Enhanced Turn-On Fluorescence Detection of Aqueous Lead Ions with Size-Shrinkable Hydrogels

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ABSTRACT: Highly sensitive detection of lead ions in water is of importance. This paper reports a new method to enhance the sensitivity of fluorescence detection of aqueous lead ions by exploiting the large volume reduction of hydrogels upon dehydration. Rhodamine-derived prefluorescent probes with high selectivity to lead ions are grafted on a carboxylated agarose hydrogel. Upon binding low-concentration lead ions, fluorescence emission is turned on. The dehydration of the hydrogel leads to a size reduction of over 40 times and an enhancement of fluorescence of 10 times at a lead-ion concentration of 10^-7 M, allowing fluorescence detection with naked eyes. Given its low cost, easy operation, and high sensitivity, the volume reduction hydrogel can be used to detect lead ions in drinking water.

1. INTRODUCTION

Lead ion is a devastating environmental toxin. Analytical methods to detect aqueous lead ions include atomic absorption spectrometry, inductively coupled plasma mass spectrometry, and optical emission spectrometry, but these methods are expensive and require trained personnel to operate them. Fluorescent methods are often used to detect lead ions in aqueous and organic solutions due to their operational readiness. However, existing fluorescent probes can optically detect lead ions at a sufficiently high concentration (>10^-5 M) because of their low sensitivity. In the case of monolayer-based detection, an even higher concentration of fluorescent probes (>10^-4 M) is needed, which is much higher than the national standard for lead levels in drinking water.

The low sensitivity in fluorescent detection is because of low concentration of metal ions in a given volume of optical excitation. Preconcentration such as extraction can be used to enrich low-concentration metal ions, but it needs more sample and extra operation steps and may lead to contamination. If the probes can be enriched after binding to metal ions, the sensitivity can be enhanced without altering optical illumination and fluorescent detectors. One way of increasing local concentration of fluorescent probes is to take advantage of size-changeable hydrogels, whose volume can be reduced hundreds or even thousands of times once dehydrated. The idea of volume increase of hydrogels has been used to locate and image proteins, while it has not been used to enhance metal ion detection when the volume of hydrogel decreases. Agarose gel shrinks more significantly after dehyration than other hydrogels such as polyacrylamide gel and gelatin, while agarose lacks active moieties (i.e., amine, carboxyl, or thiol groups) and cannot be modified directly. Agarose can be activated to form carboxylated agarose (CA) by oxidizing its primary alcohol groups, and CA can form a stable hydrogel at room temperature.

This article describes a new method to detect aqueous lead ions at low concentrations with turn-on fluorescence on a size-shrinkable hydrogel (Figure 1). A prefluorescent molecule will be immobilized on the hydrogel. Once selective binding to lead ions occurs, the fluorescent signal will be turned on. By shrinking the volume of the hydrogel where fluorescence probes are chemically linked, the sensitivity of detection will be enhanced, indicating that the fluorescent quench at a high fluorophore concentration does not play a significant role. The prefluorescent probe used here is a derivative of rhodamine, which is grafted onto the CA backbone through an amine

Figure 1. Scheme of enhanced turn-on fluorescence detection of lead ions with a dehydrating hydrogel.

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moiety. Once they bind to target metal ions, the molecules undergo a structural change from a spirocyclic lactam to an open-ring amide, resulting in pink fluorescence emission. The agarose hydrogel shrinks upon dehydration, and the density of fluorescent probes increases in the gel, causing fluorescence signals to be enhanced.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Materials. Agarose powder was obtained from IBI scientific. Rhodamine 6G (99%) was obtained from Acros Organics. Lead perchlorate trihydrate [crystalline, Pb(ClO₄)₂], acetonitrile (99%), tris(2-aminoethyl)amine (97%), dichloromethane (CH₂Cl₂), methanol (>99.8%), and perfluorooctyltrichlorosilane (PFTOS, 97%) were obtained from Alfa Aesar. Tetramethyl-1-piperidinoloxyl (98%, TEMPO), sodium bromide (>99.0%, NaBr), sodium hypochlorite solution (10−15%, NaClO), anhydrous sodium sulfate (NaSO₄), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (>97%, EDC), and sodium hydroxide (NaOH) were obtained from Sigma. Polydimethylsiloxane (PDMS) was obtained from Corning. Phosphate-buffered saline (PBS) was obtained from VWR.

2.2. Agarose Carboxylation. Agarose was treated with a TEMPO–NaBr–NaClO system to oxidize primary alcohol groups to CA as follows: 1 g of agarose powder was dissolved in 80 mL of water at 90 °C. After adjusting the pH to 11 with 1 M aqueous NaOH, the solution was cooled down to room temperature with stirring, and 22 mL of an aqueous solution containing 0.02 g of TEMPO, 0.3 g of NaBr, and 2 mL of NaClO was added dropwise into the agarose solution, and the pH of the solution was maintained at 10−11 by adding 1 M aqueous NaOH. The oxidation reaction was completed in 1.5 h. The solution was precipitated by adding a mixture of 200 mL of isopropyl alcohol and 20 mL of acetone. The precipitate was washed twice with ethanol, and the oxidized agarose was dialyzed for 48 h and lyophilized to remove the solvent.

2.3. Grafting Turn-On Fluorescent Probes on Oxidized Agarose. A rhodamine-based turn-on fluorescent probe was prepared according to the literature. A total of 80 mg of oxidized agarose was dissolved in 10 mL of PBS, followed by adding 40 mg of EDC to activate carboxyl groups at 80 °C for 1 h. The modified agarose was mixed with 20 mg of raw agarose to form a hydrogel, which was immersed in deionized water overnight. To graft the turn-on probe, the rinsed hydrogel was mixed with 40 μL of 2 mg/mL turn-on fluorescent probe in acetonitrile at 80 °C. After reaction for 1 h, the agarose was rinsed in a mixed acetonitrile and water solution (volume ratio of 20:100) 3 times, followed by centrifugation and evaporation.

2.4. Templated Hydrogel Formation. In order to generate hydrogels with defined geometry, a bottomless well made of elastic PDMS was fabricated by using a polyacrylic well (with desired shape and dimension) as the mold. The bottomless well was firmly bonded to plasma-treated glass substrates. The fluorescent probe-immobilized hydrogel at a mass ratio of agarose and water of 1:99 was then injected into the PDMS template and taken out by separating the PDMS template and glass substrate after gelation.

2.5. Metal Ion Detection. Aqueous solutions of a variety of metal ions were prepared by dissolving corresponding salts in water with concentrations ranging from 10⁻¹ to 10⁻⁷ M. The templated hydrogel was immersed in the solution of each metal ion at 60 °C for 30 min, followed by rinsing with acetonitrile–water (1:10). The hydrogel was dehydrated on a PFTOS-modified hydrophobic glass substrate and dried at 70 °C for 30 min.

The fluorescent images of hydrogels after catching metal ions were taken using an Olympus BX51 fluorescence microscope. The fluorescence intensity at each pixel of an image was derived by using ImageJ software. The zeta potential change of the hydrogel before and after modification was measured with a NANO ZS ZEN3600 zeta potential analyzer (Malvern Instruments). Fourier transform infrared (FTIR) spectra of hydrogels were collected with a spectrometer (Bruker Vertex 70) combined with a Hyperion 1000 microscope.

3. RESULTS AND DISCUSSION

Figure 2A shows the procedures of modifying agarose, where the primary hydroxyl groups of agarose are oxidized to carboxyl groups in CA, followed by grafting prefluorescent probes through the carboxyl–amine reaction. The prefluorescent probes do not produce fluorescence because carbon–nitrogen bonds destroy the conjugation structure in rhodamine and prevent it from fluorescence emission. Metal ions can bind to the amine group since the lone pair electrons of an amine group have a strong affinity to the outer shell of the metal ion. Once bound to a target cation, the molecule will undergo a
structural change from a spirocyclic lactam to an open-ring amide, which results in fluorescence in pink. The zeta potential of the aqueous solution of pristine agarose is nearly neutral (−2 mV), and oxidized agarose is negatively charged (−16.8 mV) due to formation of carboxyls. FTIR spectra indicate the vibrational peaks of carboxyl groups of oxidized agarose at the wavenumbers of 1400 and 1600 cm⁻¹ (Figure 2B). The zeta potential increases slightly (−9 mV) after immobilization of prefluorescent probes due to attachment of amines. The FTIR spectrum shows the vibrational peak of benzene on rhodamine at 1500 and 2900 cm⁻¹, which confirms successful immobilization of prefluorescent probes.

Figure 3A shows the digital images of metal ion solutions without (upper) and with (middle) UV (365 nm) excitation, as well as with prefluorescent probes and UV light (lower). Each cuvette contains one type of 10⁻² M metal ion including iron, copper, calcium, cobalt, and lead (left to right). The fluorescent probe is found to be highly selective to lead ions. Fluorescent spectroscopy (Figure 3B) shows that the maximum emission of the probe–lead pair is at 530 nm. The fluorescent intensity of the probe–lead pair is 10 times stronger than those of pairs formed by the probe and other ions. Figure 3C shows the time-dependent fluorescent intensity of the prefluorescent probe after mixing with lead ions (10⁻³ M, pH of 7). The fluorescent intensity of the mixture increases with time, and reaches a plateau after 15 min. Figure 3D shows that the pH of the solution affects the fluorescent intensity. The fluorescent intensity is negligible when the pH is greater than 10 and when the lead ion is precipitated as lead oxide in an alkaline solvent, while the fluorescent probe is not selective to lead ions in a solution with a pH below 4. Therefore, the operational pH range of the fluorescent probe is between 4 and 10. In addition, the shrinking ability of the CA hydrogel is tested by volume measurement. Figure 4A shows the dry volume of CA as a function of agarose mass ratio in the original hydrogel (volume of 15 mL), where 97.5% volume reduction has been achieved when 1% by weight of agarose hydrogel is used. This volume reduction could enrich molecules in dry agarose to a 40-times higher density theoretically.

Figure 4B shows that fluorescence emission from wet agarose treated with 10⁻⁴ M lead ions is hard to see under UV light, while it is obvious in dried agarose. Figure 4C (right) shows the fluorescence from the dried hydrogel observed with microscopy, where the fluorescence intensity is uniform across the whole gel. In contrast, the fluorescence of the hydrogel with physically adsorbed rhodamine shows uneven distribution after drying (Figure 4C left), suggesting that chemically immobilized fluorescence molecules undergo the same volume change as the hydrogel itself and do not aggregate during drying. Figure 4D shows that a linear relation exists between fluorescence intensity and the concentration of fluorescence probes in the hydrogel, where an excess amount of lead ions is added to ensure all prefluorescent probes are lighted. Therefore, the fluorescence intensity should be exponential to the level of lead ions.

Figure 5A shows the fluorescence images of lighted hydrogels with different loads of lead ions before (upper) and after drying (middle) taken at the same UV excitation and exposure time (100 ms), confirming that the hydrogel dehydration enhances fluorescence. Meanwhile, the cubic hydrogel dries to achieve a rectangular shape because water evaporation occurs preferentially at the sides of the rectangle (due to large contact areas with air), which produces a mass transfer of the wet hydrogel from the center to the edge (Figure 5B). The detection sensitivity to lead ions is enhanced to 10⁻⁷ M by comparing the fluorescence contrasts of the edge of the dry hydrogel and background. The fluorescence intensity at the edge of a dried rectangular-shaped gel is derived by ImageJ. The intensity of fluorescence depends on the amount of the fluorescence probe and geometry of the dried hydrogel. Figure 5B shows an exponential dependence of the fluorescence intensity on the lead ion level (from 10⁻⁷ to 10⁻³ M), where red and black bars are fluorescent intensities of hydrogels before and after dehydration, respectively. The enhancement ranges from 3 times at low concentrations to more than 10 times at high concentrations, compared to those of wet hydrogels. Figure 5C shows the fluorescence intensity of the hydrogel in a vertical direction as a function of thickness during the dehydration process, where the hydrogel is confined inside a Petri dish to allow evaporation only through the top surface. The fluorescent intensity increases by 20% during dehydration, which is much smaller than the lateral direction. The relatively small increase in fluorescence is due to the fact that the number of fluorescence probes in the vertical direction does not increase much after dehydration. Therefore, the fluorescence intensity should be exponential to the level of lead ions. The US Environmental Protection Agency regulates that the lead ions in drinking water should be below 10⁻⁷ M; thus, this method allows visual examination against lead ions in drinking water.

4. CONCLUSIONS

The volume reduction of hydrogels upon dehydration is used to enhance the sensitivity of turn-on fluorescence detection of lead ions. The shrinkage of the hydrogel during dehydration can enrich molecules in dry agarose to a 40-times higher density theoretically, which is confirmed by Figure 4A. The dehydration of the hydrogel is confirmed by Figure 4B, where the fluorescence intensity of the dried hydrogel is uniform across the whole gel. In contrast, the fluorescence of the hydrogel with physically adsorbed rhodamine shows uneven distribution after drying (Figure 4C left), suggesting that chemically immobilized fluorescence molecules undergo the same volume change as the hydrogel itself and do not aggregate during drying. Figure 4D shows that a linear relation exists between fluorescence intensity and the concentration of fluorescence probes in the hydrogel, where an excess amount of lead ions is added to ensure all prefluorescent probes are lighted. Therefore, the fluorescence intensity should be exponential to the level of lead ions. Figure 5A shows the fluorescence images of lighted hydrogels with different loads of lead ions before (upper) and after drying (middle) taken at the same UV excitation and exposure time (100 ms), confirming that the hydrogel dehydration enhances fluorescence. Meanwhile, the cubic hydrogel dries to achieve a rectangular shape because water evaporation occurs preferentially at the sides of the rectangle (due to large contact areas with air), which produces a mass transfer of the wet hydrogel from the center to the edge (Figure 5B). The detection sensitivity to lead ions is enhanced to 10⁻⁷ M by comparing the fluorescence contrasts of the edge of the dry hydrogel and background. The fluorescence intensity at the edge of a dried rectangular-shaped gel is derived by ImageJ. The intensity of fluorescence depends on the amount of the fluorescence probe and geometry of the dried hydrogel. Figure 5B shows an exponential dependence of the fluorescence intensity on the lead ion level (from 10⁻⁷ to 10⁻³ M), where red and black bars are fluorescent intensities of hydrogels before and after dehydration, respectively. The enhancement ranges from 3 times at low concentrations to more than 10 times at high concentrations, compared to those of wet hydrogels. Figure 5C shows the fluorescence intensity of the hydrogel in a vertical direction as a function of thickness during the dehydration process, where the hydrogel is confined inside a Petri dish to allow evaporation only through the top surface. The fluorescent intensity increases by 20% during dehydration, which is much smaller than the lateral direction. The relatively small increase in fluorescence is due to the fact that the number of fluorescence probes in the vertical direction does not increase much after dehydration. Therefore, the fluorescence intensity should be exponential to the level of lead ions. The US Environmental Protection Agency regulates that the lead ions in drinking water should be below 10⁻⁷ M; thus, this method allows visual examination against lead ions in drinking water.
lead ions in water. The rhodamine-derived fluorescent probes grafted on CA hydrogel show excellent selectivity to lead ions and superior turn-on fluorescence upon binding lead ions. The dehydration of hydrogel leads to a volume reduction of more than 40 times and can effectively increase fluorescence emission to 10 times higher after capturing lead ions at a concentration of $10^{-7}$ M. The enhancement is at such a degree that the turn-on fluorescence upon lead ion binding can be easily seen with naked eyes. Given its low cost, straightforwardness, easy operation, and high sensitivity, this volume-changing hydrogel can be used to detect lead ions in drinking water.

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**Notes**

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Aromatization of 9,10-dihydroacridine derivatives: discovering a sensing film for selective detection of Pb2+.

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A recent study by Li et al. (11) demonstrated the potential of Rhodamine Carbon Nanodots for selective sensing of Pb2+. Their work involved the development of a facile fluorescent sensing system composed of thiol-modified magnetic particles, which were capable of selectively sensing Pb2+ ions in seawater samples by isotope dilution ICPMS.

A chemosensing monolayer on glass was used as a facile fluorescent sensing film for selective detection of Pb2+. The method described by Sharon et al. (10) involved preconcentration and fluorimetric determination of polycyclic aromatic hydrocarbons using engineered GO Amberlite XAD-16 picolylamine sorbent for the extraction with sodium dodecylsulfate.

The determination of mercury(II) and lead(II) using screen-printed electrodes modified with thiol-modified magnetic particles was reported by Goryacheva et al. (17) in their study on the preconcentration and fluorimetric determination of mercury(II) in small-volume seawater samples by isotope dilution ICPMS.

Mechanically tailored agarose hydrogels through molecular alloying with β-sheet polysaccharides were studied by Micahud et al. (30) in their work on the development of mechanically tailored agarose hydrogels through molecular alloying with β-sheet polysaccharides. The study demonstrated the potential of such materials for potential applications in tissue engineering.

Goryacheva et al. (17) investigated the preconcentration and fluorimetric determination of mercury(II) in small-volume seawater samples by isotope dilution ICPMS. Their results indicated the potential of such methods for the analysis of trace elements in environmental samples.

Chemosensor based on a rhodamine 6G derivative for the detection of Pb2+ ion was developed by Li et al. (10). The method involved the use of Rhodamine Carbon Nanodots as a sensing material on a glass substrate.

The work by Sharon et al. (10) highlighted the potential of mechanically tailored agarose hydrogels for tissue engineering applications.

In conclusion, the development of chemosensing materials and their application in the analysis of trace elements in environmental samples is an active area of research, with significant potential for future applications in environmental monitoring and health care.

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