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Highly selective and sensitive gold nanoparticle-based colorimetric assay for PO_4^{3-} in aqueous solution

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ABSTRACT

A rapid, simple and real-time colorimetric assay for detecting phosphate anions (PO_4^{3-}) in aqueous solution was developed. The method was based on a novel functionalized gold nanoparticles (MPTP-Zn-AuNPs), which was synthesized by the ligand-exchange reaction of 4′-(4-mercaptophenyl)-2,2′:6′,2″-terpyridine zinc(II) complex (MPTP-Zn) with citrate-stabilized aqueous AuNPs. The strong coordination between MPTP-Zn-AuNPs and PO_4^{3-} anion gave rise to a color change from wine-red to blue-gray that was discernible by the naked eye and an easily measurable alteration in the absorption spectrum of the particles in aqueous solution. And the new technique exhibited the high selectivity for PO_4^{3-} over 11 other anions and could be used to determine PO_4^{3-} with a detection limit of 11 ppm.

1. Introduction

Phosphate anions are one of the most important constituents of living systems. And phosphate ions and their derivatives play key roles in signal transduction and energy storage in biological systems [1]. Meanwhile, phosphate is one of the major nutrients contributing in the increased eutrophication of lakes and natural waters. Its presence causes many water quality problems including increased purification costs, decreased recreational and conservation value of an impoundments, loss of livestock and the possible lethal effect of algal toxins on drinking water. Recently, the environmental pollution especially natural water resource eutrophication caused by the excessive use of phosphate has attracted much world-wide attention. Therefore the recognition and detection of phosphate anions in aqueous solution is a challenging and highly topical area of research.

In the past few years, although many sensors for phosphate ions have been designed, most of them recognize analytes depending on hydrogen-bonding and electrostatic interactions [2]. In order to avoid the competing solvation effect caused by a highly polar medium such as water [3], the established projects usually employ organic solvents as the detection medium [4]. The resulting restrictions promoted the researchers to devise and synthesize metal complexes to bind a phosphate unit of substrates in aqueous solution on the basis of coordination chemistry. In 2002, Kim et *al.* assembled a novel colorimetric sensor which can detect phosphate ions in an aqueous solution of physiological pH, spectrophotometrically and visually, with unprecedented high selectivity towards phosphate anions over a variety of mono- and dianions [5]. Recently, Hossain et *al.* synthesized and structurally characterized a new macrocycle-based dinuclear copper complex with high selective detection for phosphate anion in water at physiological pH [6]. Last year, Schubert et *al.* reported the fluorometric sensor based on bisterpyridine metallopolymer, which can detect phosphates in tap water selectively according to increased emission intensity and/or a shift of the emission wavelength [7]. However, these established methods [8] for phosphate anions have not been widely applied because they are time-consuming and inconvenient to perform outside of the laboratory. Although a classical method for the determination of phosphate through the formation of phosphomolybdate by its reduction has been well developed, it is commonly used to the detection of phosphate in the sample of water [9]. Therefore, new strategies for addressing this issue are urgently needed.

Gold nanoparticles (AuNPs) provide high sensitivity for the detection of metal ions because they exhibit characteristic surface plasmon resonance (SPR) absorption properties, which are highly dependent on size, shape and interparticle distance [10–12]. Of particular interest are the AuNP-based colorimetric sensors, which capitalize on the color change that arises from the interparticle plasmon coupling upon the aggregation of AuNPs (changing color from red to purple or blue) [13]. The controlled assembly and

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disassembly of gold nanoparticles has been a subject of great interest over the past decade due to the prospective applications in the field of ions detection [14], biosensing [15], catalysis [16] and surface enhanced Raman spectroscopy [17]. In a previous study, we have reported a novel, highly selective detection of Cr(III) in aqueous solution based on a gold nanoparticles (AuNPs) colorimetric assay and its application for determining Cr(VI) [18]. In this paper, we decided to make some attempts to design terpyridine zinc(II) complex functionalized gold nanoparticles as an appropriate binding motif toward the phosphate unit (Fig. 1). Because such a color change induced by aggregation of AuNPs is readily discernible by the naked eye, the method described here enables on-site and real-time detection of PO_4^{3-} without any advanced instrument.

2. Materials and methods

2.1. Chemicals and materials

Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot 3H_2O$) were purchased from Alfa Aesar. 2-Acetylpyridine and 4-nitrobenzaldehyde were purchased from Energy Chemical. KClO, KPF_6 and K_3PO_4 were purchased from Aladdin. I_2, KBr, KCl, KI, KNO_3, KOAc, KOH, KSCN, K_2CO_3, $K_2Cr_2O_7$, K_2SO_4, $NaNO_2$, NaOAc, NH_4OAc, $Zn(NO_3)_2$, carbonodithioic acid *o*-ethyl ester potassium salt (1:1) and pyridine were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, Milford, MA).

2.2. Instrumentation

^1H NMR spectra of samples in d^6-DMSO were recorded on a Bruker AV-300 spectrometer with tetramethylsilane (TMS) as an internal standard. UV–vis absorption spectra were measured on an Agilent 8453 spectrophotometer in aqueous solution at room temperature. Transmission electron microscopy (TEM) was performed with a transmission electron microscope (JEM-2100, Japan). Samples for TEM characterization were prepared by applying a drop of colloidal solution to a carbon-coated copper grid.

About the synthesis of 4′-(4-mercaptophenyl)-2,2′:6′,2″-terpyridine zinc(II) complex (MPTP-Zn) modified AuNPs, please see the experimental section in Supplementary material.

2.3. Colorimetric discrimination of PO_4^{3-} from other anions

Generally, for AuNPs samples, the functionalized gold colloid (1000 μL) was diluted with deionized water (1000 μL), and then mixed with different anions solutions (1500 μL) of varying concentrations.

3. Results and discussion

3.1. Stabilization of the MPTP-Zn modified AuNPs

In the previous work, Ojida et al. found that anthracene derivatives having two sets of zinc(II)-dipicolylamine selectively bind phosphorylated chemical species so as to cause the fluorescent spectral change [19]. The UV–vis spectrum of a solution of

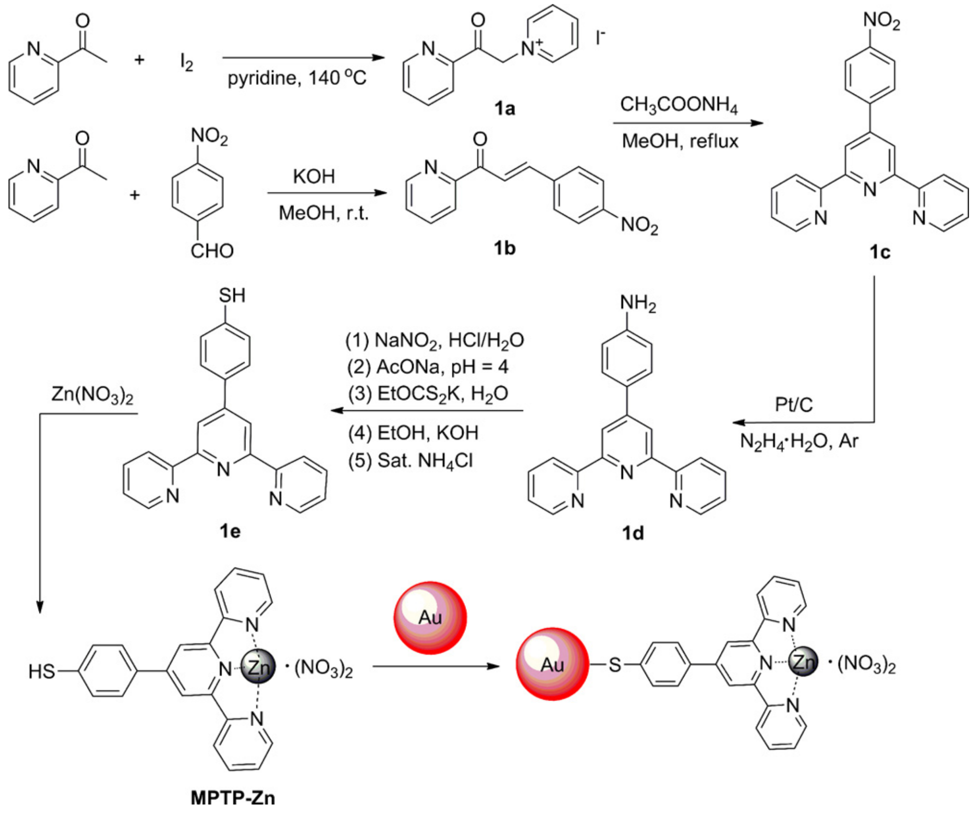

Fig. 1. The synthetic routes of MPTP-Zn modified gold nanoparticles.

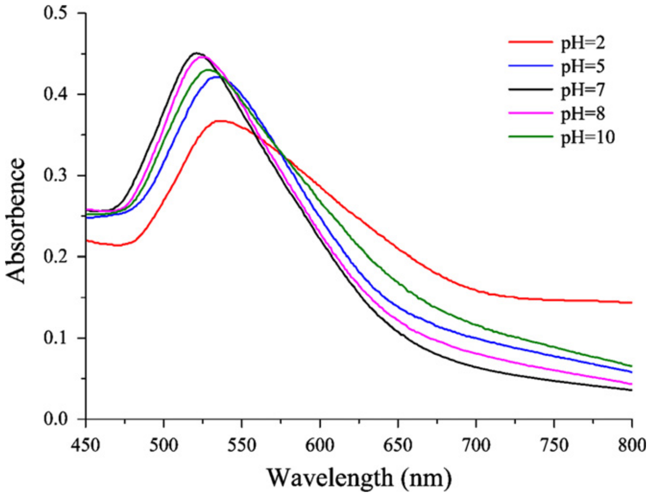

Fig. 2. UV–vis absorption spectra of MPTP-Zn-modified AuNPs under different pH conditions; spectra were recorded 30 min after adjusting to the required pH.

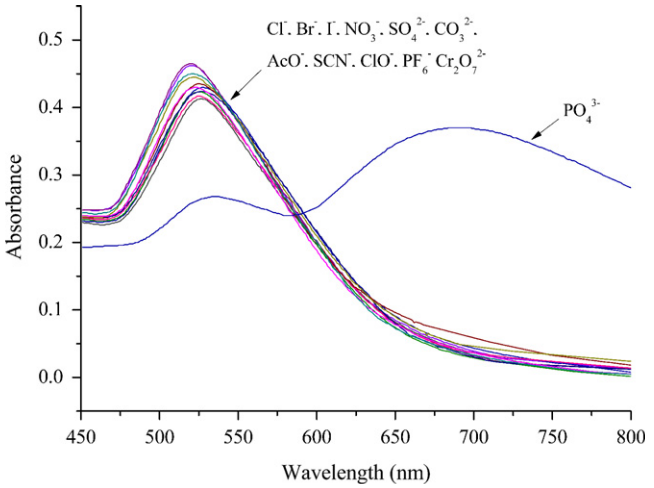

Fig. 3. UV–vis absorption spectra of MPTP-Zn-AuNPs containing 500 μM other anions compared with 200 μM PO_4^{3-}.

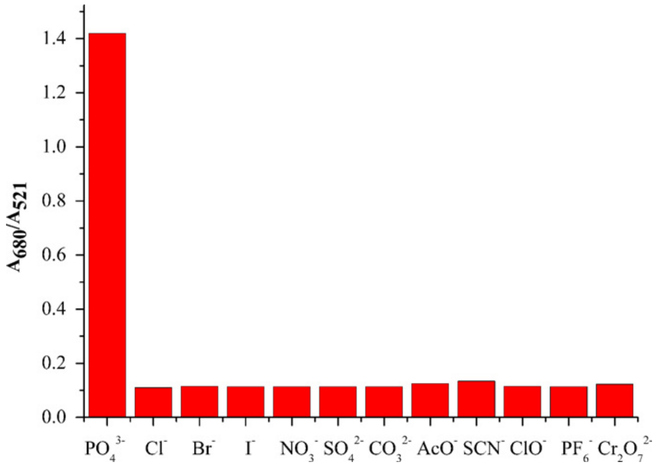

Fig. 4. The corresponding A_{680}/A_{521} ratios in the presence of different anions (500 μM) or PO_4^{3-}(200 μM).

the MPTP-Zn modified gold nanoparticles showed an absorption maximum at 521 nm, which was only slightly different from that of the unmodified gold nanoparticles (518 nm) (see Supplementary material, Fig. A.4). The surface plasmon band of the MPTP-Zn-modified gold nanoparticles remained unchanged during storage for more than 3 months [20].

We have also investigated the stability of the MPTP-Zn-modified gold nanoparticles in pH values from 2 to 10 (Fig. 2). These MPTP-Zn-modified AuNPs exhibited lower stability in solutions of pH 2–6, and over time a slight red-shift and line-broadening of the absorption band and modest precipitation were observed. However, the nanoparticles were very stable in solutions of pH 7–8 for weeks to months with no sign of aggregation. However, when the solution pH was adjusted to 8–10, the marginal red-shift was also observed with an increase of time.

3.2. Selectivity for detecting PO_4^{3-} versus other anions

On the basis of the above-established method for the synthesis of modified AuNPs, we started to explore the feasibility of developing a simple colorimetric assay for the selective detection of PO_4^{3-}. A solution of the stable modified AuNPs displayed a surface plasma resonance absorption peak at 521 nm and showed a wine-red color. When PO_4^{3-} was added to the AuNPs solution, the color changed from wine-red to blue-gray, indicating the rapid aggregation of the AuNPs. Meanwhile, the absorbance at 521 nm decreased and a new absorption band appeared at around 680 nm. The new absorbance could be attributed to the coupled plasmon absorbance of nanoparticles in close contact [21]. The associated color change caused by aggregation could be developed into a simple, rapid and field-portable colorimetric method for the visual detection of PO_4^{3-} or by detection using UV–vis spectroscopy.

To verify the specificity of the present approach, it was found that the color response was insensitive to the presence of 11 other anion species including Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}, CO_3^{2-}, AcO^-, SCN^-, ClO^-, PF_6^- and $Cr_2O_7^{2-}$, whereas upon addition of PO_4^{3-} the color showed blue gray immediately. Fig. 3 displays the UV–vis absorption spectra of the MPTP-Zn-AuNPs solutions containing different anions (500 μM) compared with that in the presence of PO_4^{3-} (200 μM), each recorded 30 min after mixing. The sensitivity and selectivity of AuNPs toward various anions was further studied by plotting the absorption intensity ratios (A_{680}/A_{521}) of MPTP-Zn-AuNPs solutions at a specified concentration of anions (Fig. 4). The PO_4^{3-}-induced value of A_{680}/A_{521} was about sevenfold larger than those seen from other anions, confirming that this phenomenon could be used as an indicator of the distinctive interaction between MPTP-Zn-modified AuNPs and PO_4^{3-}. The reason for the band-shift and color change can also be explained by a TEM study on these particles in the absence and presence of PO_4^{3-} (200 μM) (see Supplementary material, Fig. A.5). In fact, for small dispersed AuNPs, surface electrons are oscillated by the incoming light in a dipole mode. As the size of the AuNP increases, light can no longer polarize the nanoparticles homogeneously, and higher order modes at lower energy dominate, which causes a red-shift and broadening of the surface plasmon band. Furthermore, to assess the feasibility of detecting PO_4^{3-} in the presence of highly concentrated solutions of other anions, we investigated the influence of 200 μM, 500 μM, 800 μM and 1000 μM solutions of Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}, CO_3^{2-}, AcO^-, SCN^-, ClO^-, PF_6^- and $Cr_2O_7^{2-}$, respectively. The A_{680}/A_{521} ratio increased and the interference was amplified with the increasing of the concentrations of the solution of 11 other anions in the absence of PO_4^{3-} (see Supplementary material, Fig. A.6). Although the A_{680}/A_{521} ratio approached unity when the anion concentration reached 1000 μM, which may represent a critical point, the interference in determining the concentration of PO_4^{3-} was insignificant. The results listed in Table A.1 show that the A_{680}/A_{521} ratios were 1.41, 1.39, 1.40 and 1.38 when the concentrations of the 11 other anions in the presence of PO_4^{3-} were 200 μM, 500 μM, 800 μM and 1000 μM respectively. Hence, the interference in the detection of PO_4^{3-} in the presence of highly concentrated solution (up to 1000 μM) of 11 other anions did not affect the result. Effects from solutions containing one or several other anions that we thought might show interference will require further study. Considering the present technique are mainly used to determine the free PO_4^{3-} in solutions, the effects of interfering cations (Ca, Mg and Zn etc. ions) on the determination of phosphate anion are negligible. Because they can form stable complexes with phosphate anion, which are almost insolube in aqueous solutions.

Among naturally occurring phosphates, zinc(II) metal complexes is often employed to bind a phosphate unit of substrates in the enzymatic pocket [22]. On the basis of previous study, we proposed the following recognition mechanism of high selective detection of PO_4^{3-} employing the specific ligand (MPTP-Zn) stabilized AuNPs. As shown in Fig. 5, the MPTP-Zn complex with a mercapto (–SH) group served to functionalize and stabilize the gold nanoparticles. PO_4^{3-} anions in solutions were bound to the MPTP-Zn stabilized AuNPs by oxygen–zinc coordination, while the stronger coordination ability with zinc ion was not possessed by the 11 other anions. We speculated that at least two MPTP-Zn

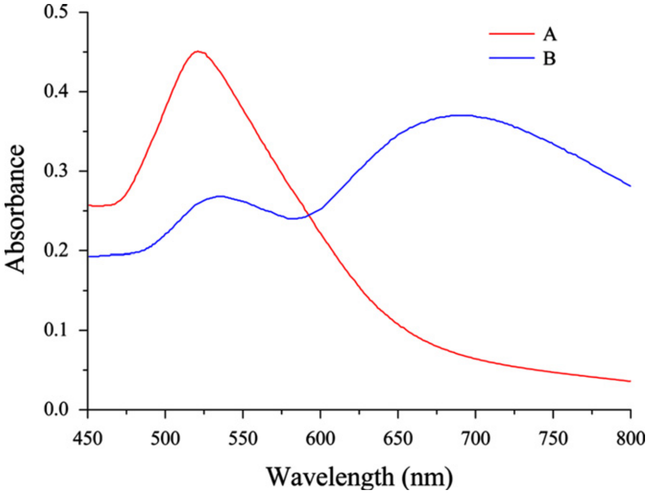

Fig. 6. UV–vis spectra of the solution of MPTP-Zn-AuNPs (A) before and (B) after addition of 200 μM PO_4^{3-}.

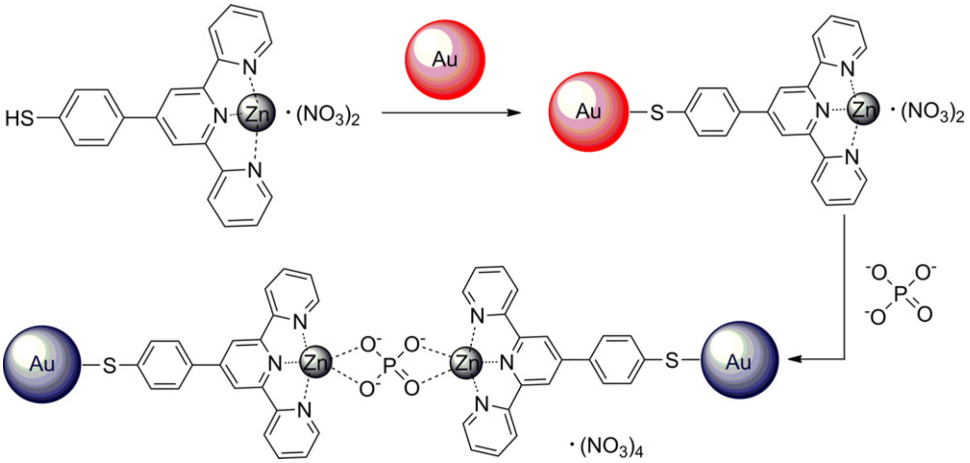

Fig. 5. Recognition mechanism of the visual detection of PO_4^{3-} based on the MPTP-Zn-AuNPs.

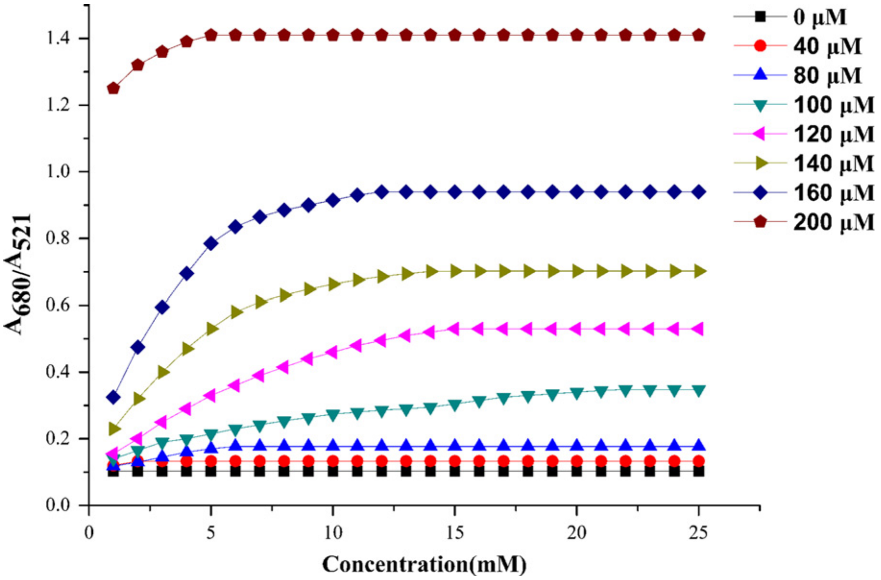

Fig. 7. Plots of the time-dependent absorption ratio (A_{680}/A_{521}) over 10 min in the presence of varying concentrations of PO_4^{3-}.

moieties, bound to different nanoparticles, could interact with PO_4^{3-}. Therefore, the gold nanoparticles were aggregated, leading to a color change (wine-red to blue gray).

3.3. Sensitivity of the response of MPTP-Zn-AuNPs to PO_4^{3-}

The color change of the MPTP-Zn stabilized gold nanoparticles induced by PO_4^{3-} could also be monitored by UV–vis spectrophotometry. In Fig. 6, it can be shown that the addition of 100 μM PO_4^{3-} led to a dramatic increase in the absorbance at 680 nm. Along with the increase of concentrations of PO_4^{3-}, an increase in the absorbance in the 680 nm region and a concomitant decrease in the intensity of the SPR peak at 521 nm were observed. These two wavelengths were chosen to represent the relative amounts of aggregated and monodisperse gold nanoparticles, respectively. Accordingly, the color of the AuNPs progressively changed from wine-red to purple and finally to blue gray. Furthermore, the intensity of the blue gray color got weaker with the amount of precipitate increasing concomitantly [23]. Control experiments only MPTP-Zn complex in conjunction with PO_4^{3-} showed no peak at 680 nm. These results confirmed the role of the gold nanoparticles in the MPTP-Zn-AuNPs assembly. For quantitative analysis of PO_4^{3-}, the ratio of the absorbance at 680 nm and 521 nm was used.

Considering that a sensor giving a fast response at room temperature would be highly desirable for on-site and real-time detection of PO_4^{3-}, we examined the aggregation kinetics of sensor with different PO_4^{3-} concentrations by monitoring the ratio of the absorbance at 680 nm and 521 nm. As shown in Fig. 7, the value of absorption ratio (A_{680}/A_{521}) increased with the reaction time increased. In the concentration range (≥ 200 μM), the absorbance ratio increased rapidly within 5 min; and for the samples with PO_4^{3-}concentrations in the range of 120–160 μM, it reached the maximum after 15 min and then remained constant with time, whereas the absorption ratio of the other low-concentration (≤ 120 μM) solutions still increased.

Under the optimized conditions, the reproducibility of the established method was evaluated by performing ten replicate measurements on a 140 μM solution of PO_4^{3-}. The results presented in Fig. A.7 (see Supplementary material) showed our method to be highly reproducible. The A_{680}/A_{521} ratio in the presence of 140 μM PO_4^{3-} was significantly higher than that for 0 μM. Because we observed only a slight increase in the absorption spectrum between 0 μM and 100 μM PO_4^{3-}, the minimum detectable concentration of PO_4^{3-} is suggested to be 120 μM. To evaluate the sensitivity of the PO_4^{3-}-dependent colorimetric sensor under the optimized detection conditions, the shift in the SPR peak of gold nanoparticles was monitored by UV–vis spectrophotometry, and the ratios of the absorbance at 680 and 521 nm were compared at various PO_4^{3-} concentrations. As shown in Fig. 8a, a concentration of PO_4^{3-} higher than 120 μM (11 ppm) induced the interparticle association of the MPTP-Zn-AuNPs, leading to a new LSPR band associated with the dipole-coupling of AuNPs. A linear relationship was found between the absorbance ratio and the PO_4^{3-} concentration of over the ranges 80–200 μM ($R^2=0.994$) (Fig. 8b).

4. Conclusions

In this paper, we have developed a novel sensor for the visual detection of PO_4^{3-} by means of the color change associated with gold nanoparticles aggregation. This method offers several advantages over the existing PO_4^{3-} detection techniques. Firstly, the method does not require expensive and complicated instrumentation, which simplifies operations and reduces the costs. Secondly, it allows detection of concentrations as low as 11 ppm to be achieved visually within 15 min, available for the rapid and sensitive detection of PO_4^{3-} in aqueous solution. Finally, this sensor exhibits excellent selectivity for PO_4^{3-} over other anions. Further studies on detecting biologically relevant phosphates such as diphosphates and adenosine-5′-triphosphate (ATP) are being conducted in our laboratory.

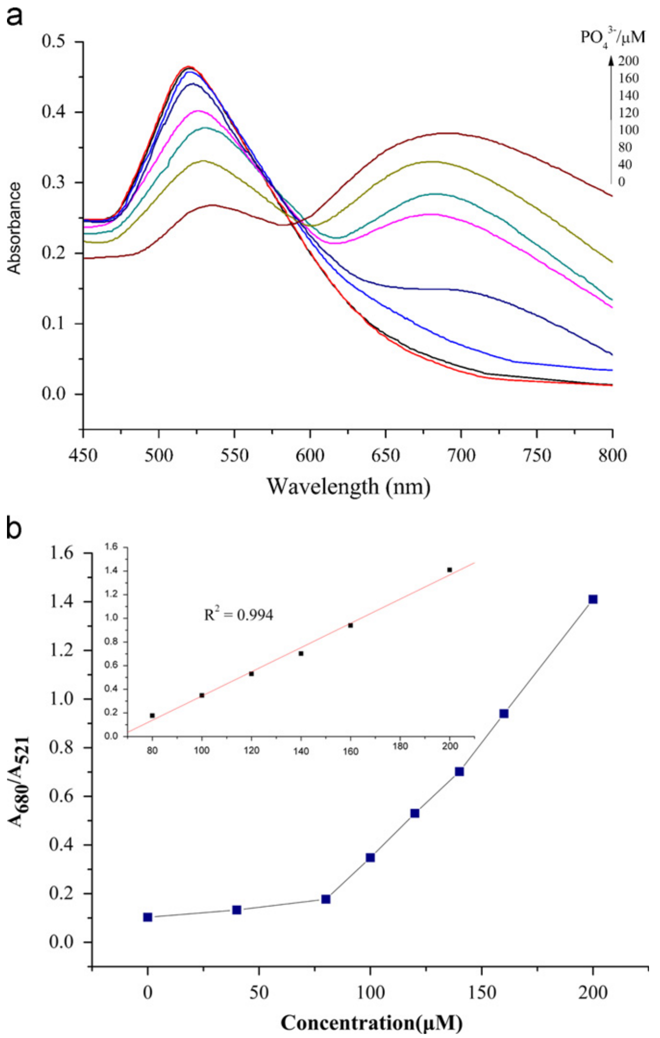

Fig. 8. (a) Concentration-dependent absorption spectra of the MPTP-Zn-stabilized gold nanoparticles in the presence of varying concentrations of PO_4^{3-}; (b) The corresponding plot of absorption ratio (A_{680}/A_{521}) versus PO_4^{3-} concentration.

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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.2012.11.064.

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