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A turn-on near-infrared fluorescent chemosensor for selective detection of lead ion based on fluophors-gold nanoparticles assembly

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Abstract

A turn-on fluorescent chemosensor of Pb^{2+} in near-infrared(NIR) region, which is based on the Pb^{2+}-tuned restored fluorescence of a weakly fluorescent fluophors-gold nanoparticles (AuNPs) assembly, has been reported. In this fluophors-AuNPs assembly, NIR fluorescent dye brilliant cresyl blue (BCB) molecules act as fluophors and are used for signal transduction of fluorescence, while AuNPs act as quenchers to quench the nearby fluorescent BCB molecules via electron transfer. In the presence of Pb^{2+}, fluorescent BCB molecules detached from AuNPs and restored their fluorescence due to the formation of chelating complex between Pb^{2+} and glutathione confined on AuNPs. Under the optimal conditions, the present BCB-AuNPs assembly is capable of detecting Pb^{2+} with a concentration ranging from 7.5×10^{-10} to 1×10^{-8} mol L^{-1} (0.16-2.1 ng mL^{-1}) and a detection limit of 0.51nM (0.11 ng mL^{-1}). The present BCB-AuNPs assembly can be used in aqueous media for the determination of Pb^{2+} unlike common organic fluorescent reagent, and also shows advantages of NIR fluorescence spectrophotometry such as less interference, lower detection limit, and higher sensitivity. Moreover, the present method was successfully applied to the detection of Pb^{2+} in water samples with satisfactory results.

Introduction

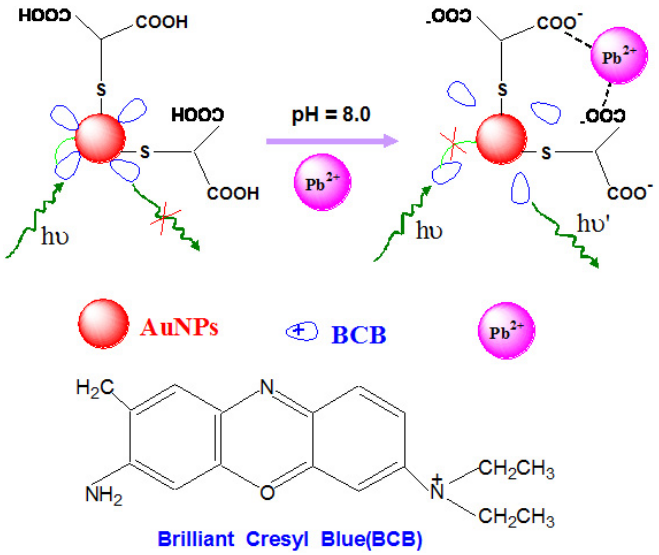
Lead ion (Pb^{2+}), generally regarded as one of the very abundant and toxic heavy metal ions in environment pollutant, is not biodegradable, and therefore easily accumulated in the environments, which leads to the contamination of soil, water, and food.[1-3] The relevant studies have demonstrated that the long accumulation of Pb^{2+} in the body is hazadous to both the central and peripheral nervous systems, and may cause many serious diseases such as muscle paralysis, memory loss, hepatic injury, lung damage, and hypertension, and so on. [1-5] For example, non-adults may suffer from permanent neurological damages, behavioral dysfunctions, and decreased IQs even at blood lead level as low as 100 ppb or 500 nM because of the non-degradation of lead ions. [1-5] In our daily lives, the main sources of lead pollution are attributed to the widespread employments and their auto-release of lead-containing products include coal combustion, gasoline, paints used in water supply systems, and lead-acid batteries. [1-5] As a result, it is of great interest to develop sensitive and reliable methods for detecting Pb^{2+} in real samples such as water, human blood, and soil.

Up to now, different classic approaches have been developed for the determination of Pb^{2+} such as inductively coupled plasma mass spectrometry (ICP-MS),[6,7] liquid-phase micro-extraction with atomic absorption spectroscopy,[8] functional nucleic acids (*e.g.* DNAzymes, aptamers)-based sensors,[1,9] dynamic light scattering technique, [10,11] electrochemical methods,[12-19] and optical methods including fluorimetric methods,[20-33] visual detection,[34] UV-vis spectrophotometry,[35] chemiluminescence,[36] and photonic crystal optrode.[37] Among these developed approaches, fluorescence-based methods have shown great advantages such as high sensitivity, simple instrumentation, and easy operation. [20-33] Fluorescence-based spectroscopy in the near-infrared (NIR) region, in which fluorescence emission generally occurs above 650 nm, is an attractive area because NIR fluorescence shows some advantages over visible-region fluorescence such as minimized background spectra interference and reduced interfering from Raman and Rayleigh scattering lights.[38-40] Obviously, based on NIR fluorescence spectroscopy, enhanced sensitivity for analytical

assay can be expected. For example, Wang group recently has reported different $NaYF_4$-based nanomaterials with NIR irradiation for assays of different analytes such as explosives, glucose, and hydrogen peroxide, demonstrating that NIR fluorescence methods show many advantages including no auto-fluorescence, low damage to samples, no photobleaching, and high sensitivity. [41-45] However, determination of Pb^{2+} with NIR fluorescence photospectroscopy has rarely been reported.[32,33]

In present study, a novel efficient NIR fluorescent chemosensor based on weakly fluorescent fluophors-gold nanoparticles (AuNPs) assembly has been developed for the determination of Pb^{2+} in aqueous solutions (Scheme 1). In this fluophors-AuNPs assembly, commercially available NIR fluorescent dye brilliant cresyl blue (BCB) moleculars act as fluophors and used for signal transduction, while AuNPs act as quenchers to quench the BCB fluorescence. As shown in Scheme 1, at pH 8.0, positively charged BCB molecules are self-assembled on negatively charged gluthione (GSH)-modified AuNPs via electrostatic interaction or Au-N bond interaction[40], which induces fluorescence quenching(turn-off) of BCB as a result of strong quenching ability of AuNPs to nearby fluophors.[40, 46-50] The fluorescence of BCB switches to "turn-on" (restore) upon addition of Pb^{2+} due to the coordination interactions between COO- group of the GSH and Pb^{2+} to form chelating complex,[51] which leads the dissociation of BCB moleculars from the surfaces of AuNPs and thus their fluorescence restore. Based on the restored fluorescence, a homogenous assay method for Pb^{2+} is proposed and the feasibility of the AuNPs-based "turn-on" fluorescence sensing for Pb^{2+} is demonstrated. Herein, BCB and AuNPs were chosen to construct weakly fluorescent(*i.e.*, low background) assembly which is desirable for sensitive turn-on assay are based on the two facts, BCB is water soluble, commercially available, and NIR fluorescent emission. In addition, AuNPs have displayed strong quenching ability to nearby fluophors through different mechanisms[40,52-55] because AuNPs processes a broad absorption band and have high extinction coefficients (*e.g.* up to 8×10^8 L mol^{-1} cm^{-1} for 13 nm AuNPs at 520 nm),[53-57] which is about 3-5 orders of magnitude higher

than common organic molecules.[48,57] These distinguished properties of BCB and AuNPs allow BCB-AuNPs assembly to form low background fluorescence for turn-on detect with enhanced sensitivity. In addition, the high water solubility of BCB-AuNPs assembly enables the assay to be performed in aqueous media without the need for organic cosolvents. As a result, the quantitative analysis of Pb^{2+} can be realized in a simple and sensitive approach.



Scheme 1. Illustration of FET-based sensor of lead ion

Experimental

Instrumentation

An LS-55 fluorescence spectrophotometer (PerkinElmer, USA) combined with a quartz cell (1 cm ×1 cm) was employed for fluorescence spectra measurements. UV-Vis absorption spectra were performed on a UV-3010 spectrophotometer (Hitachi, Japan). A Hitachi H-600 transmission electron microscopy (TEM) (Tokyo, Japan) was used for the morphological characterization of the prepared Au nanoparticles.

Materials

Chloroauric acid tetrahydrate ($HAuCl_4 \cdot 4H_2O$) was purchased from Aladdin-Reagent Company Trisodium citrate, brilliant gresyl blue, glutathione (GSH), and lead nitrate ($Pb(NO_3)_2$) were

obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The buffer solution (pH 6.0-8.5) was prepared by 0.01mol L^{-1} KH_2PO_4-Na_2HPO_4. The reagents used in this study were of analytical grade and used without any further purification. All solutions were prepared with Milli-Q purified water (>18.0 MQ) was used throughout for the preparation of solutions.

AuNPs preparation and surface modification with GSH

In this study, AuNPs were prepared with $HAuC1_4$ reduction by trisodium citrate, as described in previous reports[53-55]. Typically, into a round-bottom flask with a reflux condenser, 99.0 mL water and 1.0 mL 1% $HAuCl_4$ solution were injected under vigorous stirring, and the flask was then incubated in oil bath to reflux under stirring. While the mixture was heated to boiling state, 5.0 mL of trisodium citrate solution (1%, weight percent) was quickly injected into the flask, and then the reaction was allowed to reflux for 20 min. The color of the solution was turned to deep red from pale yellow and the solution was cooled to room temperature and then stored at 4°C in the refrigerator for further use.

The surface modification of AuNPs with GSH was also carried out according to previous literature.[53] In general, an aqueous solution of gold nanoparticle (4.0 nM) and GSH (1.0 μM) were mixed in the volume ratio of 8:1, and then pH of the resulting mixture was adjusted to 9.0 using 1.0 M NaOH. The mixture was let to react for 3 h at room temperature in dark condition under continuing stirring to ensure GSH confined onto the surface of AuNPs.

Preparation of BCB-AuNPs assembly

To investigate the behavior of fluorescence quenching of BCB by AuNPs, different concentration of AuNPs was added into the 200 μL BCB solution (100 μM) with a pH value of 8.0. The final volume of the mixture solution was adjusted to 2.0 mL with phosphate buffer solution (pH 8.0). Then the solution was equilibrated at ambient temperature for 30 min, and the fluorescence of the mixture solutions were measured at 660 nm with an excitation at 520 nm.

Procedures for the determination of Pb^{2+}

For the determination of Pb^{2+}, a mixture solution containing 200 μL of BCB solution (100 μM) and 850 μL of AuNPs (4.0 nM) was added into in a series of 10 mL volumetric flasks, and then various concentrations of Pb^{2+} solution were added, then the mixture was diluted to 2.0 mL with pH 8.0 phosphate buffer. The mixture was allowed to react thoroughly for 40 min at room temperature. The fluorescence intensities of the mixture solutions were measured at 660 nm with an excitation at 520 nm.

Results and discussion

Characterization of AuNPs

The typical TEM images of the as-prepared AuNPs are shown in Fig. S1 (Supporting Information). We can see that the shape of AuNPs are regular, monodisperse, and spherical, and the average diameter of AuNPs is about 13.0±1.3 nm. A characteristic surface plasmon resonance (SPR) peak is located at 520 nm, [53-55] as shown in UV-vis absorption spectra (Fig. S2, curve a, Supporting Information). Based on the Beer's law (A = εbc), and the extinction coefficient (ε) of 2.01×10^{8} M^{-1} cm^{-1} for about 13 nm AuNPs at 520 nm,[48,52-55] the particle concentration of the AuNPs solution was estimated to be 4.0 nM. After the modification of GSH on AuNPs, the characteristic SPR peak is slight red-shifting and located at 522 nm (Fig. S2, curve b, Supporting Information), suggesting the AuNPs are successfully modified by GSH through the Au-S covalent bond.[58]

Fluorescence quenching of BCB by AuNPs

The quenching effect of AuNPs on fluorescence of BCB was studied. Fig. 1 displays the emission spectra of BCB in presence of different concentrations of AuNPs. As shown in this figure, in the absence of AuNPs, BCB solution fixed at a concentration of 10 μM shows the characteristic emission fluorescence at ca. 660 nm,[59] while different concentrations of AuNPs ranging from 0.1 to 1.7 nM were introduced into BCB solution, the fluorescence of BCB at maximum emission were

quenched gradually with the increasing of concentration of AuNPs, accompanying with a very slight 3 nm red-shifting. In addition, when the added AuNPs was above 1.7 nM, the fluorescence intensity did not show obvious change, indicating the quenching of BCB reached at the maximum level. The maximum quenching efficiency can be calculated to be 75.1% on the basis of the formular, E_q=1-F/F_0, where F_0 and F represent the fluorescence intensity in the absence and presence of AuNPs, respectively. This result suggests that AuNPs shows strong quenching ability to the fluorescence of BCB. The spectroscopy of BCB-AuNPs assembly showed a good stability in aqueous solution for over one week. Furthermore, TEM measurements (not shown) further demonstrated that the morphology of AuNPs did not shown discernable change upon BCB molecules were confined on AuNPs surface. As demonstrated by the negligible changes of the fluorescence signal and morphology of BCB-AuNPs assembly, we believe the BCB-AuNPs assembly is favorable for their further analytical applications.

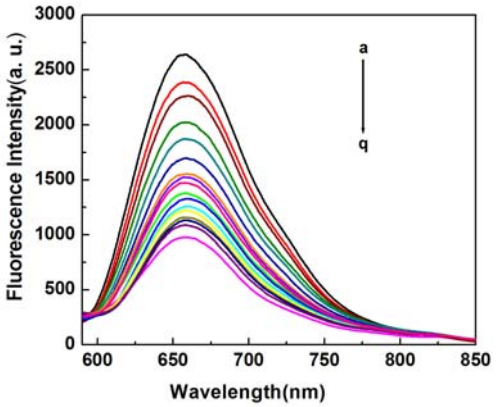


Fig. 1 The fluorescence spectra of BCB in the BCB-AuNPs system with various concentrations of AuNPs in phosphate buffer with a pH of 8.0. BCB:10 μM; AuNPs (from a to q): 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 , 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7 nM.

Previous studies have demonstrated that three typical mechanisms including fluorescence resonance energy transfer (FRET),[53-56] electron transfer (ET), [40] and inner filter effect (IFE)[5] dominate the fluorescence quenching of fluorphores by the nearby AuNPs. In this study, as shown

in Fig. S3(Supporting Information), only slight overlap between the emission spectrum of BCB and the absorption spectrum of AuNPs was observed, the observed dramatic quenching of BCB is less likely caused by energy transfer.[40, 60-62] In addition, as shown in Fig.1, the observed spectral changes of BCB in the presence of various concentrations of AuNPs with 3-nm red-shift of the emission maximum, suggested that AuNPs quenched the fluorescence of adsorbed BCB more likely through electron transfer rather than energy transfer processes.[40,46,63] Besides, the IFE may also contribute to the fluorescence quenching of BCB, but generally the contribution of IFE to quenching of fluophors is subordinate to FRET or ET.[64] In this study, electron transfer mechanism may dominate the quenching of BCB by nearby AuNPs.

Fluorescence turn-on assay of Pb^{2+} with BCB-AuNPs assembly

Fig. 2 depicts the emission spectra of BCB, BCB-Pb^{2+}, BCB-AuNPs, and BCB-AuNPs-Pb^{2+}, respectively, under the same experimental conditions. As shown in this figure, BCB solution exhibits a strong fluorescence signal at about 660 nm (curve a). When different concentrations of Pb^{2+} were added to the solution, the fluorescence intensity and peak location did not show any obvious changes (curve b, curve c), indicating that there is no interaction between Pb^{2+} and BCB. Also shown in this figure, upon AuNPs was added into the BCB solution, the fluorescence intensity of BCB was decreased dramatically (curve d) via electron transfer between BCB molecules and AuNPs. At pH 8.0, BCB molecules are positively charged[59] and GSH-modified AuNPs are negatively charged, [51] and therefore there exists the electrostatic interaction between them. On the other hand, the -NH_2 group contained in BCB molecules can react with gold.[40] We assumed that the electrostatic interaction and Au-N interaction result in the formation of BCB-AuNPs assembly. More interesting, when Pb^{2+} ions were introduced into the BCB-AuNPs assembly solution, the fluorescence of BCB was restored (curve e and f), suggesting that BCB molecules released from the surfaces of AuNPs due to the formation of chelating complex between Pb^{2+} and the COO^- of the

GSH.[51]

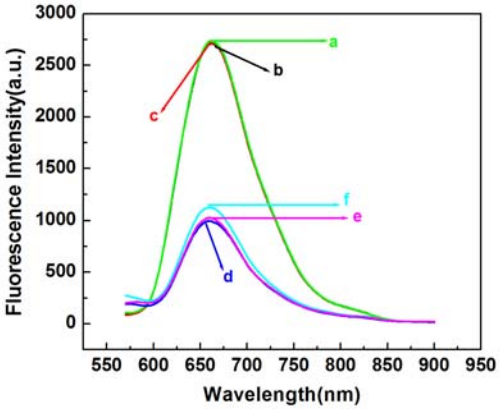


Fig. 2 Fluorescence spectra of (a) 10 μM BCB, (b) (a) + 0.25 nM Pb^{2+}, (c) (a) + 2.5 nM Pb^{2+}, (d) (a) + 1.7 nM AuNPs, (e) (d) + 0.25 nM Pb^{2+}, and (f) (d) + 2.5 nM Pb^{2+} in phosphate buffer with a pH of 8.0.

Optimization of experiment conditions for Pb^{2+} assay

The effect of pH on the BCB-AuNPs assembly was investigated. Fig. 3 displays the fluorescence difference (ΔF) of the BCB-AuNPs assembly solution in absence and presence of Pb^{2+} in the pH range from 6.0 to 8.5. As shown in this figure, it can be seen that the largest fluorescence difference is obtained at pH 8.0. As aforementioned, at pH 8.0, BCB molecules are positively charged,[59] while GSH-AuNPs are negatively charged,[51] and thus the strong electrostatic interaction between them is favorable to form compact BCB-AuNPs assembly and therefore transfer electrons between BCB molecules and AuNPs, resulting in the lowest background fluorescence of the BCB-AuNPs assembly. In addition, the negatively charged $–COO^-$ groups of GSH confined on AuNPs easily bind strongly with Pb^{2+}, leading to releasing of BCB molecules from GSH-AuNPs surface and therefore the restoration of their fluorescence. Combining these two points for considering, it is not surprising that the maximum fluorescence difference can be obtained at pH 8.0. Therefore, pH 8.0 buffer was recommended and used throughout in this study.

At room temperature, when BCB and AuNPs were mixed, the relative fluorescence intensity

reached a stable value within 30 min. In addition, upon Pb^{2+} was introduced into BCB-AuNPs assembly solution, the fluorescence was restored quickly and reach a stable value within 7 min. In this study, a incubation time of 40 min was adopted for Pb^{2+} assay.

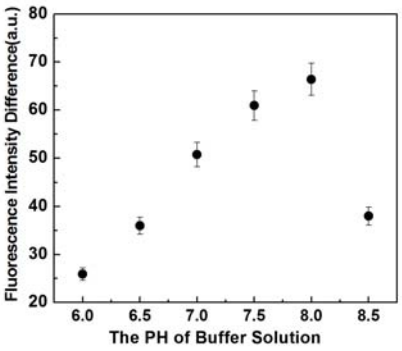


Fig. 3 Effect of pH of buffer solution on the fluorescent enhancement efficiency of the BCB-AuNPs system in the presence of Pb^{2+}. The concentrations of BCB, AuNPs and Pb^{2+} are 10 μM BCB, 1.7 nM and 1.5 nM, respectively.

NIR fluorescent detection Pb^{2+} with BCB-AuNPs assembly

Under the optimum conditions, the proposed BCB-AuNPs assembly system for the determination of Pb^{2+} was evaluated. Fig. 4 displays the fluorescence spectra of BCB-AuNPs assembly system in the presence of different concentrations of Pb^{2+}. As shown in this figure, the fluorescence intensity of BCB-AuNPs restored gradually with the increasing of concentrations of Pb^{2+}, indicating the more Pb^{2+} was added, the more BCB molecules were released from the surface of AuNPs and as thus the fluorescence was restored gradually. The plot of the relatively enhanced fluorescence intensity, $\Delta F = F\text{-}F_0$, against the concentration of added Pb^{2+} is shown in Fig 4. Herein, F and F_0 represent the fluorescence intensity in the presence of different concentrations of Pb^{2+} and absence of Pb^{2+}, respectively. It is clear that the enhanced fluorescence intensity (ΔF) exhibits a linear response to Pb^{2+} concentration in the range of 7.5×10^{-10}-1.0×10^{-8} M (0.16-2.1 ng mL^{-1}) with a correlation coefficient of 0.998. The limit of detection (3σ) for Pb^{2+} is 0.51 nM (0.11 ng mL^{-1}).

Where σ represents the standard deviation of eight blank measurements. These analytical parameters are better than or comparable to those reported in literatures, as shown in Table S1(Supporting Information). The reproducibility of the present system was also evaluated by measurements of 5.0 nM Pb^{2+} and the relative standard deviation (RSD) for 11 repeated measurements was found to be 1.93%, indicating that the response of the BCB-AuNPs assembly system to Pb^{2+} assay is highly reproducible.

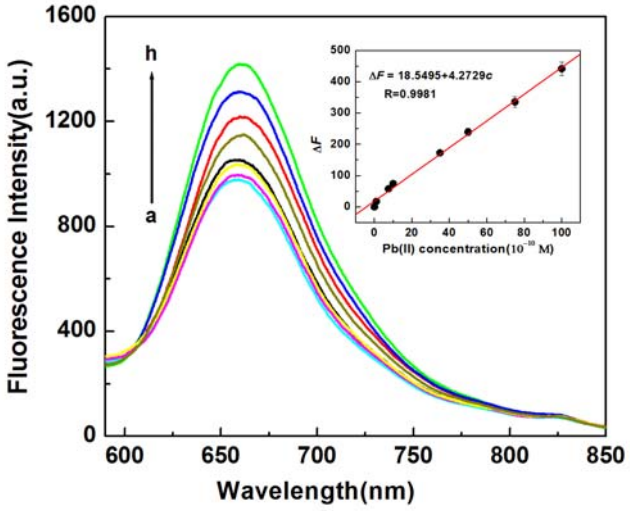


Fig. 4 Fluorescence spectra of BCB-AuNPs system in presence of different concentrations of Pb^{2+} (From a to i: 0, 1×10^{-10}, 7.5×10^{-10}, 1×10^{-9}, 3.5×10^{-9}, 5×10^{-9}, 7.5×10^{-9}, 1×10^{-8} M). Inset: the enhanced fluorescence intensities as a function of concentration of Pb^{2+}. BCB:10 μM, AuNPs: 1.7 nM, pH=8.0.

Analysis of Pb^{2+} in Real samples with the proposed BCB-AuNPs assembly system

Prior to assaying Pb^{2+} in real samples, the effect of potential interfering substances such as coexistence of common ions on the determination of Pb^{2+} was investigated. We examined the fluorescence response of the present system to Pb^{2+} at a concentration of 5.0 nM in the presence of different interfering substances. The effects of main relevant metal ions on the fluorescence intensity of the present system for 5 nM Pb^{2+} were studied and the results showed that the potential

coexisted ions induced less than ± 5% interference with the detection of the Pb^{2+} (Table S2, Supporting Information). These results suggested that BCB-AuNPs system possessed a good selective fluorescence response toward Pb^{2+}, and also indicated that a turn-on fluorescent method for the determination of Pb^{2+} could be developed by the present system.

To evaluate the applicability of the method, we use this method to detect real different samples. Certain amounts of Pb^{2+} standard solution were directly spiked into the real samples, and the results of real samples by standard addition method are summarized in Table 1. As shown in Table 1, excellent recoveries in the range from 95.0% to 114.0% were obtained for all samples, and the results are also good agreements with classical ICP-AAS method, suggesting that the proposed method is reliable and suitable for real applications.

Table 1. Results of determination of Pb^{2+} in real samples[a]

	The proposed method				ICP-AAS			
Water samples	Original (Mean ± SD) / ng mL^{-1}	Spiked / ng mL^{-1}	Found (Mean ± SD) / ng mL^{-1}	Recovery (Mean ± SD) / %	Original (Mean ± SD) / ng mL^{-1}	Spiked / ng mL^{-1}	Found (Mean ± SD) / ng mL^{-1}	Recovery (Mean ± SD) / %
Waste water (form dye mill)	1.61±0.03	0.2	1.80 ± 0.01	95.0 ± 5.0	1.59±0.01	0.2	1.78±0.01	95.0 ± 0.0
		0.3	1.93±0.02	106.6± 6.7		0.3	1.90±0.02	103.3 ± 3.3
Tap water	0.73±0.04	0.5	1.30±0.01	114.0± 2.0	0.67±0.03	0.5	1.25±0.03	116.0 ± 0.0
		1.0	1.68±0.03	95.0± 3.0		1.0	1.62±0.02	95.0 ± 1.0

[a] all the data are the mean for five repeated measurements

Conclusions

In summary, we have developed a convenient turn-on NIR fluorescent method for sensitive detection of Pb^{2+} with the BCB-AuNPs assembly in aqueous solutions. Based on the chelating interaction between Pb^{2+} and $–COO^-$ groups confined on the surface of AuNPs, the BCB molecules release from the BCB-AuNPs assembly and therefore their fluorescence restore. By virtue of the restored fluorescence, a turn-on type fluorescent chemosensor for Pb^{2+} was developed. The

proposed method was successfully applied to the determination of Pb^{2+} in different water samples including waste water and tap water, suggesting the present method has the potentially practical applications in environmental analysis.

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