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Sensitive and selective determination of NO_2^- ion in aqueous samples using modified gold nanoparticle as a colorimetric probe

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ABSTRACT

A sensitive and selective colorimetric method for determination of nitrite ion in aqueous samples was developed using 1-(2-mercaptoethyl)-1, 3, 5-triazinane-2, 4, 6-trione-functionalized gold nanoparticles (MTT-GNPs). The nitrite ion seems to be used as a "molecular bridge", which can form NH—N and NH—O hydrogen bonds with the MTT-GNPs, shorten the interparticle distance, and induce the aggregation of the MTT-GNPs. This aggregation results in a dramatic change from wine-red to purple-gray color. Therefore, the concentration of nitrite ion in environmental samples can be quantitatively detected using the MTT-GNPs sensor by the naked eyes or UV–vis spectrometer. Moreover, investigations have revealed the sensitivity of the detection could be clearly improved by modulating pH of the solution, which led to a more rapid color change in the optimized GNPs system. The absorption ratios (A_{790}/A_{535}) of the modified GNPs solution exhibited a linear correlation with nitrite ion concentrations and the limit of detection was 1 ppm. This cost effective sensing system allows for the rapid and facile determination of the concentration of NO_2^- ions in aqueous samples.

1. Introduction

When nitrite ions are used as preservatives for meat and fish products, they can react with secondary or tertiary amines present in the body resulting in the formation of carcinogenic nitrosamines, which enhances the possibility of cancer [1,2]. Furthermore, nitrite ions in the blood can react with iron(II) of hemoglobin forming methemoglobin, which lacks the ability to carry oxygen and causes fetal methemoglobin anemia, resulting in cyanosis [3]. Thus, the importance of the quantitative determination of nitrite ions in water, food, and agricultural products is widely recognized and significant, since a high concentration can lead to potential risks to human health. Specifically, the United States Environmental Protection Agency (US EPA) and World Health Organization (WHO) have established the maximum limit of nitrite ions in drinking water as 3 mg L^{-1} and 1 mg L^{-1}, respectively [3,4].

A variety of analytical methods have been reported for the determination of nitrite ions including electrometry [5,6], chromatography [7–10], capillary electrophoresis [11], spectrophotometry [12,13], and spectrofluorimetry [14], but most of these methods require complicated instruments and professional staff. Hence, exploring an accurate, rapid, and convenient analytical method to detect nitrite ions still remains a challenge for analytical chemists.

With developments in nanotechnology, various novel colorimetric assays have been exploited. Gold nanoparticles (GNPs) have been extensively used as colorimetric probes for different sensing and biosensing applications owing to their unique size-dependent and interparticle-distance dependent optical properties and accompanying color changes. GNP-based colorimetric methods have been reported for the detection of anions [15,16], heavy metal ions [17–21], DNA [22], bacteria [23], and cancerous cells [24], among others. Lu's group [25] has developed 1-(2-mercaptoethyl)-1,3,5-triazinane-2,4,6-trione (MTT)-stabilized GNPs as a colorimetric probe for melamine detection in milk, based on the hydrogen-bonding between modifiers and melamine. Therefore, the development of on-site, real-time, and low-cost melamine sensing has become increasingly attractive. Furthermore, MTT can be easily conjugated to GNPs through the –SH group. Inspired by these works, we have examined the potential of MTT-modified gold nanoparticles as a novel and sensitive probe for the

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determination of negatively charged ions in aqueous samples, since the NH of the MTT molecule could bind anions via hydrogen bonding. Here, we have tested the MTT-GNPs with various anions, which are F^-, Cl^-, Br^-, SO_4^{2-}, PO_4^{3-}, NO_3^-, and NO_2^-. The NH of MTT-GNPs could only be cross-linked with nitrite ions, accompanying clear color change (from wine-red to purple gray). Moreover, the GNP-based system has a better sensitivity for the detection of nitrite ions in acidic solution. Also, the absorption ratio (A_{790}/A_{535}) of the modified GNPs in the pH-optimized system exhibits a linear correlation with the nitrite concentration, allowing the determination of the nitrate concentration in aqueous samples within 30 min. Therefore, an optimized MTT-GNP system as a novel, on-site, real-time, and low-cost nitrite ion sensor has a wide range of practical applications.

2. Experimental

2.1. Materials

Gold(III) chloride hydrate, sodium citrate, ethyl chloroformate, acetone, and potassium thiocyanate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methylene chloride and HCl were purchased from J. T. Baker (Phillipsburg, NJ, USA). F^-, Cl^-, Br^-, SO_4^{2-}, PO_4^{3-}, NO_3^-, and NO_2^- were purchased from AccusStandard (New Haven, CT, USA). pH paper was purchased from Whatman International Ltd. (Maidstone, UK). HCl and NaOH were purchased from Samchun Chemical (Gyeong Gi-Do, Korea). Sodium chloride was purchased from Junsei Chemical (Tokyo, Japan). Distilled water used in this experiment was obtained through a Milli-Q water purification system from Millipore (Bedford, MA, USA) and was used throughout the study.

2.2. Apparatus

The absorption spectra of the GNPs were recorded at room temperature using a UV–vis–NIR spectrophotometer 5000 (Varian Inc., Palo Alto, CA, USA). UV–vis was measured in the range of 400–800 nm using quartz cells of 1 mm path length. Measurement of particle size distributions were carried out using a Zetasizer (Malvern Instruments Ltd., Worcestershire, UK). The image and the diameter of the MTT-GNP and the nitrite-induced aggregation of MTT-GNP were measured from a micrograph using a transmission electron microscope (TEM) CM30 (Philips, NC, USA) and an atomic force microscope (AFM) XFM XE-100 (Park systems, Suwon, Republic of Korea). TEM specimens were obtained by depositing a dispersion of the GNPs and evaporating the solvent. The concentrations of nitrite ion in aqueous solutions were measured using ion chromatography (IC) (Metrohm, Riverview, FL, USA).

2.3. Preparation of MTT and GNPs conjugated with MTT

MTT was synthesized according to literature procedure [26], and then MTT-conjugated gold nanoparticles were prepared by a ligand-exchange reaction between MTT and citrate-stabilized gold nanoparticles. We synthesized ~33 nm gold nanoparticles by the reduction of $HAuCl_4$ using sodium citrate. Generally, 50 mL of 1 mM $HAuCl_4$ as heated to reflux with stirring, and then 5 mL of 38.8 mM sodium citrate was added rapidly. The solution was boiled for another 30 min to give a wine-red colored solution. The ligand-exchange reaction was performed at room temperature by mixing 0.5 mL of the prepared gold colloids with 1 mL of aqueous 1 mM MTT under stirring. Volumes of 0.08, 0.3, 0.5, 0.7, 0.9, and 1.1 mL of 1 mM MTT were added to 0.5 mL of GNPs. We did not observe any color change for the MTT-GNP solution upon addition of MTT, indicating that the self-aggregation of MTT-GNPs did not occur.

3. Results and discussion

Nanomaterials often display unique physical properties that are not observed in the same materials on a larger scale. The coherent collective motion of electrons on the surface in noble metals such as gold or silver arises when the size of the particles are on the order of a few nanometers. Gold nanoparticles strongly absorb in the visible region of light, when the frequency of the incident electromagnetic field is resonant with the coherent oscillation of the electrons on the surface of the nanoparticle. In nanoparticles, this phenomenon is called surface plasmon resonance (SPR). A strong absorption peak of SPR appears at about 520 nm in the UV–vis spectra of gold nanoparticles. This is the reason why gold nanoparticles display a wine-red color in solution. The SPR peak frequency is influenced by the size and shape of the nanoparticles, the refractive index of the surrounding medium, and the degree of aggregation [27–29].

We synthesized MTT, a thiol-functionalized cyanuric acid [26], and prepared ~33 nm MTT-GNPs using the ligand-exchange reaction of GNPs with MTT [25]. The GNP size under these preparation conditions is larger than that (12 nm) of MTT-stabilized GNPs synthesized in an earlier study [25]. The mean size of the GNPs was easily affected by the amount of sodium citrate and other preparation conditions. It is known that the GNP size affects the surface plasma absorption maxima. The λ max values of GNPs of sizes 15 and 33 nm were 519 and 535 nm, respectively. However, this change in λ max as a function of GNP size seems not to influence the results and reliability of the experiments [27–29]. The gold nanoparticles conjugated with MTT were well dispersed and displayed uniform wine-red color due to the strong SPR at 535 nm.

Following the synthesis of the MTT-GNPs, we developed a simple colorimetric assay for the selective detection of anions. The control solution that did not contain any anions showed the wine-red color, and each solution with the addition of F^-, Cl^-, Br^-, PO_4^{3-}, SO_4^{2-}or NO_3^- ions (5 ppm) did not show any color change. However, the solution with nitrite ion gradually changed from a wine-red to a purple-gray color as displayed in Fig. 1(A). The UV–vis absorption spectra of the MTT-GNPs solutions were acquired 30 min. after the addition of each anion, and are displayed in Fig. 1 (B). λ_{790} does not seem to be λ max as shown in Fig. 1(B), and λ max could be a little longer than 800 nm. The reason why we chose A_{790} as λ max is because the radiation source of the UV–vis spectrometer used in this experiment only covers the range 200–800 nm, and the absorbance curves of the UV–vis spectra drop vertically at 800 nm. But we are certain that this choice does not make any difference to the relative absorbance ratios of the two λ max values.

The sensitivity and selectivity of GNPs towards various anions was further studied by plotting the absorbance ratios (A_{790}/A_{535}) of the MTT-GNPs solution in the presence of each anion as shown in Fig. 1(C).

The selectivity of the optimized sensor for nitrite ions was evaluated by comparison of the absorbance ratio (A_{790}/A_{535}) with that of solutions containing similar anions (F^-, Cl^-, Br^-, PO_4^{3-}, SO_4^{2-}, and NO_3^-). It was found that GNPs conjugated with MTT responded selectively to nitrite, as indicated by the dramatic increase in the absorbance ratio (A_{790}/A_{535}). Thus, we successfully identified the aggregation of nanoparticles by nitrite ion in GNPs conjugated with MTT. The absorbance at 535 nm decreased and a new band with an absorption at 790 nm developed, and could be attributed to the coupled plasmon absorbance by closer contact

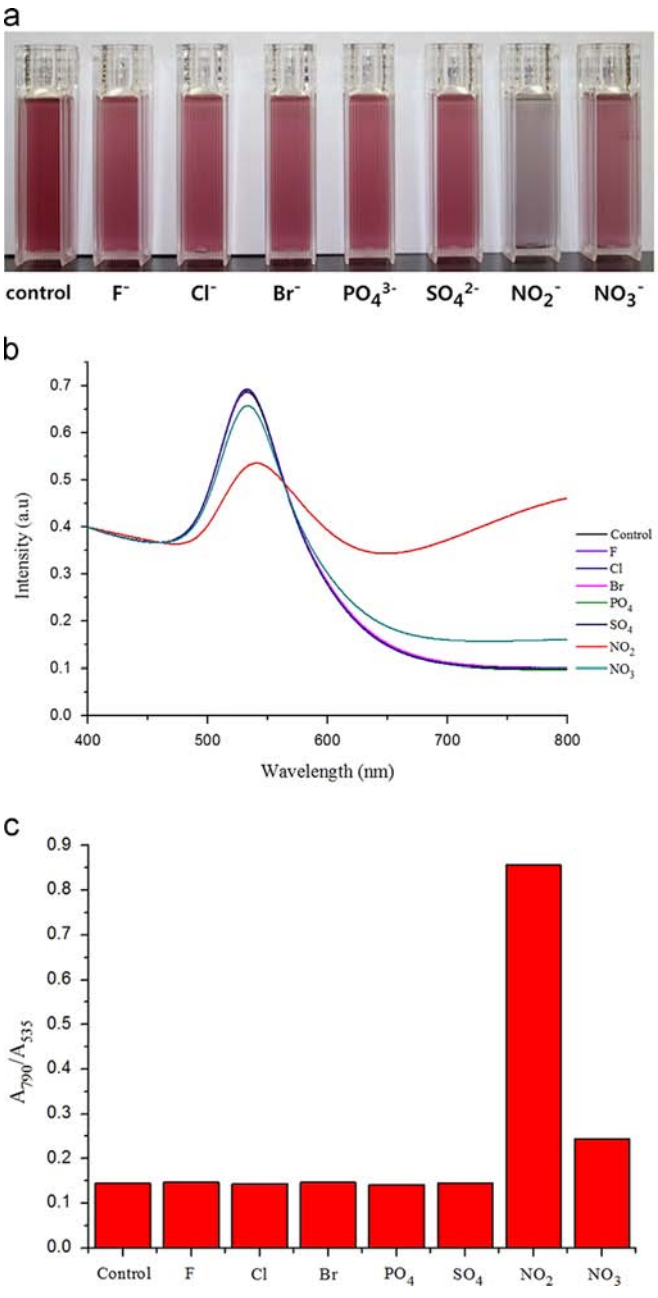

Fig. 1. (A) Visual color change of the MTT-stabilized GNPs upon addition of 3 ppm anionic ions (F^-, Cl^-, Br^--, PO_4^{3-}, SO_4^{2-}, NO_2^-, NO_3^-) (B) UV–vis absorption spectra of the MTT-stabilized GNPs upon addition of 3 ppm anionic ions (F^-, Cl^-, Br^-, PO_4^{3-}, SO_4^{2-}, NO_2^-, NO_3^-) (C) The corresponding absorbance ratios (A_{790}/A_{535}) in the various anions including nitrite ion.

with other nanoparticles due to aggregation. The absorbance ratio (A_{790}/A_{535}) induced by the NO_2 ion was about six-fold greater than those of other anions, which indicated the distinct interaction between MTT-GNPs and NO_2^-. Furthermore, MTT-GNPs show an excellent selectivity for NO_2^- ions. The unique selectivity seems to be attributed to the high specificity of three recognition sites for hydrogen bonding between nitrite ions and MTT [25].

The associated color change caused by aggregation in the presence of nitrite ions could be developed into a simple, rapid, and field-portable colorimetric method for the visual detection of NO_2^- or by detection using UV–vis spectroscopy. The conditions for GNP aggregation have been optimized by controlling the relative amount of NO_2^-, the pH, and the concentration of MTT-GNP solutions and reaction times.

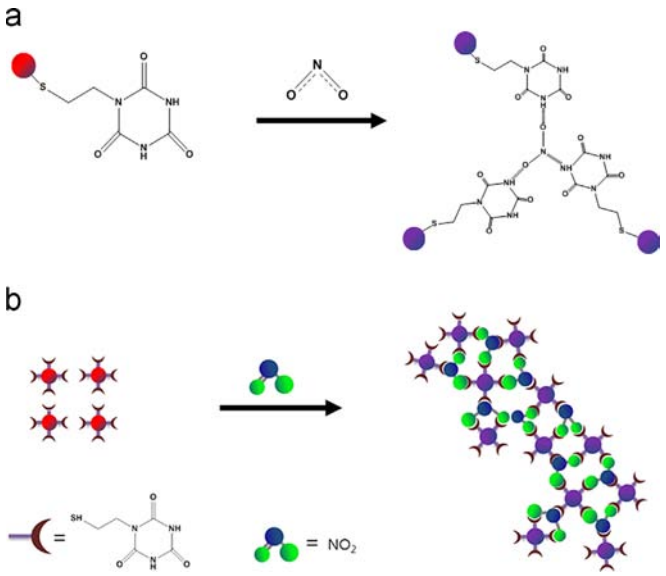

Fig. 2. A schematic diagram of the colorimetric gold nanoparticle sensor to detect nitrite ion using GNPs conjugated with MTT. (A) Hydrogen-bonding interaction between nitrite ion and MTT bound to GNPs. (B) A schematic diagram for GNPs aggregation of MTT-GNPs reacted with nitrite ion and its color change. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Fig. 2 presents a schematic diagram of the colorimetric gold nanoparticle sensor to detect nitrite ions using GNPs conjugated with MTT, and Fig. 2(a) shows a hypothetical structure presenting the hydrogen-bonding interaction between diaminopyridine and nitrite ions [30–32]. This excellent selectivity for NO_2^- ions was predicted to be due to the high selectivity of triple hydrogen bonding recognition between MTT and NO_2^- ions. In addition, Fig. 2(B) displays a schematic diagram for the aggregation of MTT-GNPs upon reaction with nitrite ions and the color change from the wine-red color of MTT-GNPs to the purple gray color due to the shortening of the interparticle distance.

The size distribution of GNPs conjugated with MTT was measured by a Zetasizer instrument, Zetasizer instruments shown in Fig. 3(A), and the average size was 33 nm. The wine-red color of MTT-GNPs in the absence of nitrite ion, and the purple gray color of GNPs-MTT in the presence of nitrite ion are shown in Fig. 3(B). Images of the MTT-GNPs (Fig. 3(C)) and the nitrite-induced aggregation of MTT-GNPs (Fig. 3(D)) were acquired using TEM and atomic force microscopy (AFM). The TEM images reveal that the size of each MTT-GNPs is about 33 nm (similar to what was measured by the Zetasizer) and is dramatically reduced in MTT-GNPs in the presence of NO_2^- as shown in Fig. 3(D). The band shift and color change in the presence of NO_2^- ions can also be explained by TEM. Specifically, surface electrons oscillated by light cannot homogeneously polarize the nanoparticles for small dispersed GNPs, and higher order modes at lower energies dominate, which can cause a red shift and broadening of the surface plasmon band.

The performance of the developed NO_2^- sensor could be strongly influenced by the pH of the solution, since the aggregation of the MTT-GNPs induced by the NO_2^- ions is generated by hydrogen bonding as shown in Fig. 2(A). Media pH not only influences the interaction between MTT-GNPs and NO_2^-, but also affects the stability of GNPs. The effect of pH was carried out using a pH range of 3.0–13. The results of the experiment (Fig. S1 in supporting information) showed that the absorption ratio (A_{790}/A_{535}) at low pH values (3.0–5.0) increases dramatically. This is probably due to the fact that when the hydrogen bond strength between NH of MTT and NO_2^- increases in strong acidic media,

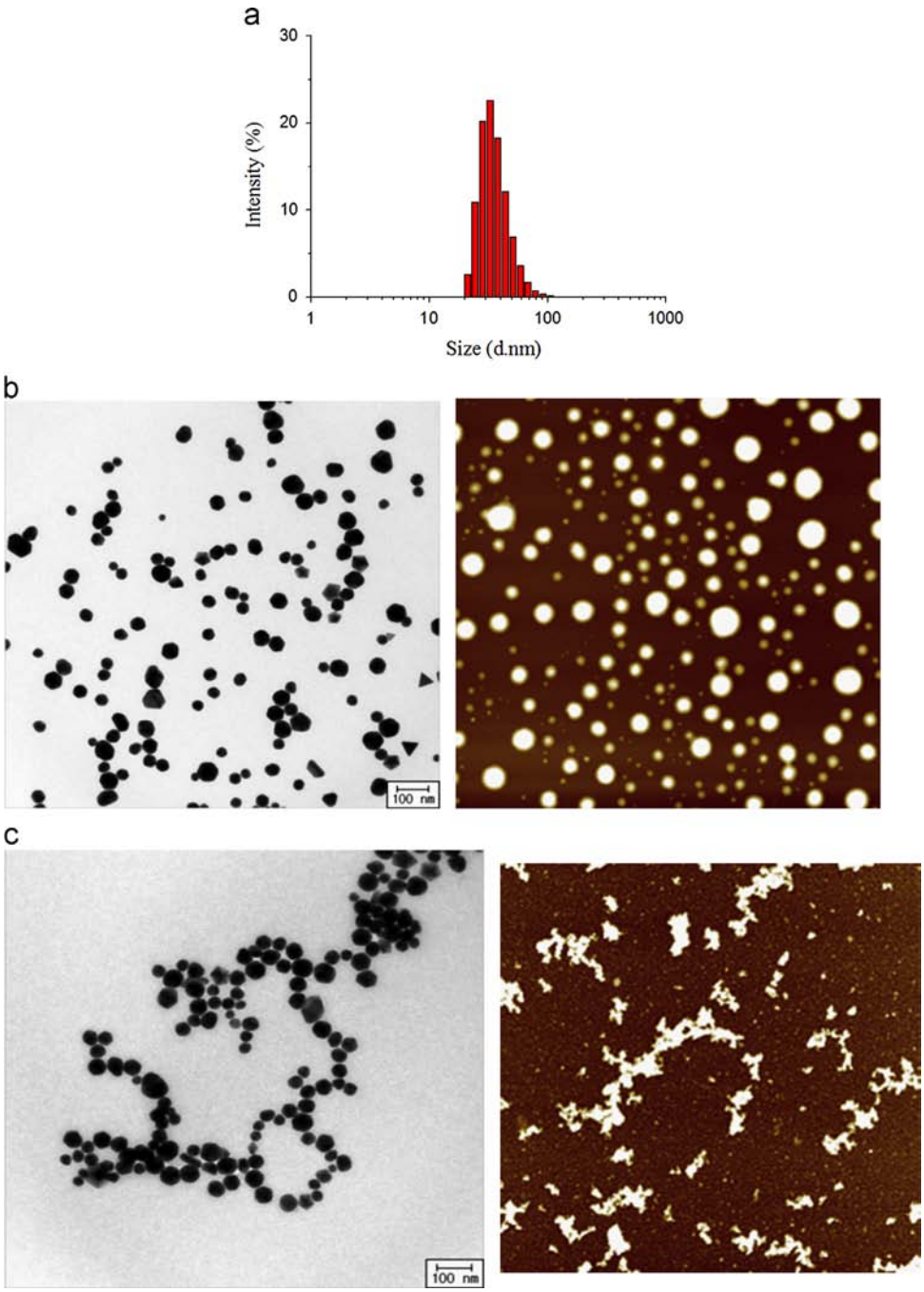

Fig. 3. (A) The size distribution of MTT-GNPs. The mean diameter is ~32.6 nm. (B) TEM (left) and AFM (right) images of MTT-GNPs in the absence of nitrite ions, respectively. (C) TEM (left) and AFM (right) images of MTT-GNPs in the presence of nitrite ions, respectively.

the aggregation of MTT-stabilized GNPs is induced. Therefore, the optimum pH of the media was determined to be 5.0.

In addition, in order to find out the optimum conditions for GNP aggregation, various concentrations of MTT-GNP solutions were tested and their UV–vis spectra and absorbance ratios (A_{790}/A_{535}) were monitored. We found that the changes have nothing to do with the concentrations of these species, but were induced by the NO_2^- ions (Fig. S2 in Supporting information).

The color change induced by NO_2^- ions can be monitored by UV–vis spectrometry, and these color changes depend on the concentration of nitrite ions as shown in Fig. 4(A). An increase in the absorbance in the 790 nm region and a concomitant decrease in the intensity of the SPR peak at 535 nm were observed along with the increase of NO_2^- concentration (0, 1, 2, 3, 4 and 5 μL mL^{-1}) in MTT-GNP solutions as shown in Fig. 4(B). The color of the GNPs changed progressively from wine-red to purple, and finally to a purple-gray color in accord with the increase in NO_2^- concentration. These color changes seem to represent the relative amounts of aggregated and monodisperse gold nanoparticles. We examined the aggregation kinetics of MTT-GNPs reacting with various concentrations of NO_2^- by monitoring the absorbance ratios (A_{790}/A_{535}), since a sensor with a fast response at room temperature would be highly desirable for on-site and real-time detection. It was found that the reaction times were inversely proportional to the concentration of nitrite ion. That is, concentrations of nitrite ion, 1, 2, 3, 4 and 5 ppm, required the reaction times of 100, 70, 50, 35, 30 min, respectively (Fig. S3 in Supporting information).

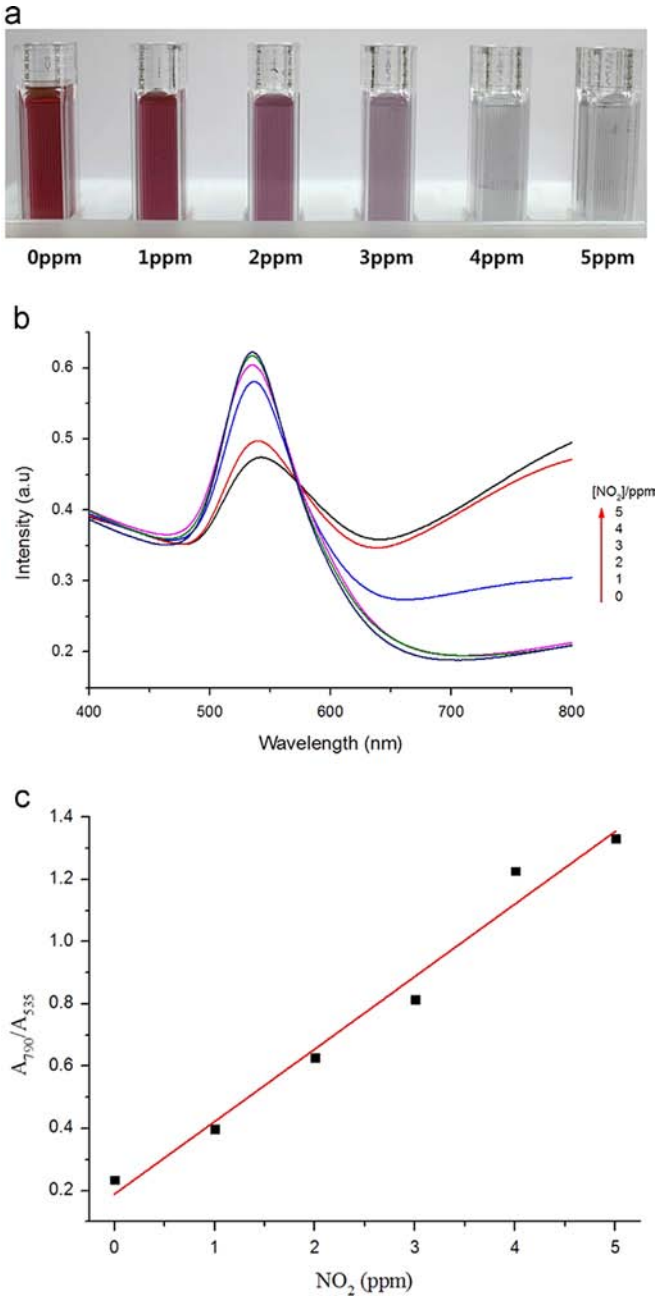

Fig. 4. (A) Visual color change of GNPs functionalized with 1 mM MTT solution upon addition of five different concentrations of nitrite ions using the optimized condition (from left to right; 0, 1, 2, 3, 4 and 5 ppm). (B) UV–vis absorption spectra of GNPs functionalized with 1 mM MTT in the concentration of 1–5 ppm nitrite ion. (C) The corresponding A_{790}/A_{535} ratios in UV–vis absorption spectra of GNPs-MTT as a function of the concentration of nitrite ion($y=0.2330x+0.0517$, $r^2=0.9734$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The absorbance ratios (A_{790}/A_{535}) were measured as a function of NO_2^- concentration for the quantitative analysis of NO_2^-, and are shown in Fig. 4(C). Linear regression analysis of the calibration curve was performed and good linearity (correlation coefficient, $r^2=0.9737$) was obtained at all concentrations. The limit of detection (LOD) of this colorimetric probe was 1 μL mL^{-1} within 30 min reaction time, similar to that of IC. This detection limit meets the guideline of the US EPA and WHO for drinking water. The concentration (3 and 5 μL mL^{-1}) of nitrite ions in distilled water were measured by the MTT-GNP colorimetric probe and IC. As shown in Table 1, the analytical results by the developed colorimetric sensor are similar to those monitored by IC. Furthermore, the determination of NO_2^- ions has been performed using tap and pond water from a garden of our research institute and the concentrations of NO_2^- were not detected within the LODs (1 and 0.1 μL mL^{-1}) by neither the colorimetric GNPs probe nor IC as shown in Table 2.

Therefore, this use of this colorimetric GNP-based probe for the detection of nitrite ions in aqueous samples could be better than instrumental methods in terms of the cost, simplicity, and time.

Table 1
Concentrations of nitrite ion spiked in distilled waters were measured by MTT-GNPs colorimetric probe and ion chromatography.

Content of nitrite (μl mL^{-1}) added in distilled water		
Added	Detected by colorimetric probe	Detected by ion chromatography
3.00	2.97 ± 0.32	3.01 ± 0.32
5.00	5.31 ± 0.32	5.01 ± 0.34

Table 2
Analytical results for the detection of real samples in tap water and pond water at KIST.

Sample	Content of nitrite (μL mL^{-1})	
	By colorimetric probe	By IC
Tap water	< 1	< 0.1
Pond water	< 1	< 0.1

4. Conclusion

In this paper, we have developed a novel sensitive and selective sensor for the visual detection of NO_2^- by means of the color change associated with gold nanoparticles aggregation. This method offers several advantages over the existing NO_2^- detection techniques. First, the method does not require expensive or complicated instrumentation, which simplifies operations and reduces the associated costs. Second, it allows detection of concentrations as low as 1 ppm to be achieved visually within half an hour, resulting in the rapid and sensitive detection of NO_2^- in aqueous solution at the levels allowed in drinking water by the US EPA and WHO. Finally, this sensor exhibits excellent selectivity for NO_2^- over other anions.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.02.030.

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