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Sensitive and selective detection of trivalent chromium using Hyper Rayleigh Scattering with 5,5'-dithio-*bis*-(2-nitrobenzoic acid)-modified gold nanoparticles

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Abstract

Hyper Rayleigh Scattering (HRS) and absorption spectral assays using surface-modified gold nanoparticles (AuNP) have been developed for sensitive and selective detection of trivalent chromium (Cr^{3+}) from other metal ions including hexavalent chromium (as $\text{Cr}_2\text{O}_7^{2-}$). Gold nanoparticles of 13 nm, covalently attached with 5,5'-dithio-*bis*-(2-nitrobenzoic acid) (AuNP-DTNBA), is used as a probe for both the absorption and HRS assays. AuNP-DTNBA is able to detect Cr^{3+} at 20 ppb level at pH 6.0 using absorption spectral change of the AuNP-DTNBA. Visible color change can be observed when mixed with 250 ppb of Cr^{3+} , while there is no color change when mixed with 2 ppm level of some of the most common metal ions such as $\text{Cr}_2\text{O}_7^{2-}$, Hg^{2+} , Ba^{2+} , Fe^{3+} , Pb^{2+} , Na^+ , Zn^{2+} , Cd^{2+} , Co^{2+} , Mn^{2+} , Ca^{2+} , and Ni^{2+} . However, a color change is observed when mixed with Ni^{2+} , Zn^{2+} , and Cd^{2+} at a concentration higher than 2 ppm. The detection limit for the HRS assay is on a remarkable 25 ppt level, and there is no detectable HRS signal at 2 ppm level for $\text{Cr}_2\text{O}_7^{2-}$, Hg^{2+} , Ba^{2+} , Fe^{3+} , Pb^{2+} , Na^+ , Zn^{2+} , Cd^{2+} , Co^{2+} , Mn^{2+} , Ca^{2+} , and Ni^{2+} .

Keywords

Gold nanoparticles; trivalent chromium; 5,5'-dithio-*bis*-(2-nitrobenzoic acid); Hyper Rayleigh Scattering; pH dependence; colorimetric

Introduction

Trace analysis of chromium in the environment is of interest because of the effects it has on human health. Chromium commonly exists in chromium III (Cr^{3+}) and VI ($\text{Cr}_2\text{O}_7^{2-}$) oxidation states. Contamination from steel manufacturing, electroplating, tanning industries, and wood treatment are some of the routes to reach the environment [1–3]. It enters the body from inhalation, ingestion, and skin absorption [4]. Comparatively, hexavalent chromium is highly toxic and carcinogenic because of its oxidative potential [1, 3, 5]. Trivalent chromium, on the other hand, is used as a dietary supplement in the form of chromium picolinate to enhance the activity of insulin, which is essential for metabolism of carbohydrates, fats, and proteins [6]. However, once Cr^{3+} is inside the cell, it binds to DNA and causes DNA strand breakage, DNA-protein crosslink, and oxidative DNA base modifications, which can lead to mutation and cancer if the damaged DNA is not correctly

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repaired [4, 7–9]. Hence, there is a need to develop rapid, sensitive, and reliable methods for chromium detection.

Taking advantage of the remarkable optical properties of gold nanoparticles (AuNP), many research groups have used AuNP for sensing [10–16]. Detection of both hexavalent and trivalent chromium using colorimetric, immunoassays, and amperometric techniques has been reported [10–13, 17–19]. Zhou et al. constructed an AuNP labeled with 5-thio-2-nitrobenzoic acid and horseradish peroxidase and detected Cr^{3+} at pH 7.4 using colorimetry, and achieved a detection limit of $0.04 \mu\text{M}$ (2 ppm) [12]. Dang et al. used AuNP modified with 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNBA) for Cr^{3+} detection at pH 7.5 and achieved a detection limit of 94 ppb [13]. Jena et al. developed an AuNP electrode to detect amperometric $\text{Cr}_2\text{O}_7^{2-}$ with a great detection limit of 0.1 ppb [17]. The proposed mechanism for Cr^{3+} detection by AuNP-DTNBA is that the free carboxylate groups of DTNBA selectively binds Cr^{3+} and causes the AuNP-DTNBA to aggregate, creating new absorption bands of the AuNP. The potential problem is the selectivity of DTNBA for Cr^{3+} from other co-existing heavy metal ions. Although the authors addressed this problem in their publication [13], our tests showed that the pH dependence is more complicated due to the concentration of the buffer and the concentration of the co-existing metal ions. At pH 7.5, there were interferences from Ni^{2+} , Zn^{2+} , Cd^{2+} , and Fe^{3+} . In this paper, we report an improved colorimetric method as well as present a new two-photon Hyper Rayleigh Scattering (HRS) assay for better selective and sensitive detection of trivalent chromium using DTNBA modified AuNP.

AuNP-based HRS method has been recently reported as a sensitive and effective technology for detection of various trace chemicals from our research group [14, 16, 20–22]. HRS takes advantage of the light scattering properties of AuNPs that is highly particle size dependent. HRS exhibits 2 to 3 orders of magnitude higher sensitivity than colorimetric assays. The HRS method we developed in this study can detect trivalent chromium at concentrations as low as 25 ppt. With careful tuning of the pH, selective binding of Cr^{3+} to the AuNP-DTNBA complex resulted in a rapid color change that is visible at a concentration of Cr^{3+} as low as 250 ppb.

Experimental

Reagents and Materials

Chloroauric acid (HAuCl_4), sodium citrate tribasic (TSC), 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNBA), ethanol (200 proof), CrCl_3 , HEPES disodium salt, and all other metal salts were purchased from Sigma-Aldrich (St Louis, MO). Spring Valley Chromium Picolinate supplemental tablets were purchased from a local store. All samples were prepared with nanopure water ($18.2 \mu\Omega$).

Equipment

UV-Vis absorption spectra were measured using an Ocean Optics (Model CHEMUSB4-UV-VIS) spectrophotometer. The concentration of chromium picolinate supplement pill was confirmed with a Perkin Elmer Optima 3000 ICP-OES. The size of the AuNP was determined using a JEOL JEM-1011 Transmission Electron Microscope (TEM), which has magnifications of 50 to 1,000,000 under accelerating voltage of 40 to 100 kV.

Gold nanoparticle (AuNP) synthesis

AuNP of 13 nm (absorption maximum 520 nm) was synthesized using our method published before [15, 16]. To obtain a concentrated AuNP solution, 50 mL of the AuNP solution was centrifuged for 90 min at 9000 rpm. After centrifugation, 45 mL of the supernatant was

discarded, and the AuNP was suspended in the remaining 5 mL of supernatant. The concentration of the AuNP was determined spectrophotometrically [23].

Modification of AuNP with DTNBA

The AuNP was diluted 10 times with the pH 6.0, 7.5, or 9.0 HEPES buffer (5 mM) to achieve the final AuNP concentration of 1 nM. The color of the buffered AuNP solution was red. A 50 μ M DTNBA solution was prepared fresh in ethanol before use. AuNP-DTNBA complex was prepared by mixing the solutions of 1 nM AuNP in HEPES buffer of different pHs and 50 μ M DTNBA in ethanol in an 8:2 v/v ratio at room temperature. They were used for colorimetric and HRS experiments. These AuNP-DTNBA solutions at pH 6.0, 7.5, and 9.0 were stable for 10 days at room temperature. Using pHs below 6.0 or above 9.0, precipitation occurred.

Colorimetric Experiments

Stock solutions of Cr^{3+} and other metal ions were prepared in nanopure water at pH 6.0, 7.5, and 9.0 with the addition of HEPES buffer. A 675 μ L aliquot of the above AuNP-DTNBA solutions at pH 6.0, 7.5, and 9.0 was added with different concentrations of Cr^{3+} or 2 ppm of Hg^{2+} , Ba^{2+} , Ni^{2+} , Fe^{3+} , Pb^{2+} , Zn^{2+} , $\text{Cr}_2\text{O}_7^{2-}$, Cd^{2+} , Na^+ , Co^{2+} , Mn^{2+} , Ca^{2+} , and Pb^+ solutions to see if any of these ions can cause a color change. The final volume was adjusted to 750 μ L with the corresponding HEPES buffers. The mixtures were allowed to react over night.

Titration experiments were conducted with AuNP-DTNBA solution in quartz cuvettes by addition of Cr^{3+} solutions with final concentrations between 0 to 20 ppm. The absorption spectra or the HRS data were recorded for the resultant solutions.

Extraction of Cr^{3+} from dietary supplements

One chromium picolinate tablet, which has 100 ppm of chromium based on the nutrition information on the label, was sonicated in 10 mL of water for an hour at 70 °C. Once the samples were cooled, they were centrifuged for 15 min at 10,000 rpm. After centrifugation, the supernatant was removed, and an aliquot was analyzed by ICP-MS to determine the concentration which matched the concentration given.

Hyper Rayleigh Scattering (HRS)

Solutions of 3.5 mL of the AuNP-DTNBA solution in a quartz cuvette with different concentrations of Cr^{3+} or other heavy metal ions were prepared. HRS intensities were measured in solutions using an Nd-YAG pulse laser, shown in Figure 1. The laser delivers a wavelength of 1064 nm with pulse duration of 7 ns at a repetition rate of 10,000 kHz to shine normal to the detector [15, 20, 24–26]. In order to improve signal-to-noise ratio, a concave mirror and a 532 nm interference filter were used before the detector. All experiments were performed on a vibrationless air floating table. Signals were amplified 3000 times and processed through a high speed 2 GHz oscilloscope. To reduce noise, the collected signals were averaged by an electronic boxcar averager. The HRS light was separated from its linear counterpart by a high-pass filter and a monochromator, and detected with a cooled photomultiplier tube (PMT). The pulses were counted with a photon counter. To make sure that only the second-harmonic signal is collected by PMT, a 3 nm interference filter and a monochromator were used in front of PMT. After exposure of 5–10 min to the laser, there has been no noted photothermal damage of AuNP-DTNBA observed [16, 20, 21, 25].

Results and Discussion

PH-dependence for the selective interaction of AuNP-DTNBA with Cr³⁺

AuNP-DTNBA complexes were dispersed in a 5 mM HEPES buffer and the pH was adjusted to pH 6.0, 7.5, and 9.0 by HCl or NaOH. It should be noted that citrate-coated AuNPs are not stable below pH 6.0 and above pH 9.0. Figure 2A shows the absorption spectrum of AuNP, AuNP-DTNBA, and AuNP-DTNBA in the presence of 250 ppb of Cr³⁺. The first cuvette (Figure 2B left) contains only AuNP, which has a wavelength of 520 nm and a diameter of 13 nm and corresponds to the TEM image in Figure 2C. The second cuvette (Figure 2B middle) contains AuNP-DTNBA, whose absorption shifts slightly to the red at 523 nm. AuNP-DTNBA has similar size and shape as the unmodified AuNP (Figure 2D). Once Cr³⁺ (250 ppb) is added to the AuNP-DTNBA solution, an instant color change from wine red to dark blue is observed (Figure 2B right), and a new band with absorption maximum at 630 nm appears. Addition of Cr³⁺ to AuNP-DTNBA solution induced aggregation, although the individual AuNPs can still be observed and their diameters remain about the same (TEM in Figure 2E). This indicates that Cr³⁺ forms coordination bonds with carboxylate groups from adjacent AuNP-DTNBA complexes [13]. We also observed from the TEM data that the aggregation is directly proportional to Cr³⁺ concentration.

As for pH dependence, we tested AuNP-DTNBA prepared at pH 6.0, 7.5, and 9.0 of 5 mM HEPES buffer. At pH 6.0, color change was observed with the naked eye when added with Cr³⁺ as low as 250 ppb; at pH 7.5, color change was only observed with > 6 ppm of Cr³⁺; while at pH 9.0, there was no color change with as high as 100 ppm of Cr³⁺. It is a known that Cr³⁺ complexes with the carboxylates at pH 6 in buffered solutions [27]. The same trend is observed in the present study. From Figure 3, AuNP aggregation and color change can distinctively be observed only at pH 6.0. This clearly indicates that by a simple pH tuning, a ppb level sensitivity of Cr³⁺ could be detected using AuNP-DTNBA colorimetrically.

We further tested selectivity of AuNP-DTNBA against other metal ions such as Ni²⁺, Zn²⁺, Pb²⁺, Fe²⁺, Cd²⁺, Mn²⁺, Al³⁺ and Cr₂O₇²⁻ at pH 6.0 and 7.5. pH 9 was not used because no change in color was observed even when 100 ppm of Cr³⁺ was added. As seen at pH 7.5, there are weak to strong absorbance changes when added with 20 ppm of Ni²⁺, Zn²⁺, Cd²⁺, and Fe³⁺ (Figure 4), meaning there are potential interferences. At pH 6.0, Ni²⁺ causes a strong spectral change, while Cd²⁺ and Zn²⁺ cause weaker spectral changes, due to the interaction with AuNP-DTNBA. All other metal ions do not cause any spectral changes, indicating that no aggregation resulted due to the presence of these ions. Therefore, pH 6.0 is better than 7.5 for selective and sensitive detection of chromium. This is different from the result by Dang et al [13].

Hyper Rayleigh Scattering (HRS)

To improve sensitivity for Cr³⁺ detection, HRS was used since it determines a small change in the size of the nanoparticles. Figure 5A shows that the HRS intensity of AuNP-DTNBA dramatically increases when Cr³⁺ is added; until at concentration of approximately 20 ppb, the intensity levels off at higher concentrations. The leveling off is due to saturation binding of the AuNP-DTNBA with Cr³⁺. Since environmental samples have many co-existing metal ions, we performed the HRS assay with addition of other common metal ions. Figure 5B shows there is no HRS signal increase with the addition of 2 ppm of Hg²⁺, Ba²⁺, Ni²⁺, Fe³⁺, Pb²⁺, Zn²⁺, Cd²⁺, Na⁺, Co²⁺, Mn²⁺, Ca²⁺, and Pb⁺ except Cr³⁺ and Cr₂O₇²⁻. A careful examination with Cr₂O₇²⁻ shows that the increase of HRS signal is negligible in the concentration range of less than 150 ppb (Figure 5). The TEM images of AuNP-DTNBA show there is no AuNPs aggregation in the presence of Hg²⁺, Ba²⁺, Ni²⁺, Fe³⁺, Pb²⁺, Zn²⁺,

$\text{Cr}_2\text{O}_7^{2-}$, Cd^{2+} , Na^+ , Co^{2+} , Mn^{2+} , Ca^{2+} , and Pb^+ , except Cr^{3+} . A HRS intensity change can even be observed with the addition of 25 ppt of Cr^{3+} , and colorimetric change can be observed with addition of 250 ppb of Cr^{3+} . As a result, the HRS assay is two orders of magnitude more sensitive than the colorimetric assay.

Figure 6 shows the absorption spectra of AuNP-DTNBA in the presence of Cr^{3+} . As the concentration of Cr^{3+} increases, the 520 nm peak decreases and a peak at 630 nm increases. This is manifested as the solution color change. The TEM images show that in comparison to 50 ppb versus 250 ppb of Cr^{3+} , the presence of the higher concentration of Cr^{3+} causes more aggregation.

Sensitive detection of Cr^{3+} in a vitamin supplement

To test the practical use for both the colorimetric and HRS assays for real-life samples, a vitamin supplement containing Cr^{3+} was tested. Figure 7 shows the HRS signal change of AuNP-DTNBA when it is added with a Cr^{3+} standard, a $\text{Cr}_2\text{O}_7^{2-}$ standard, and samples from a chromium picolinate vitamin. Up to the addition of 180 ppm $\text{Cr}_2\text{O}_7^{2-}$ standard to the AuNP-DTNBA solution, there is no significant change of the HRS signal, in contrast to the great changes of the HRS signal upon addition of Cr^{3+} . The Cr^{3+} dependent HRS intensity change is very similar to the standard samples, indicating the applicability and robustness of our assay for real-life samples. It demonstrates that this HRS technique can be used to detect Cr^{3+} with concentrations as low as 25 ppt in environmental samples.

Conclusion

Sensitive and selective colorimetric and HRS assays using DTNBA-modified AuNP have been developed for Cr^{3+} detection. It is imperative to note that the pH plays a critical role and pH 6, instead of pH 7.5, was proven to be essential for detection of low concentrations of Cr^{3+} without interference from other heavy metal ions. The detection limits for both the colorimetric and HRS assays are 250 ppb and 25 ppt, respectively.

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Abbreviations

AuNP	Gold nanoparticles
DTNBA	5,5'-dithio- <i>bis</i> -(2-nitrobenzoic acid)
AuNP-DTNBA	gold nanoparticle covalently linked with 5,5'-dithio- <i>bis</i> -(2-nitrobenzoic acid)
HRS	Hyper Rayleigh Scattering
PMT	Photomultiplier Tube

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Highlights

- Optimized pH (6.0) to achieve selectivity using modified gold nanoparticles for absorption/colorimetric analysis of Cr^{3+}
- Hyper Rayleigh Scattering assay for detection of Cr^{3+} at 25 ppt level
- Demonstrated the HRS method for Cr^{3+} detection in chromium picolinate pills

Figure 1.
Experimental set-up for TPS assay

Figure 2. (A) Absorption spectra of AuNP, AuNP-DTNBA, AuNP-DTNBA + 160 ppb Cr³⁺, (B) Color of solutions (left to right): AuNP, AuNP-DTNBA, AuNP-DTNBA + Cr³⁺; (C-E) TEM images of AuNP, AuNP-DTNBA, AuNP-DTNBA + Cr³⁺.

Figure 3.

Absorption spectra of AuNP-DTNBA + 1 ppm Cr^{3+} at pH 6, AuNP-DTNBA + 5 ppm Cr^{3+} at pH 7.5, AuNP-DTNBA + 10 ppm Cr^{3+} at pH 9. Insert: respective colorimetric images of AuNP-DTNBA + Cr^{3+} at (A) pH 9.0, (B) pH 7.5, and (C) pH 6.0.

Figure 4. Absorption spectral changes of AuNP-DTNBA solution with 20 ppm metal ions at pH 6.0 (left) and pH 7.5 (right). With addition of 20 ppm of Cr^{3+} , AuNP-DTNBA precipitates.

Figure 5.

(A) Concentration dependent HRS intensity change for Cr^{3+} in the range of 0 – 500 ppb.

Insert: HRS intensity change in the range of 0 – 2 ppb of Cr^{3+} . (B-F) TEM images of AuNP-DTNBA after the addition of 2 ppm of Hg^{2+} , Ni^{2+} , Cr^{3+} , Pb^{2+} , and Cd^{2+} , respectively.

Figure 6. Titration of AuNP-DTNBA solution with Cr^{3+} (0, 10, 25, 50, 100, 150, 200, 250 ppb). Insert: Absorbance change at 630 nm. TEM images of AuNP-DTNBA in the presence of 50 ppb Cr^{3+} (left) and 250 ppb Cr^{3+} (right).

Figure 7.
HRS intensity for $\text{Cr}_2\text{O}_7^{2-}$ standard, Cr^{3+} standard, and chromium pill sample.