Engineering grey Nanosystem as Activatable Ratio-colorimetric Probe for Detection of Lead Ions in Preserved Egg

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Abstract

A grey system based on the principle of complementary colors was constructed as an activatable probe for sensitive and specific Ratio-colorimetric detection lead ions (Pb\(^{2+}\)) in complex food matrix. This grey system was prepared through mixing purple-red AuNPs-capped glutathione (GSH) and green-blue sulfonated pigment green 7 (SPG7), named SPG7/AuNPs probe. In the presence of Pb\(^{2+}\), the strong chelation of Pb\(^{2+}\) with GSH could trigger the aggregation of AuNPs, leading to the color activation of SPG7. Hence, the absorbance ratio \(A_{523\text{ nm}}/A_{628\text{ nm}}\) of AuNPs at ~ 523 nm and SPG7 at ~ 628 nm could be used for highly-specific reporting of Pb\(^{2+}\) levels with a low detection limit of 0.33 \(\mu\)g/L. Moreover, this probe exhibited promising practical applications in real preserved egg samples with recoveries of 89.2% to 107.5% and relative standard deviations (R. S. D.) in the range of 0.28% to 2.12%, revealing its great potential in harmful substance detection.

Keywords: Gold nanoparticles; Color complementarity; Ratio-colorimetric; Lead ions; Preserved egg;
1. Introduction

Preserved egg (also known as Songhuadan) is traditional Chinese flavored egg product. Due to the unique flavor and certain medicinal value, preserved egg is consumed by consumers in more than 30 countries around the world. However, preserved egg is made by wrapping duck eggs with a mixture of calcium oxide, lead oxide, tea, soda ash, potassium carbonate and salt for 4-6 weeks at room temperature, easily causing that Pb$^{2+}$ enters the inside through the eggshell. It has been proved that Pb$^{2+}$ can cause adverse health effects in humans, especially children, when Pb$^{2+}$ is exposed to the human body, causing abdominal pain, diarrhea, vomiting, headache, heart palpitations, vasospasm and liver and kidney damage. In view of these facts above, it is urgent to develop a simple, rapid, low-cost, sensitive, and specific assay for Pb$^{2+}$ monitoring in preserved eggs, avoiding chronic accumulation poisoning caused by eating preserved eggs with excessive Pb$^{2+}$ for a long time.

To date, a large number of sensitive techniques including atomic absorption spectrometry, inductively coupled plasma mass spectrometry, DNA biological sensor, anodic stripping voltammetry, fluorescence, have been developed toward Pb$^{2+}$ detection. Although these assays can monitor Pb$^{2+}$ at a low concentration, some inherent issues are unavoidable such as expensive equipment requirements, multiple assay steps, complex probe preparation, and etc. Alternatively, the colorimetric assays have also been used for Pb$^{2+}$ detection because of point-of-care analytical detection in terms of their miniaturization, low cost, and no aid of complex instruments. For example, Wang et al. developed a label-free 17E DNAzyme-based probe to detect Pb$^{2+}$ using unmodified AuNPs, with 500 nM detection limit. Using the specific binding of different bio-molecules to AuNPs, Knecht and co-workers selected mercaptoundecanoic acid (MUA) as a binding ligand to achieve colorimetric detection of Pb$^{2+}$. Beside, Nazeeruddin et al. prepared GSH-AuNPs self-assembled sensors for the analysis of Pb$^{2+}$ which produced by halogenated lead-halide perovskite solar cells (PSCs) with a detection limit of 15 nM. In particular, Tseng and co-workers developed a colorimetric detection of Pb$^{2+}$ by controlling the particle size, particle size distribution of gallic acid-capped gold nanoparticles (GA-AuNPs) and minimizing electrostatic repulsion between each GA-AuNP, the lowest detection limit is 10 nM. Despite the great success and the ongoing increase of interest with AuNPs-based colorimetric assays, most of them were dependent on AuNPs as the starting color indicator, which may somehow lower signal-to-background ratios and hence weaken the detection sensitivities.

To overcome these shortcomings of the colorimetric assays-based AuNPs above, we have herein developed a novel active Ratio-colorimetric SPG7/AuNPs probe-based the color complementary principle, which was obtained by the mixture of AuNPs stabilized with anionic ligand glutathione (GSH-AuNPs) with sulfonated pigment green 7 (SPG7) under the optimal experiment conditions and revealed its high convenience, sensitivity, and specificity for Pb$^{2+}$ detection in preserved eggs. We demonstrated that two kinds of solutions with the complementary color i.e., the mixture of purple-red AuNPs and green-blue SPG7 absorbed a couple of complementary light of green-blue and purple-red, resulting in the generation of grey solution. SPG7 was selected for the preparation of probes due to its chemical stability and special color. Not only could it be used as an internal standard to correct the level of lead ions, it also made color changes easier to identify. After incubation with Pb$^{2+}$, the strong chelation of Pb$^{2+}$ to GSH could effectively trigger the aggregation of AuNPs and liberate the green-blue color of SPG7. As a result, an obvious color of green-blue was observed, which have been successfully used for simple, rapid, low-cost, sensitive and specific detection of Pb$^{2+}$ in preserved egg.

2 Materials and methods

2.1 Reagents and apparatus
The main chemical reagents used in this work were chloroauric acid hydrated (\( \text{AuCl}_3 \cdot \text{HCl} \cdot 4\text{H}_2\text{O} \): Nanjing Chemical Reagent CO., Nanjing, China), L-glutathione (GSH, J&K Scientific Ltd., Beijing, China), lead nitrate (\( \text{Pb (NO}_3 \) \( \_2 \)), aluminum chloride (\( \text{AlCl}_3 \)), ferric chloride (\( \text{FeCl}_3 \)), magnesium chloride (\( \text{MgCl}_2 \)), barium chloride (\( \text{BaCl}_2 \)), potassium carbonate (\( \text{K}_2\text{CO}_3 \)), manganese sulfate (\( \text{MnSO}_4 \)), mercury chloride (\( \text{HgCl}_2 \)), cadmium chloride (\( \text{CdCl}_2 \)), calcium chloride (\( \text{CaCl}_2 \)), sodium chloride (\( \text{NaCl} \)) and zinc sulfate (\( \text{ZnSO}_4 \)) (Sigma-Aldrich). The purity of all metal salts was 99.99%. The Britton-Robison buffer with a pH of 6.5 was made by mixing phosphoric acid (\( \text{H}_3\text{PO}_4 \)), orthoboric acid (\( \text{H}_3\text{BO}_3 \)), acetic acid (\( \text{CH}_3\text{COOH} \)) and sodium hydroxide (\( \text{NaOH} \)). Concentrated nitric acid (\( \text{HNO}_3 \)) and perchloric acid (\( \text{HClO}_4 \)) were purchased from Sigma-Aldrich. Preserved eggs were purchased from a local supermarket (Hefei, China). Pigment green 7 was obtained from J&K Scientific Ltd., Beijing, China. 4-hydroxybenzenesulfonic acid hydrate was purchased from Aladdin Industrial Corporation, Shanghai, China. The water used throughout was deionized water (D. I. water).

Transmission electron microscopic (TEM) images of nanoparticle were acquired with a JEM-2100 transmission electron microscope (JEOL, Ltd., Japan). Absorption spectra were recorded with a UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan) at room temperature. Raman spectra were conducted on a portable BWTeki-Raman@Plus (Model #BWS465-785S) equipped with a standard BCR100A accessory. A 90 Plus/BI-MAS equipment (Brook haven, USA) was used to obtain the dynamic light scattering (DLS) spectra. A Zetasizer (Nano-Z, Malvern, UK) was applied to measure the zeta potential of nanoparticle.

2.2 Preparation of GHS-AuNPs

The synthesis of GHS-AuNPs was referred to the previous method with some modification. First, 0.03 g trisodium citrate dehydrate and 100 mL D. I. water was first added into a cleaned flask under stirring (400 rpm), and the mixture solution was heated to 100°C. After that, \( \text{AuCl}_3 \cdot \text{HCl} \cdot 4\text{H}_2\text{O} \) (250 mM 100 \( \mu \text{L} \)) was added rapidly into the above mixture solution, and the resulting mixture was continuously stirring at 400 rpm for 7 min to observe an purple-red solution, followed by stirring and cooling to room temperature on an ice-water bath. Afterward, the 0.4 mg GSH was added into the solution with stirring at 650 rpm for 1 h, and the original GHS-AuNPs was obtained and stewed overnight at room temperature. Finally, the original GHS-AuNPs was transferred into a 10 KD Millipore, centrifuged (8000 rpm), and washed with D. I. water for three times. The concentration of the purified GSH-AuNPs was 2.08 nM and stored for further use.

2.3 Preparation of the as-proposed colorimetric SPG7/AuNPs probe

Firstly, pigment green 7 (Figure S1) was pre-treated with 4-hydroxybenzenesulfonic acid hydrate to obtained SPG7. Subsequently, 140 \( \mu \text{L} \) 0.1% SPG7 was added into the 500 \( \mu \text{L} \) of 2.08 nM AuNPs for 1.0 min incubation at room temperature, a grey solution of SPG7/AuNPs probe was obtained.

2.4 Detection of \( \text{Pb}^{2+} \) using the Ratio-colorimetric SPG7/AuNPs probe in aqueous solution

2.4.1 Drawing of standard curve. Briefly, in 1.5 mL EP tube, 640 \( \mu \text{L} \) SPG7/AuNPs (1.41 nM determined by AuNPs) in Britton-Robison buffer (pH=6.5) was incubated eight different concentrations (1.0 \( \mu \text{g/L} \), 10.0 \( \mu \text{g/L} \), 100.0 \( \mu \text{g/L} \), 1.0 mg/L, 10.0 mg/L, and 100.0 mg/L, 0.2 g/L, 0.5 g/L) of Lead nitrate standard solutions (400 \( \mu \text{L} \)) under slight shaking to acquire sample groups. For the determination of \( \text{Pb}^{2+} \) concentration in sample solutions, three parallel tests were performed for each sample. Meanwhile, the control group consisted of 11 blank samples prepared by adding 400 \( \mu \text{L} \) of ultrapure water to 640 \( \mu \text{L} \) SPG7/AuNPs in 1.5 mL EP tube under the same conditions. After 1.0 min, the absorption spectra were recorded at room temperature using a UV-Vis spectroscopy. \( A_{523 \text{ nm}}/A_{628 \text{ nm}} \) and standard deviation of all samples and control groups were calculated, followed by a linear
2.4.2 Selective experiment. Selectivity is one of the most crucial characteristics of an applied probe. First, metal cation salt solutions having a same concentration of 1 mg/L were prepared, including aluminum chloride (AlCl$_3$), ferric chloride (FeCl$_3$), magnesium chloride (MgCl$_2$), barium chloride (BaCl$_2$), potassium carbonate (K$_2$CO$_3$), manganese sulfate (MnSO$_4$), mercury chloride (HgCl$_2$), cadmium chloride (CdCl$_2$), calcium chloride (CaCl$_2$), sodium chloride (NaCl) and zinc sulfate (ZnSO$_4$). In order to investigate the selectivity of the colorimetric SPG7/AuNPs probe for Pb$^{2+}$, we incubated 640 $\mu$L SPG7/AuNPs with 400 $\mu$L 100 mg/L some other metal ions under the same conditions, and then the absorption spectra were obtained with the same UV-Vis spectroscopy. The test was repeated three times to reduce the accidental error.

2.5 Detection of Pb$^{2+}$ using the Ratio-colorimetric SPG7/AuNPs probe in commercial preserved eggs

2.5.1 Pretreatment of actual samples. The traditional craft preserved egg and lead-free craft preserved egg were randomly purchased from the supermarket in the city of Hefei, China. For the detection of Pb$^{2+}$ in commercial preserved eggs, perchloric acid digestion was applied in the process of pretreatment. Briefly, divide 9.0000 g of preserved egg protein in a preserved egg into three equal parts, preserved egg protein (3.0000 g) was first dispelled with 10 mL mixture solution composited with HNO$_3$ and HClO$_4$ (V : V = 20 : 1) at 220 °C for 5 h, finally, about 3 mL of clear solution was obtained. It was worth noting that in the process of digestion, the loss of Pb$^{2+}$ and the error of experimental results should be avoided by prohibiting the complete evaporation of the solution. After cooling to room temperature, the volume of the solution was adjusted to 10 mL, and the pH of the digestive juice was regulated to 6.5 with Britton-Robison buffer.

2.5.2 Detection of prepared samples. For the detection of prepared samples, we used the method of spike-recovery. 200 $\mu$L different concentrations of Pb$^{2+}$ (0.0 $\mu$g/L, 1.0 $\mu$g/L, 10.0 $\mu$g/L, and 100.0 $\mu$g/L, respectively) were added to 200 $\mu$L the pre-treated sample to form the spiked sample. Subsequently, 640 $\mu$L SPG7/AuNPs (1.41 nM determined by AuNPs) mixed with 400 $\mu$L spiked sample for incubation of 1.0 min. Afterward, all preserved egg samples were preformed to acquire the absorption spectra. Calculating $A_{523\text{nm}}/A_{628\text{nm}}(Y)$ based on the absorption spectrum, and then according to linear equation, the concentration of Pb$^{2+}$ (X) was acquired. By calculating the recovery rate of each sample, we could evaluate the accuracy of the analysis results.

(Figure 1)

3 Results and discussion

3.1 Design of the as-proposed Ratio-colorimetric SPG7/AuNPs probe for Pb$^{2+}$ detection

Figure 1 displayed the design of Ratio-colorimetric SPG7/AuNPs probe for Pb$^{2+}$ detection. In detail, AuNPs stabilized with anionic ligand glutathione (GSH-AuNPs) was selected as the specific nanoparticles for interaction with Pb$^{2+}$, attributing to the strong chelation of GSH with Pb$^{2+}$. Moreover, the GSH-AuNPs with a characteristic absorption peak at ~ 523 nm possesses a nice surface plasmon resonance property (SPR), suggesting the possibility for developing a colorimetric method to visual sensing in food and environment analysis. It was well-known that two complementary colors mixed in a proper proportion could generate a neutral color. SPG7 had two absorption peaks at ~ 401 nm and ~ 628 nm, given such unique property, which was chosen as the complementary compound for simple physical mixing with GSH-AuNPs under the optimized conditions, resulting in the formation of a grey SPG7/AuNPs probe. In Figure 1b, the changed color of the probe added with Pb$^{2+}$ (0.0 $\mu$g/L ~ 0.5 g/L) was showed in CIE L*a*b* color space. Upon interaction with Pb$^{2+}$, the strong chelation of Pb$^{2+}$ with GSH could trigger the aggregation of GSH-AuNPs and liberate the color from SPG7(Figure 1c). As a
result, the increase color of SPG7 could be used for reporting the Pb\textsuperscript{2+} level.

(Figure 2)

3.2 Preparation and characterization of the activatable Ratio-colorimetric SPG7/AuNPs probe

It has been reported that the stable AuNPs stabilized with GSH was prepared through a reduction method.\textsuperscript{14} To testify the existence of GSH on the surface of the prepared AuNPs as a stabilizer, the absorption spectra of bare AuNPs before and after modification with GSH were first investigated. As shown in Figure 2a, there was a slightly red-shift for the characteristic absorbing peak of AuNPs, probably indicating a strong interaction of AuNPs with GSH.\textsuperscript{19} Afterward, the appearance of Au-S bond at \( \sim 296 \text{ cm}^{-1} \) and disappearance of S-H bond at \( \sim 2524 \text{ cm}^{-1} \) in the Ramon spectrum of GSH-AuNPs revealed that GSH was successfully modified to the surface of AuNPs (Figure 2d).\textsuperscript{11} Transmission electron microscopic (TEM) image showed that the as-prepared GSH-AuNPs was spherical and monodisperse (Figure 2b), which was displayed an average hydrodynamic size of 13.5 \( \pm \) 0.8 nm in aqueous solution acquired by the dynamic light scattering analysis (DLS, Figure 2c). In view of these results, we then investigated the preparation process of SPG7/AuNPs probe by titration experiments. We tried to mix GSH-AuNPs and SPG7 in different proportions, the result showed that the grey SPG7/AuNPs solution was composited with 140 \( \mu \text{g} \) 0.1% SPG7 and 500 \( \mu \text{L} \) of 1.41 nM AuNPs (Figure S2). The negligible effect on the morphology and size (an average hydrodynamic size of 14.3 \( \pm \) 1 nm) confirmed that there was no chemical binding between AuNPs and SPG7 (Figure 2e&2f). In addition, the probe storing in a glass bottle at 4\(^{\circ}\)C had good stability within one month. Therefore, the Ratio-colorimetric SPG7/AuNPs probe was obtained by a simple physical mixing of GSH-AuNPs with SPG7, revealing a simple, time-save, and low-cost preparation process.

(Figure 3)

3.3 Sensitivity of Pb\textsuperscript{2+} detection by SPG7/AuNPs probes in aqueous solution

To explore the applications of this Ratio-colorimetric SPG7/AuNPs probe, we investigated the colorimetric response of SPG7/AuNPs to Pb\textsuperscript{2+}. According to previous literatures, in the absence or presence of Pb\textsuperscript{2+}, GSH-AuNPs could maintain stable and uniform distribution when the pH was in the range of 4.0 ~ 8.0. Hence, we conducted all experiments in Britton-Robison buffer (pH=6.5). The SPG7/AuNPs probe originally exhibited a grey (Figure 3a). For SPG7, the absorbance at 420 nm was slightly reduced, but no displacement occurred. The intensity and position at 628 nm were constant, indicating that it was more accuracy to quantify the concentration of Pb\textsuperscript{2+} using the peak intensity at 628 nm. After incubation with Pb\textsuperscript{2+}, a green-blue originated from SPG7 was gradually observed, resulting from the aggregation of AuNPs that induced by Pb\textsuperscript{2+} (Figure 3b). Encouraged by this, we then studied the sensitivity of this proposed colorimetric SPG7/AuNPs probe for reporting Pb\textsuperscript{2+} by monitoring its absorbance ratio of \( A_{523\text{ nm}}/A_{628\text{ nm}} \) after incubation with vary concentration of Pb\textsuperscript{2+} for 1.0 min. As showed in Figure 3c, after adding to 400 \( \mu \text{L} \) Pb\textsuperscript{2+} with different concentration (1 \( \mu \text{g/L} \) – 0.5 g/L), a gradually declined absorption intensity at 523 nm was observed, and the SPR of AuNPs gradually shifted from 523 nm to 570 nm. An isosbestic point at around 560 nm upon the varied concentration of Pb\textsuperscript{2+} suggested one complex was formed in the process. At the same time, rely on stable absorbance at 628 nm of SPG7, a linear relationship between \( A_{523\text{ nm}}/A_{628\text{ nm}} \) and the Pb\textsuperscript{2+} concentration was observed from 1 \( \mu \text{g/L} \) – 100 mg/L (\( Y=0.8697−0.0200 \text{X}, R^2=0.9935 \)) (Figure 3d). Therefore, we believed that this probe could be used for detecting Pb\textsuperscript{2+} with a minimum detectable concentration (0.33 \( \mu \text{g/L} \)), the detection limit was calculated from LOD=3\( \sigma \)/s, where \( \sigma=0.0022 \) was the standard deviation of the blank signal and s=0.02008 was the slope of the standard curve. When the concentration of Pb\textsuperscript{2+} was lower than 100 mg/L, there were small aggregates formed by several nanoparticles evenly dispersed in the
solution. In the range of 1 µg/L – 100 mg/L, the chelation of Pb²⁺ with GSH followed a linear relationship. However, when the concentration of Pb²⁺ was higher than that, the small aggregates expanded further and sank due to gravity, the A₅₂₃ nm/A₆₂₈ nm decreased sharply. It showed that the effective monitoring range of the probe was 1 µg/L – 100 mg/L. At a specific Pb²⁺ concentration, particularly at 1 µg/L, larger standard deviation was exhibited, this was due to the fact that 1 µg/L was close to the LOD and there are more oscillations around the detection limit. Analogously, the variation at 100 mg/L was owed to the differences in aggregation ability of the GSH-AuNPs. Before our research, a number of methods have been established to detect Pb²⁺ in various samples, traditional quantitative methods include the inductively coupled plasma mass spectrometry (IPC-MS), the atomic absorption spectroscopy (AAS) and the atomic fluorescence spectrometry (AFS). Compared to the designed probe, these methods require expensive and complicated instruments and take a long time. Recently, colorimetric methods have achieved great development in Pb²⁺ detection. However, the common disadvantages of these methods were complicated preparation, strong background and high detection limit. The detection limits of Pb²⁺ were as follows: 14 µg/L for Carbon dot, 132.5 µg/L for DNAzymes-AuNPs, 3 µg/L for Gallic acid-AuNPs and so on (Table 1), these results are higher than the LOD of our method. As shown in Figure 3e, digital photo showed the perfect gradient color changed from grey to green-blue accompanied, and the clear detection result strongly proved the sensitivity of the probe even if small changes (slope) occurred. Meanwhile, optical photographs presented by 96-well plates also demonstrated that this system allows a high-throughput rapid detection. Hence, we have proved that our activated colorimetric strategy of Pb²⁺ achieved a higher sensitivity (0.33 µg/L) by contrast with other reported methods, which might be more accurate for Pb²⁺ detection in environment and complicated food samples.

(20)

For the detection of actual samples, selectivity was an important factor in assessing the quality of a probe. Thence, we excavated the selectivity of Pb²⁺ over other metal ions, such as aluminum chloride (AlCl₃), ferric chloride (FeCl₃), magnesium chloride (MgCl₂), barium chloride (BaCl₂), potassium carbonate (K₂CO₃), manganese sulfate (MnSO₄), mercury chloride (HgCl₂), cadmium chloride (CdCl₂), calcium chloride (CaCl₂), sodium chloride (NaCl) and zinc sulfate (ZnSO₄), each sample was tested in triplicate. After incubating metal ions (400 µL 100 mg/L) with 640 µL SPG7/AuNPs probe for 1 min, the absorption spectra of all samples were measured and the A₅₂₃ nm/A₆₂₈ nm were calculated, we could clearly see from Figure 4a that, among all the metal ions, only Pb²⁺ could induce obviously aggregation of AuNPs and activation of SPG7’s color, meanwhile, causing a significant A₅₂₃ nm/A₆₂₈ nm weaken (Figure 4b). Other metal ions had slight change compared to the blank group, which may be due to the non-specific aggregation of gold nanoparticles caused by salt. Based on the aggregation kinetics of GSH-AuNPs after addition of metal ions, different metal ions chelate with GSH at different speed. Other metal ions had bigger error than Pb²⁺ due to the difference in detection time and the slow chelation rate. The corresponding optical photo as a more intuitive evidence also intimated that the assay probe has very high specificity toward Pb²⁺ (Figure. 4c), in addition to being blue-green after incubation with lead ions, probes incubated with other ions were still grey.

(Figure 4)

3.4 Colorimetric detection of Pb²⁺ in preserved eggs

As mentioned above, the grey SPG7/AuNPs revealed negligible response to other metal ions, indicating a possibility for precise and specific detection of Pb²⁺ in preserved eggs. Therefore, we applied standard addition
method to test the content Pb$^{2+}$ in two types of preserved eggs, one for traditional preserved eggs and one for lead-free preserved eggs. In this study, we used a universal perchloric acid digestion method to pre-treat samples to remove interfering substances such as proteins, fats, carbohydrates, etc, details described in the experimental section. Known quantities of Pb$^{2+}$ were spiked into the samples of preserved eggs to obtain the necessary concentrations. The recovery rate was calculated by the following equation:

$$R = \frac{V_2 \cdot C_2 - V_1 \cdot C_1}{V_s \cdot C_S}$$

$V_1, C_1$……the volume (μL) and mass concentration (μg/L) of unspiked samples.

$V_2, C_2$……the volume (μL) and mass concentration (μg/L) of the sample after the spike.

$V_s, C_S$……the spiked volume (μL) and mass concentration (μg/L).

The test results in Table 2 showed that the range of spiked recovery was 89.2% – 107.5%, which reflected the better accuracy of the designed probe for Pb$^{2+}$ analysis. Relative standard deviation (R.S.D.) of samples was 0.28% – 2.12%, implied that this approach had a high degree of precision. The SPG7/AuNPs could be directly used for the determination of Pb$^{2+}$ in the commercial preserved eggs. The results showed that the content of Pb$^{2+}$ was 2.41 mg/kg in tradition craft preserved egg and 0.46 mg/kg in lead-free craft preserved egg respectively. At the same time, due to the inherent advantages of this method, the interference of other impurities on the UV-vis absorption spectra was reduced.

(Table 2)

4 CONCLUSION

In summary, based on combining the color complementarity principle and advantages of GSH-AuNPs, we for the first time developed a grey nanoprobe for Pb$^{2+}$ detection in preserved eggs. Due to the unique characteristic of the Ratio-colorimetric probe, the background of this colorimetric method was significantly reduced, at the same time, the concentration of SPG7 was used to correct the concentration of Pb$^{2+}$, making the detection results more efficient and accurate. These advantages made it possible to be a colorimetric sensing platform for the detection of Pb$^{2+}$. This method not only extended the application of AuNPs, but also raised a novel idea of optical detection, the successful application in the actual preserved egg products illustrated that this method had a great potential in food and environmental analysis applications.

ASSOCIATED CONTENT

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Supporting information

Molecular structure of Pigment Green 7 (Figure S1), zeta position of GSH-AuNPs, SPG7 and complex (Figure S2), and optimization experiment of SPG7 and GSH-AuNPs ratio (Figure S3).

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Table 1. Comparison of reported colorimetric sensors for the detection of Pb\(^{2+}\).

| Samples        | Probe                                      | Linear range of Pb\(^{2+}\) / (µg/L) | R\(^2\)  | LOD / (µg/L) | Reference          |
|----------------|--------------------------------------------|--------------------------------------|---------|--------------|-------------------|
| Table sugar    | Carbon dot                                 | 100-500                              | 99.6%   | 14           | Ansi, V. A. etc\(^{26}\) |
| Orange juice   | Biomass reinforced graphene oxide (BGO) membrane | 500-10\(^4\)                          | 99.6%   | 0.84         | Wang, J. etc\(^{27}\) |
| Apple juice    | Alginate-LDH-S hydrogel                    | 500-10\(^4\)                          | 99.7%   | 0.39         | Ang, J. etc\(^{21}\) |
| Water          | DNAzymes-AuNPs                             | 132-662                              | *       | 132.5        | And, J. etc\(^{22}\) |
| Water          | DNAzymes-AuNPs                             | 33.1-1324                            | *       | 33.1         | And, J. etc\(^{28}\) |
| Drinking water | Gallic acid-AuNPs                          | 3.3-331.21                           | 99.8%   | 3            | Huang, K. etc\(^{23}\) |
| Water          | DNAzymes-AuNPs                             | 0.993-331.21                         | *       | 0.99         | Wang, Z. etc\(^{29}\) |
| Preserved egg  | SPG7/AuNPs                                 | 1-10\(^5\)                           | 99.3%   | 0.33         | Our method         |
Table 2. Labeling of Pb\(^{2+}\) in preserved eggs with grey probes in traditional craft preserved egg and lead-free craft preserved egg. (n = 3)

| Samples                      | Pb\(^{2+}\) spiked (µg/L) | Pb\(^{2+}\) measured (µg/L) | Recovery (n=3, %) | R. S. D. (n=3, %) |
|------------------------------|---------------------------|-----------------------------|-------------------|-------------------|
| Traditional craft preserved egg | 0                         | 7.22                        | *                 | 1.08              |
|                              | 10                        | 17.07                       | 98.7%             | 0.37              |
|                              | 100                       | 96.41                       | 89.2%             | 1.21              |
|                              | 1000                      | 1082.30                     | 107.5%            | 0.98              |
| Lead-free craft preserved egg | 0                         | 0.71                        | *                 | 0.60              |
|                              | 10                        | 10.27                       | 95.6%             | 0.28              |
|                              | 100                       | 105.51                      | 104.8%            | 2.12              |
|                              | 1000                      | 968.05                      | 96.7%             | 1.35              |
Figure 1. General design of the as-proposed Ratio-colorimetric SPG7/AuNPs probe for Pb$^{2+}$ detection. (a) The process of probe preparation. (b) CIE L*a*b* color space (c) Detect Pb$^{2+}$ using the proposed probe.
Figure 2. Characterization of AuNPs and SPG7/AuNPs probe in aqueous solution. (a) UV-vis spectra of AuNPs and GSH-AuNPs. (b) TEM image of the GSH-AuNPs (scale bar: 50 nm). (c) The corresponding DLS of the prepared GSH-AuNPs (13.5 ± 0.8 nm). (d) Raman spectra of pure GSH and GSH-AuNPs. (e) TEM image of the mixture which was composed of GSH-AuNPs and SPG7 (scale bar: 50 nm). (f) The corresponding DLS of the mixture (14.3 ± 1 nm).
Figure 3. Colorimetric detection of Pb$^{2+}$ in aqueous solution. (a) UV-vis spectra of the GSH-AuNPs (i), SPG7 (ii), and the mixture without (iii) and with Pb$^{2+}$ (iv). The inset showed photo images of four solutions, respectively. (b) The TEM image in the presence of Pb$^{2+}$ (scale bar: 100 nm). (c) UV-vis absorption spectra of SPG7/AuNPs probe in the presence of different concentrations of Pb$^{2+}$ (1 μg/L – 0.5 g/L). (d) Plot of $A_{523\text{ nm}}/A_{628\text{ nm}}$ value of GSH-AuNPs as a function of the concentration of Pb$^{2+}$; The linear relationship showed a plot of $A_{523\text{ nm}}/A_{628\text{ nm}}$ versus the concentrations of Pb$^{2+}$ in the range of 1 μg/L – 100 mg/L. The error bars represented standard deviations based on three independent measurements. (e) The corresponding photo images of SPG7/AuNPs probe containing different concentrations of Pb$^{2+}$ (1 μg/L – 0.5 g/L).
Figure 4. Selectivity of the SPG7/AuNPs for Pb\textsuperscript{2+} sensing in aqueous solution. (a) UV-vis spectra of varieties metal ions (Blank, Al\textsuperscript{3+}, Fe\textsuperscript{3+}, Mg\textsuperscript{2+}, Ba\textsuperscript{2+}, K\textsuperscript{+}, Mn\textsuperscript{2+}, Hg\textsuperscript{2+}, Cd\textsuperscript{2+}, Ca\textsuperscript{2+}, Na\textsuperscript{+}, Zn\textsuperscript{2+}, Pb\textsuperscript{2+}) detected by the SPG7/AuNPs probe; (b) The corresponding column chart. Error bars indicate standard of the three independent measurements. (c) The corresponding optical photo.