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Naked-eye sensor for rapid determination of mercury ion

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ABSTRACT

A naked-eye paper sensor for rapid determination of trace mercury ion in water samples was designed and demonstrated. The mercury-sensing rhodamine B thiolactone was immobilized in silica matrices and the silica matrices were impregnated firmly and uniformly in the filter paper. As water samples flow through the filter paper, the membrane color will change from white to purple red, which could be observed obviously with naked eye, when concentration of mercury ions equals to or exceeds 10 nM, the maximum residue level in drinking water recommended by U.S. EPA. The color change can also be recorded by a flatbed scanner and then digitized, reducing the detection limit of Hg^{2+} down to 1.2 nM. Moreover, this method is extremely specific for Hg^{2+} and shows a high tolerance ratio of interferent coexisting ions. The presence of Na^{+} (2 mM), K^{+} (2 mM), Fe^{3+} (0.1 mM), Zn^{2+} (0.1 mM), Mg^{2+} (0.1 mM), Ni^{2+} (50 μM), Co^{2+} (50 μM), Cd^{2+} (50 μM), Pb^{2+} (50 μM), Cu^{2+} (50 μM) and Ag^{+} (3.5 μM) did not interfere with the detection of Hg^{2+} (25 nM). Finally, the present method was applied in the detection of Hg^{2+} in mineral water, tap water and pond water.

1. Introduction

Mercury is a widely distributed pollutant with broad toxicological profiles and exists in various forms (metallic, inorganic and organic salts). Inorganic mercuric ion is one of the most stable state of mercury [1] and is posing serious threat to the health of human beings. There are numbers of reports on the permanent damage of mercury to kidneys, digestive system, and nervous system [2]. Many countries and organizations have regulated the upper limit of Hg^{2+} for drinking water. For example, the United States Environmental Protection Agency (EPA) has set a maximum limit of 10 nM in drinking water [3]; whereas, the European Union and China permit a level of 5 nM Hg^{2+} in drinking water [4,5]. Many high-performance lab instruments can determine Hg^{2+} precisely down to sub ppb level, for example, atomic fluorescence spectrometry (AFS) [6], atomic absorption spectrometry (AAS) [7], inductively coupled plasma-mass spectrometry (ICP-MS) [8] and other electrochemical [9–13] and optic methods [14,15]. Extremely high sensitivity and specificity can be achieved by these methods. However, the routine running cost is very high, and special technical skills are also required for machine operation and sample pretreatment. In addition, some methods, especially the electrochemical methods, have obvious cross-talk interference among some ions. Thus, a rapid and simple method remains desirable for detection of Hg^{2+}.

Colorimetric sensor is one attractive approach for rapid and simple determination of Hg^{2+} [16–19], owing to its facile operation and simplicity. The color change can be easily caught by the naked eye and no special equipment is required. Besides those colorimetric sensors, some test strip methods based on DNA-functionalized gold nanoparticles [20–24] have also been developed for the one-step naked-eye detection of Hg^{2+}. These methods show great potential in rapid detection of Hg^{2+}, but further study is still needed to improve the stability, to simplify the strip operation conditions, and to prevent false results [20,21]. Compared to DNAzyme-AuNPs, molecule chemosensors are more stable [25–28].

Recently, we reported a reliable method for detection of trace Hg^{2+} by naked eye [29]. After Hg^{2+} preconcentration by dispersive liquid–liquid microextraction (DLLME), Hg^{2+} reacted with a highly selective molecule chemosensor and color change could be observed by naked eye. The preconcentration procedure was not straightforward enough for field Hg^{2+} screening. It would be necessary to develop a simple and single-step method for the determination of trace mercury with high sensitivity and selectivity. In recent years, a novel colorimetric determination method named colorimetric solid phase extraction (C-SPE) [30–33] has been developed. It complexes and concentrates analytes on a reagent-impregnated SPE membrane, and the resulting color change is then measured directly on the surface by a hand-held diffuse reflection spectra (DRS). By utilizing this technology, the

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elution step can be completely eliminated and the direct analysis of analyte on the surface of adsorbent-matrix is realizable. Due to its simplicity and rapidity for routine analyses, C-SPE provides another alternative for greener sample pretreatment.

In this paper, we developed a naked-eye paper sensor for rapid determination of Hg^{2+}. Hg^{2+} selective probe, rhodamine B thiolactone [36,37], were entrapped on porous silica matrix, and silica layer was impregnated in a filter paper. The water samples flowed through the membrane and the Hg^{2+} ions were captured by the rhodamine B thiolactone in silica matrices. The porous silica also acts as a preconcentrator for Hg^{2+} to improve the sensitivity effectively. The color change of the sensor membrane could be observed directly with naked eye at concentration level of 10 nM Hg^{2+} in water samples, and could be recorded by a home flatbed scanner [34,35] instead of DRS.

2. Experimental

2.1. Chemicals and materials

In this experiment, all regents were of analytical grade and used as received without further purification. Tetraethoxysilane (TEOS) and [3-(2,3-epoxypropoxy)]trimethoxysilane were obtained from ABCR GmbH & Co. KG and phenyltriethoxysilane (PTES) was from Alfa Aesar. Ascorbic acid and concentrated nitric acid (ultra pure, Hg≤0.0000005%) were from Jingchun Industry Co., Ltd. (Shanghai, China). Rhodamine B was from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Rhodamine B thiolactone was synthesized from rhodamine B and thiourea by two steps [36,37]. Deionized water was Wahaha deionized water (Hangzhou, China). The medium-speed qualitative filter paper from Hangzhou Special Paper Industry Co., Ltd. (Hangzhou, China) of Φ 7 mm was used as the substrate for silica matrices.

The standard stock solution of Hg^{2+} was prepared by dissolving the appropriate amount of mercury chloride salts in 2% (v/v) diluted HNO_3. Other metal ions, such as Cu^{2+}, Pb^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+}, Zn^{2+}, Mg^{2+}, K^{+}, Na^{+} and Ag^{+}, were in the form of their nitrate salts.

2.2. Preparation of sol–gel membrane

The sol–gel colloid formulation was prepared by mixing 1.2 mL of TEOS, 1.0 mL deionized water and 2.0 mL ethanol. The pH was adjusted to 2 by using 0.1 M hydrochloric acid to catalyze the hydrolysis of the silica precursors. After stirring for 3 h, 1.5 mL of 5 mM rhodamine B thiolactone was added and then the mixture was stirred for another 0.5 h. The filter paper was dipped in the sol–gel colloid solution for 5 min. After withdrawn from the solution, the membrane was dried in the air for 30 min (membrane 1). Membrane 2 and 3 were prepared in the same way as above, except that 0.15 mL PTES or [3-(2,3-epoxypropoxy)]trimethoxysilane was added in the silica precursors.

2.3. Sample collection and preparation

Mineral water bought from local supermarkets, tap water obtained from our laboratory and pond water from Dalian Institute of Chemical Physics were used as real water samples. The tap water was collected after flow of 5 min and the pond water was collected below 20 cm of water surface. All water samples were treated by the addition of 1.5 mL concentrated nitric acid per 1 L of water and kept in glass bottles. The sampling bottles had been cleaned with deionized water, dilute nitric acid and deionized water, in sequence. Water samples were stored at 4 °C and in dark. The water samples filtered with 0.45 μm cellulose acetate membrane (Millipore, Billerica, USA) were analyzed within 48 h after sampling. To avoid the oxidation of rhodamine B thiolactone probe in membrane, water samples were treated with ascorbic acid (5.7 μM) and then adjusted to pH 5 with 1.0 M acetate/acetic acid buffer solution.

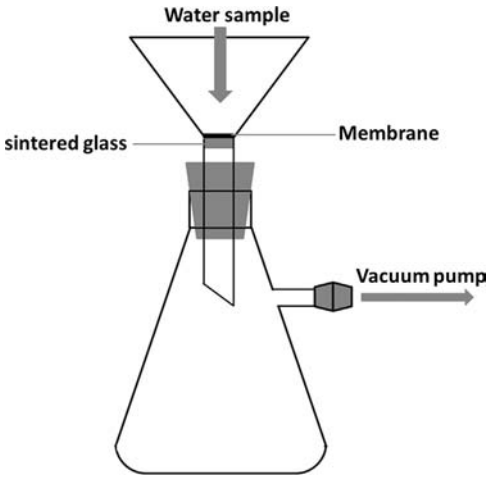

Fig. 1. The experimental set-up for mercury ion testing.

2.4. Procedures and data analysis

The mercury-sensing membrane was fixed on a sintered glass filter, as shown in Fig. 1. When the vacuum pump worked, water samples were pumped through the membrane at flow rate of about 30 mL min^{-1}. Then the membrane was picked and used for sensing experiments. Records of the membrane colors were performed on a commercial flatbed scanner. The blank-image was obtained with deionized water without Hg^{2+}. The average red, green and blue (RGB) values of the membranes were obtained from the center of the membranes (Φ 5 mm, 50% of the total size) using Adobe Photoshop™ software.

3. Results and discussion

3.1. Colorimetric response of Hg^{2+}

This colorimetric Hg^{2+} detection method is based on the complexation reaction between Hg^{2+} and rhodamine B thiolactone [36,37], and the formation of purple red product (Fig. 2). The silica matrix was chosen as the host material because of its large surface area, good stability and the adjustable hydrophobicity/-philicity. When water samples passed through the membrane, the filtration enhanced the complexing interaction probability between Hg^{2+} and rhodamine B thiolactone. The membrane coated with silica matrix serves as both a preconcentrator and a chromogenic reactor for Hg^{2+}. Therefore, the sensitivity of this method was improved significantly.

The colorimetric response change between sample-image and blank-image was normally expressed using Euclidean distance (ED), which was defined by the following formula.

$$ED = \sqrt{(\Delta R)^2 + (\Delta G)^2 + (\Delta B)^2}$$

In this experiment, the red and blue channels showed no significant color change while the green channel contributed almost 99% of the total color change. Therefore, the color signal change of green channel, instead of ED, was used for quantitative analysis throughout this experiment.

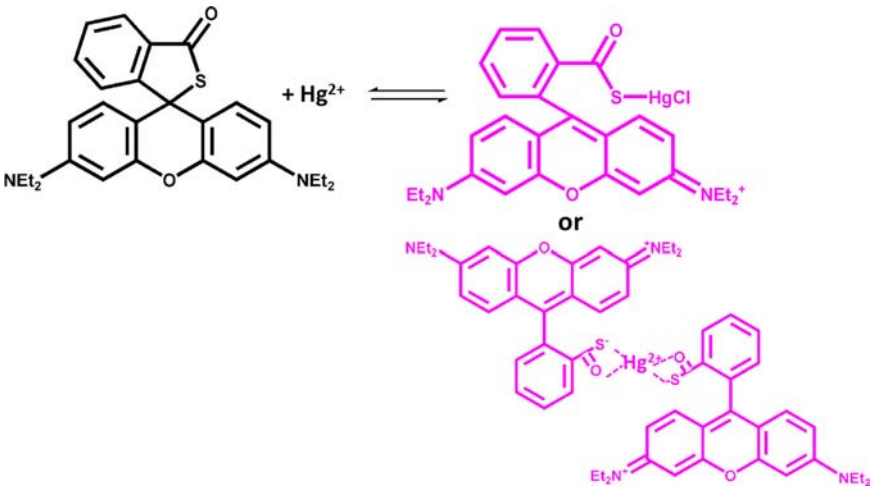

Fig. 2. The complexing reaction between rhodamine B thiolactone and mercury ion.

3.2. Optimization of testing conditions

3.2.1. Selection of sol–gel colloid

In this experiment, inorganic silica materials and organically hybrid materials were both studied for silica matrix. We found that the inorganic colloid (membrane 1) and ormosils (membrane 2, phenyl hybrid; membrane 3, [3-(2, 3-epoxypropoxy)] hybrid) both could be impregnated firmly and uniformly in the filter paper. More importantly, leaching of Hg^{2+} indicator could also be avoided. In this experiment, it was found that membrane 1 showed higher colorimetric response than other membranes. A possible reason was suggested. The introduction of phenyl or 3, [3-(2, 3-epoxypropoxy)] group would increase the hydrophobicity of membranes, so the hydrophobic interaction between Hg^{2+} indicator and organic silica matrices was stronger. In addition, the water permeation into silica gel was weakened, and the complexing chance between hydrophilic Hg^{2+} and rhodamine B thiolactone were decreased. As a result, the enrichment of Hg^{2+} was reduced and the colorimetric response decreased. Therefore, membrane 1 was chosen throughout this experiment.

3.2.2. The volume of water samples

To select an appropriate volume of water samples, calibration curves were obtained using the plot of colorimetric response against volume of water samples from 50 to 300 mL (Fig. 3). The colorimetric response was enhanced with increased sample volume, as a result of the increase of total amount of Hg^{2+} enriched in the membrane. As shown in Fig. 3, the colorimetric response was saturated even at 200 mL of water sample at concentration of 50 nM, while the relationship between colorimetric response and sample volume of 25 nM kept linear up to 200 mL. Therefore, the sample volume should be adjusted depending on the desired detection range of Hg^{2+}. Considering of the sensitivity and appropriate linear range for Hg^{2+} at concentration around 10 nM, sample volume of 200 mL was chosen.

3.2.3. Sample pH

In the complexing reaction between Hg^{2+} and indicator, pH plays a unique role. To study the effect of pH, Hg^{2+} samples at pH 3, 4, 5, 6.3, 7, and 10.6 were prepared. The change of colorimetric response in green channel (ΔG) at various pH values was shown in Fig. 4. It was revealed that the colorimetric response reached the highest at a pH of about 5 and reduced with the decrease of pH. This result was in good agreement with what reported that a pH range from 5 to 8 was suitable for Hg^{2+} detection with rhodamine

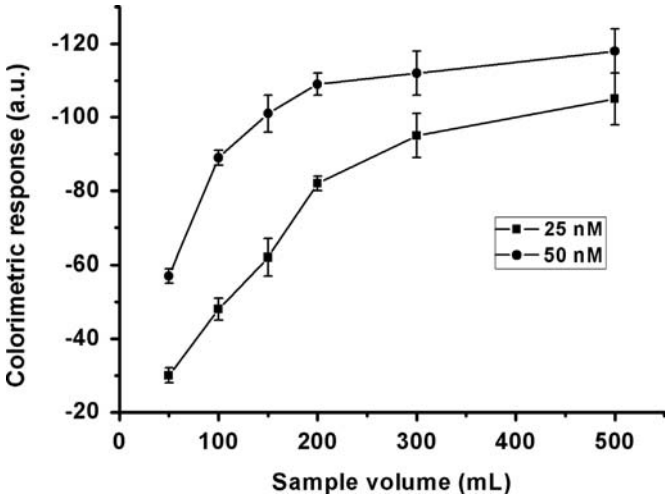

Fig. 3. Influence of sample volume upon the colorimetric response in the green channel of the rhodamine B thiolactone-Hg^{2+} complex compound. The concentration of Hg^{2+} was 25 nM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

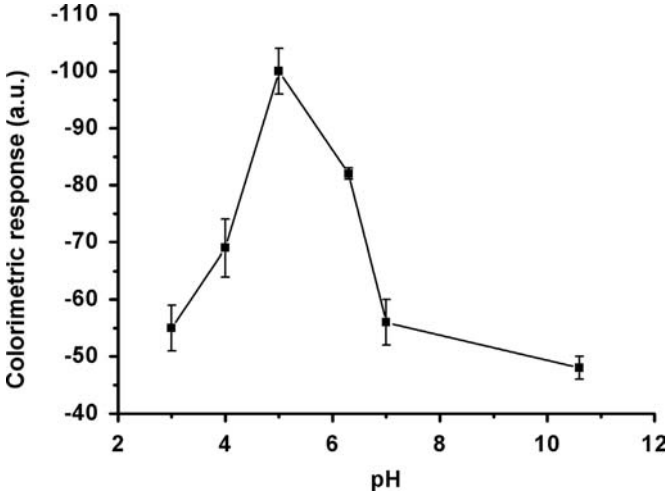

Fig. 4. Influence of pH upon the colorimetric response in the green channel of the rhodamine B thiolactone-Hg^{2+} complex compound. The concentration of Hg^{2+} was 25 nM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

B thiolactone [34,35]. In alkaline solutions, metal hydroxide species (soluble $M(OH)^+$ and/or insoluble precipitate of $M(OH)_n$) were formed and the total amount of Hg^{2+} decreased, leading to the reduction of colorimetric response. Therefore, pH 5 was chosen in this experiment.

3.3. Analytical performance

Under the above optimized conditions, the concentration of Hg^{2+} detectable by the color change with the naked eye was evaluated. A series of sensor papers exposed to different Hg^{2+} solution were displayed in Fig. 5, and the color changes with Hg^{2+} concentration were drawn in Fig. 6. As shown in Fig. 5, the color of the membrane changed from white to purple red at the Hg^{2+} concentration of 10 nM. In this way, water samples with concentration of Hg^{2+} near the upper limit of safe level can be found immediately only by naked eye. It means that water samples containing Hg^{2+} at or over 10 nM can be discriminated rapidly and effectively without instruments.

In this way, the naked-eye sensor provided a rapid method for the qualitative determination of water samples with concentration of Hg^{2+} over 10 nM. In addition, the quantitation of Hg^{2+} could also be achieved by the use of a plat scanner. In the condition of the absence of more accurate instruments, it could be used as a supplementary method. Standard Hg^{2+} samples with concentration from 2 nM to 100 nM were prepared for calibration. Colorimetric response of R, G and B channel were compared. As shown in Fig. 6, the colorimetric response of R and B channel had no significant change with the concentration of Hg^{2+}. The colorimetric response of G channel and Hg^{2+} concentration had a good linear correlation in the range of 2–30 nM with a squared correlation coefficient (R^2) of 0.9960. The limit of detection (LOD) was estimated using three times standard deviation defined as the following formula of $C_L = 3S_B/m$ (C_L, S_B, and m are the limit of detection, standard deviation of six measurements of the blank sample, and the slope of the calibration curve, respectively), and was found to be 1.2 nM, which is one eighth of the upper limit of Hg^{2+} for drinking water mandated by U.S. EPA guidelines. The repeatability of this method was also evaluated by assaying five standard solutions with 10 nM Hg^{2+}. A relative standard deviation (RSD) of 7.8% illustrated the good repeatability of the method. Generally, the method was sensitive and stable enough to detect trace Hg^{2+} in environmental water samples, even in drinking water samples.

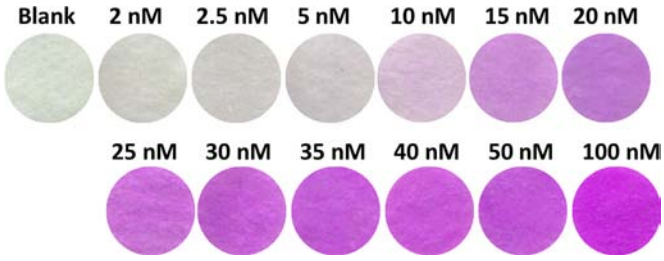

Fig. 5. Sequential color profile as the function of Hg^{2+} concentration.

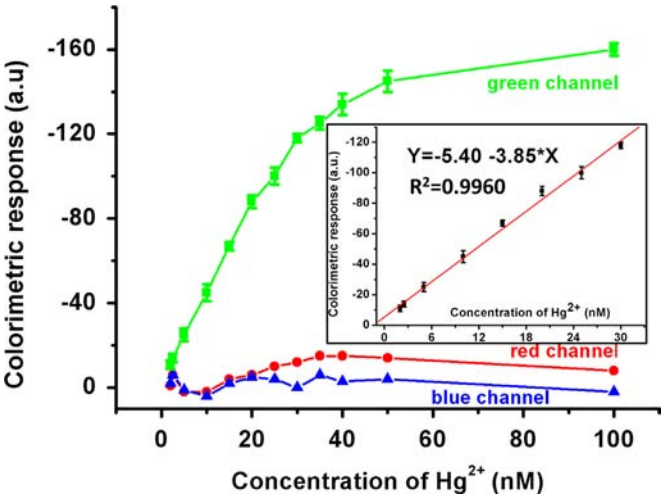

Fig. 6. The relationship between the colorimetric response in red, green and blue channel and the concentration of Hg^{2+}. The insert is the linear relationship between the colorimetric response in green channel and Hg^{2+} at the concentration range from 2 to 30 nM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Interference of potential metal ions

In environmental water samples, various metal ions are ubiquitous, and most foreign metal ions have much higher concentration than Hg^{2+}. Therefore, it is necessary to investigate the selectivity of the sol–gel membrane doped with rhodamine B thiolactone as a colorimetric sensor. The detection specificity was evaluated under the same conditions by adding different metal ions. Corresponding changes of colorimetric response are illustrated in Fig. 7. It is obvious that only Hg^{2+} could cause significant colorimetric response change. Relative to the colorimetric response of Hg^{2+} in the green channel, all these interfering coexisting ions showed the response less than $\pm 8\%$. The tolerance ratio of interfering coexisting ions to Hg^{2+} (C_M/C_{Hg}) was determined by using solutions with a constant concentration of Hg^{2+} at 25 nM and varied concentrations of coexistent ions, including Ag^+, Cu^{2+}, Pb^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+}, Zn^{2+}, Mg^{2+}, K^+ and Na^+. When a criterion of 90–110% recovery of Hg^{2+} was adopted, the metal ions could be considered as noninterference. For the studied metal ions including K^+, Na^+, the proposed method exhibited very high tolerance to 80,000, and the tolerance ratio reached 4000 for Fe^{3+}, Zn^{2+}, and Mg^{2+}. No interference was found when the C_M/C_{Hg} ratio was up to 2000 for Cu^{2+}, Pb^{2+}, Cd^{2+}, Co^{2+} and Ni^{2+}. Furthermore, Ag^+ had interference from 0.25 μM upwards and the tolerance ratio was up to 140 when masked with 1 mM chloride ion. The excellent selectivity of this method can be

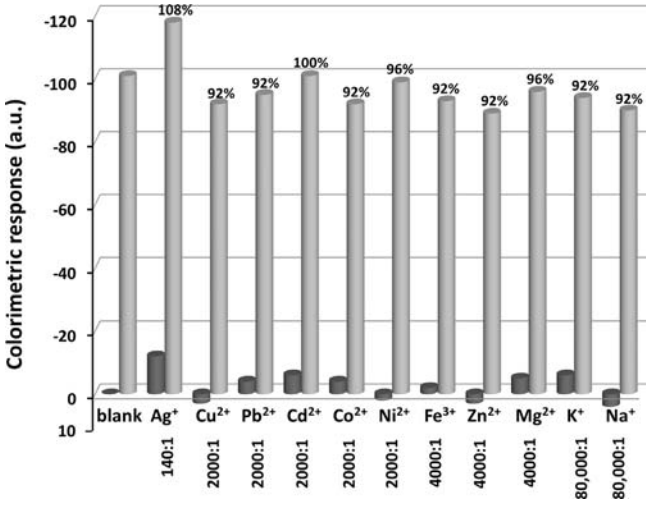

Fig. 7. The colorimetric response to various cations (green channel). The dark columns represent the colorimetric response change of different concentrations of interfering ions. The gray columns represent the colorimetric response change of interfering ions upon the subsequent addition of 25 nM of Hg^{2+}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

expected by the high selectivity of rhodamine B thiolactone probe for Hg^{2+} [36,37].

3.5. Membrane stability

Rhodamine B thiolactone is susceptible to oxygen when exposed to air. To improve the membrane stability, 1.0 mM of ascorbic acid as a water-soluble reductant was tested to avoid the oxidation of this indicator. In addition, the membrane stability also was tested by keeping in a tightly sealed bottle filled with nitrogen. Fig. 8 demonstrates the colorimetric response change of the membranes monitored at appropriate intervals up to 40 days. The membranes exposed in open air changed to pink even after 1 day due to the oxidation of rhodamine B thiolactone. When the membranes were stored in ascorbic acid solution or bottles filled with nitrogen, the colorimetric response change was minimized remarkably up to 40 days. Therefore, the membranes could be stored in ascorbic acid solutions or containers without oxygen. For the long-stored Hg^{2+} sensor paper, the slight color change may lead to some increase in mercury concentration observed. In this situation, the blank imagine should be recorded first. After the sensor paper was exposed to water samples, the sample imagines were also recorded. The contrast image should be used for the assessment of Hg^{2+} concentration.

3.6. Method application

The applicability of the proposed method was evaluated by determination of Hg^{2+} in three real water samples including mineral water, tap water, and pond water. When water samples were pumped through the membranes, no significant color change could be observed. The method was further tested for quantitative determination of Hg^{2+}. The colorimetric response of G channel also had no change and no Hg^{2+} was detected in such water samples. The result was later verified by ICP-MS. As shown in Table 1, Hg^{2+} in all water samples was below 1.2 nM. When spiked with 10 nM of Hg^{2+}, the measured Hg^{2+} amounts in mineral water and tap water agreed very well with the spiked level. It indicates that such a method is applicable to the quantification of Hg^{2+} in aqueous samples. It was found that the spiked recovery of pond water was only 41%. The reason may be suggested that some compounds, which could have reaction with Hg^{2+}, resisted in pond water and the amount of free Hg^{2+} decreased.

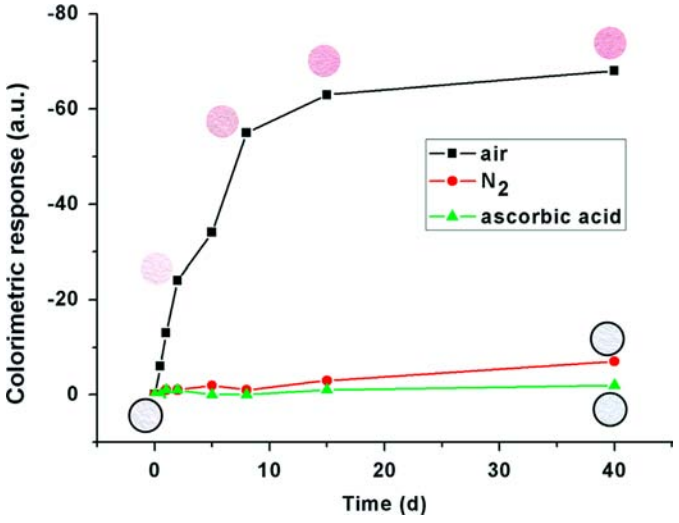

Fig. 8. Stability monitoring of the membranes kept in open air, 1.0 mM of ascorbic acid and nitrogen.

Table 1
Analytical results of Hg^{2+} in water samples and of the spike-recovery tests.

Sample	This method		ICP-MS
	Spiked (nM)	Found (nM, mean ± SD)	Found (nM, mean ± SD)
Mineral water	0	ND[a]	0.075 ± 0.015
	10	9.4 ± 0.7	
Tap water	0	ND	0.110 ± 0.010
	10	10.2 ± 0.6	
Pond water	0	ND	0.044 ± 0.007
	10	4.1 ± 1.2	

[a] Not detected.

4. Conclusion

In this paper, a convenient, sensitive and selective colorimetric sensor was successfully developed for the naked-eye discrimination and quantification of Hg^{2+}. The Hg^{2+} indicator (rhodamine B thiolactone) was immobilized in the silica matrices with filter paper as substrate. When samples containing Hg^{2+} were filtered through the membrane, trace Hg^{2+} could be enriched and colorated simultaneously, and the color change from white to purple red could be discriminated clearly by naked eye, or recorded by a home-use flatbed scanner. For the quantitative determination of Hg^{2+}, the colorimetric sensor exhibits a linear range of 2–30 nM. Either by the naked eye or in combination with a flatbed scanner, 10 nM of Hg^{2+} can be detected, which just meets with the EPA guideline for the maximum allowable mercury level in drinking water. This colorimetric sensor also shows good selectivity for Hg^{2+}. Furthermore, this membrane also has a sufficiently long life when kept free from oxygen. The developed naked-eye paper sensor was an advantageous alternative for the portable and easy Hg^{2+} measurements with high reliability.

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