. 3b shows the response of the Hg^{2+} sensing device towards various concentrations of Hg^{2+} in PBS

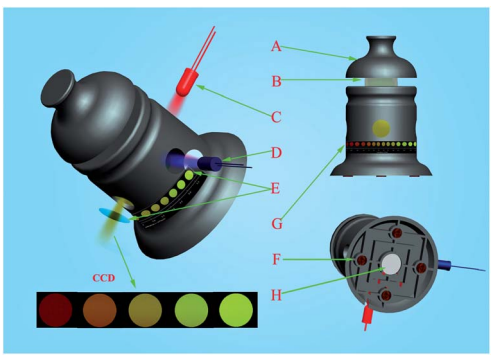

Fig. 1 Schematic diagram of Hg^{2+} colorimetric device: (A) colorimetric device, (B) colorimetric cuvette, (C) background light, (D) excitation light, (E) filter, (F) base, (G) colorimetric card, (H) micro-batteries.

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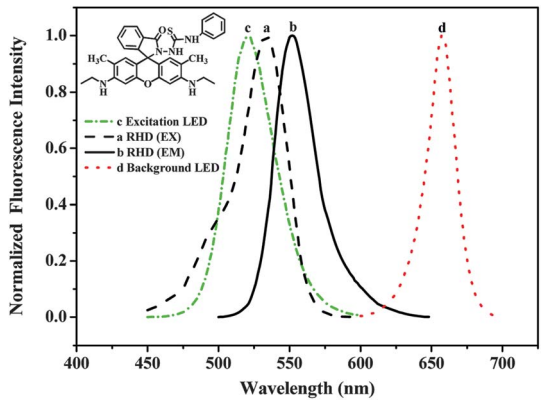

Fig. 2 Normalized excitation and emission spectra: (a) and (b) RHD + Hg^{2+} in 0.05 M PBS (dashed line and solid line), (c) excitation LED (green line), (d) background LED (red line).

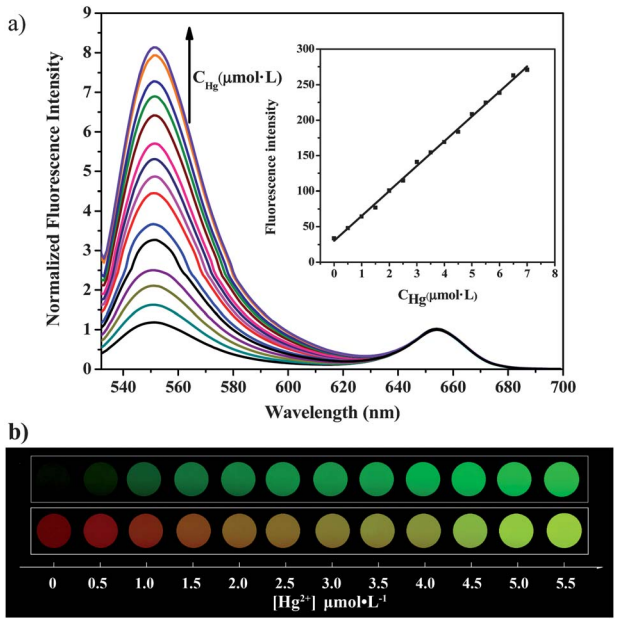

Fig. 3 (a) Response of the colorimetric device to different Hg^{2+} concentrations. Hg^{2+} concentration (from the top to the bottom curve at 553 nm) the inset figure shows the changes of fluorescence intensity of RHD upon addition of Hg^{2+} (0.5–5.5 × 10^{-6} M) in PBS (pH 7.4). (b) Color images with background light (lower row) and without background light (upper row) in different concentrations of Hg^{2+} captured by a CCD camera.

(PBS/methanol 4 : 1 pH 7.4). The results indicated that the colorimetric device had a good response to the concentration change of Hg^{2+}. The color gradually changed from red to yellow-green with increasing Hg^{2+} concentration, and displayed a distinguishable color change with a resolution up to 5 × 10^{-7} M Hg^{2+}. The detection range was found to be from 0.5 × 10^{-6} M to 5.5 × 10^{-6} M. The responses of the colorimetric device in terms of their fluorescence spectra in different Hg^{2+} concentrations were also investigated (Fig. 3a). The inset figure in Fig. 3a revealed that there was a good linear dependence of the fluorescence intensity on the concentration of Hg^{2+}, and the linearity was found to be from 0.5 to 5.5 × 10^{-6} M, with a correlation coefficient of 0.997. (The ratio of the intensity of background light to RHD fluorescence was 8.13 : 1.)

According to our previous studies,[25,28] the suitable ratio of the intensity of background light to RHD fluorescence would be helpful to improve the detection limit of Hg^{2+}. An obvious color change could be displayed with a suitable reference light intensity; therefore, an LED (λ_{max}, 520nm) was used as the excitation source in the colorimetric device, and an intensity of the red background light was simultaneously adjusted. Finally, the suitable ratio of the intensity of background light to RHD fluorescence intensity was found to be 7.56 : 1. Fig. 4 shows the responses of the colorimetric device towards Hg^{2+} concentrations ranging from 0.5 to 5 × 10^{-7} M and the color images change from red to dark-yellow with a resolution up to 5 × 10^{-8} M. The proposed colorimetric Hg^{2+} sensing device could be conceived and have great potential applications in the rapid, semi-quantitative analysis of Hg^{2+} in water samples.

A short response time between fluorescent reagents and target molecules is a great help for rapid determinations. A typical desulfurization reaction used in a Hg^{2+} chemodosimeter requires relatively long reaction times. However, the reaction of the RHD responsible for these changes reaches completion well within the time frame (<1 min) of these measurements. The response time of the Hg^{2+} colorimetric device depended on the fluorescent probe characteristics. Fig. 5 shows the Hg^{2+} sensing results when the colorimetric device was exposed to different concentrations of Hg^{2+} (0, 2.5 and 5.0 × 10^{-7} M) at pH 7.4.

It was observed that no obvious color change could be found if the reaction time was over 1 min, indicating that the reaction reached completion well within the time frame (<1 min) in accordance with the previous reports.[29,30] A 1 min system response time was chosen in this study since it yielded stable responses for a wider Hg^{2+} concentration range.

For practical applications, an important consideration for a Hg^{2+} sensing approach is its selectivity in the presence of other competitive ions. Under the optimum conditions, the effect of the Hg^{2+} sensing device upon the addition of co-existing metal ions (10 equiv.) was investigated in pH 7.4 PBS. As shown in Fig. 6a, only a weak background fluorescence signal could be obtained without Hg^{2+}, but when 2.5 × 10^{-7} M Hg^{2+} was added, there was an obvious fluorescence emission increase. 10 equiv. of other metal ions showed no significant effect on the fluorescence properties of the RHD. Correspondingly, as shown in Fig. 6b, marked color changes upon the addition of Hg^{2+} could be clearly identified, while the selected co-existing metal ions did not cause detectable changes in the appearing color. The result was in accordance with the previous reports,[31] revealing that the RHD had a specific response to Hg^{2+} over other prevalent toxic metal ions in the environment.

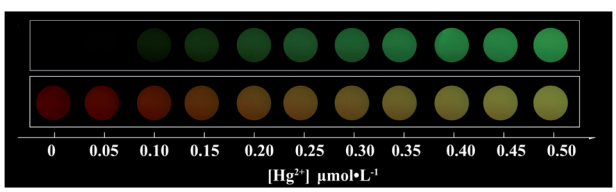

Fig. 4 Color changes with background light (lower row) and without background light (upper row) with different concentrations of Hg^{2+} (range from 0.5 to 5 × 10^{-7} M) in PBS.

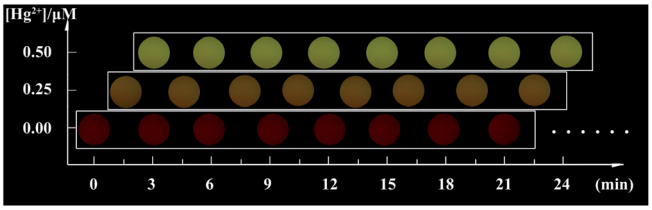

Fig. 5 Circular color images at Hg^{2+} concentrations of 0, 2.5 and 5.0×10^{-7} M.

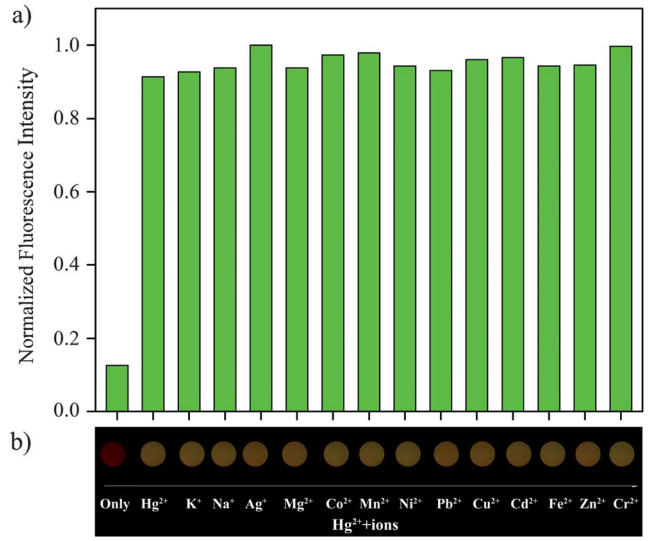

Fig. 6 (a) Normalized fluorescence intensity of RHD (1×10^{-5} M) and Hg^{2+} (2.5×10^{-7} M) in the presence of different metal ions (10 equiv.) in pH 7.4 PBS. (b) Color images of co-existing ion effect in the pH 7.4 PBS.

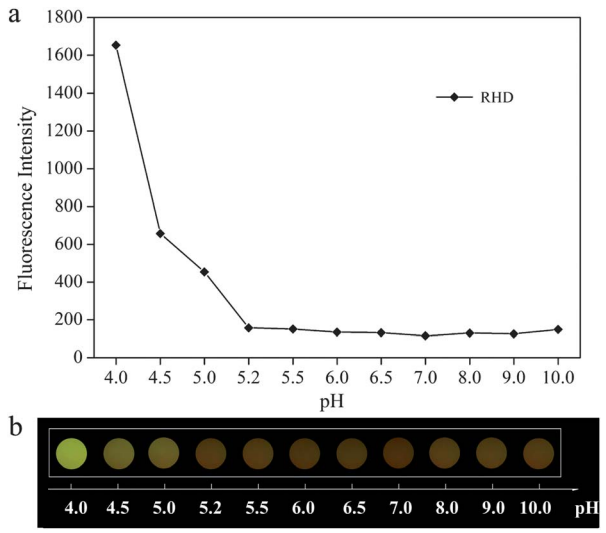

Fig. 7 (a) Fluorescence intensity of RHD (1×10^{-5} M) and Hg^{2+} (2.5×10^{-7} M) at different pH values ranging from 4.0 to 10.0. (b) Color changes of RHD (1×10^{-5} M) in different pH solutions.

The fluorescence intensity is generally influenced by the ambient environment, such as temperature and pH in particular. Therefore, the influence of pH on the colorimetric device was investigated prior to other aspects of the study because H^+ plays an important role in the fluorescence process. The fluorescence intensities of RHD containing 2.5×10^{-7} M Hg^{2+} were detected in the pH range from 4.0 to 10.0, and the results are presented in Fig. 7a. The corresponding color changes obtained from the sensing device were shown in Fig. 7b. With the pH increased from 4.0 to 5.2, in accordance with the reports,[32,33] the fluorescence intensity decreased enormously due to protonation-induced ring opening of the spirolactam in the RHD. The fluorescence intensity showed no significant change when the pH was above 5.2. The corresponding color change from green-yellow to green-brown (pH 4.0 to 5.2) was in accordance with the fluorescence intensity change. Under the consideration of water samples and sensing stability, a PBS of pH 7.4 was selected.

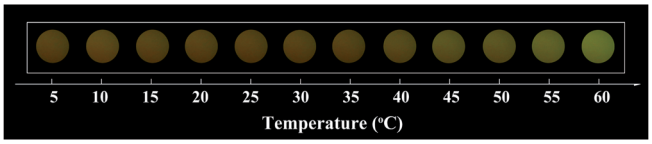

Fig. 8 Color changes of RHD (1×10^{-5} M) at different temperatures.

Table 1 Hg^{2+} detection in water samples using the colorimetric device and cold atomic fluorescence mercury meter (CAFM)

Samples	Added (10^{-7} M)	Hg^{2+} sensor (10^{-7} M)	CAFM (10^{-7} M)[a]	Color
Lake water	0	0	—	
Lake water	2.5	2.5	2.34 ± 0.01	
Lake water	5.0	5.0	4.86 ± 0.01	
Tap water	0	0	—	
Tap water	2.5	2.5	2.54 ± 0.01	
Tap water	5.0	4.5	4.86 ± 0.01	
Wastewater	0	0	—	
Wastewater	2.5	2.5	2.44 ± 0.01	
Wastewater	5.0	5.0	4.75 ± 0.01	

[a] CAFM were measured by three times.

Temperature usually affects the fluorescence efficiency of a fluorophore. Generally, a higher temperature results in a lower fluorescence intensity due to the lower fluorescence quantum yield and non-radiative processes related to thermal agitation (collisions with solvent molecules, intermolecular vibrations and rotations, *etc.*).[34] The temperature effect ranging from 5 to 60 °C was investigated under the optimal conditions. As shown in Fig. 8, there was no obvious color change when the temperature was below 40 °C. An obvious color change could be observed when the temperature was above 40 °C. Considering the stability and convenience of the sensing device, we selected room temperature for the Hg^{2+} sensing applications.

The colorimetric sensing device was applied to detect Hg^{2+} in several water samples (wastewater, lake water and tap water samples

that were collected from around Xiamen University). The detection results for the selected samples were compared with those obtained from commercial equipment, cold atomic fluorescence mercury meter (CAFM). As shown in Table 1, Hg^{2+} concentrations could be well read out directly *via* the Hg^{2+} colorimetric device, and be in good accordance with the results from CAFM. This result indicated that the developed approach provided a more convenient and easier method to identify Hg^{2+} concentrations within several minutes *via* a simple standard colorimetric card.

Conclusions

We have demonstrated a simple colorimetric device for Hg^{2+} sensing using a dual color system. The approach presented good selectivity against various other metal ions, low-cost and high sensitivity to Hg^{2+}. Experimental results showed that the normalized fluorescence intensity was linearly enhanced with Hg^{2+} concentration increasing from 0.5×10^{-7} to 5.0×10^{-7} M, with a resolution up to 5×10^{-8} M. In addition, the sensing approach was also successfully applied in the detection of Hg^{2+} in water samples.

Acknowledgements

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