UV-VISIBLE SPECTROSCOPY DETECTION OF IRON(III) ION ON MODIFIED GOLD NANOPARTICLES WITH A HYDROXAMIC ACID

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The present work describes the preparation of gold nanoparticles (AuNPs) functionalized with hydroxamic acid and the use of them in UV-visible spectroscopy detection of iron(III) ions. The prepared AuNPs were thoroughly characterized by using UV-visible spectroscopy, TEM, and 1H NMR techniques. The newly synthesized hydroxamic acid-AuNPs are brown in color due to the intense surface plasmon absorption band centered at 527 nm. In the presence of Fe(III), the surface plasmon absorption band is centered at 540 nm. However, the sensitivity of hydroxamic acid-AuNPs towards other metal ions such as Mg(II), Ca(II), Ag(I), Cu(II), Mn(II), Cr(II), Ni(II), Co(II), Fe(II), Hg(II), and Pb(II) can be negligible. This highly selective sensor allows a direct quantitative assay of Fe(III) with a UV-visible spectroscopy detection limited to 45.8 nM.

Keywords: nanoparticles, surface plasmon resonance, hydroxamic acid-AuNPs, UV-visible spectroscopy, nanosensor, iron.

Introduction. It is well known that iron is found in surface and drinking water and plays a vital role in biological systems, and also it is the most abundant metal ion in the human body. Many proteins use iron for oxygen transport, electron transport, and as a catalyst in oxido-reductase reactions [1]. In other words, an excess of iron ions in a living cell can catalyze the production of reactive oxygen via the Fenton reaction, which can damage lipids, nucleic acids, and proteins. Serious diseases such as Alzheimer’s, Huntington’s and Parkinson’s result from the toxicity of iron ions [2, 3], so the concentration of iron becomes a parameter for the quality of drinking water [4].

Several analytical methods have been used for the determination of iron in water such as HPLC methods [5], atomic absorption [6], electrochemical methods [7], and voltammetry [8]. These and many other techniques often require quite expensive and time-consuming sample pretreatment, including pre-concentration and matrix separation. Therefore, due to the lack of suitable iron indicators, the development of cost-effective sensors for the easy and fast detection of metal ions with the naked eye without resorting to any expensive instruments is still an active research area [9, 10].

Recently, noble metal nanoparticles designed as UV-visible spectroscopy sensors can provide an important method of detection, which allows a direct analysis of the substrate simply with the naked eye. Gold nanoparticles have also been intensively studied because of their potential uses in nano electronic devices [11, 12], medical diagnosing [13], as catalyst [14], for drug delivery [15], and as a nanosensor [16, 17]. Today UV-visible spectroscopy sensors have drawn more and more attention as a means to decrease experimental costs and simplifying the sensing process [18, 19]. In particular, the design and synthesis of gold nanoparticles (AuNPs) functionalized with targeted functional molecules, due to their unique optical properties (i.e., surface plasmon resonance absorption and resonance light scattering), have attracted much attention, and they have been extensively used as modern ultrasensitive detection and imaging probes [20–22]. Based on this unique property of gold nanoparticles, functionalized AuNPs have been extensively explored as probes for sensing/imaging a wide range of analytes/targets, such as alkali metal ions [23], copper(II) [24], mercury(II) [25], lead(II) [26], pollutant aromatic amines [27], and lectins [28].

In this work, we present a new UV-visible spectroscopy assay for detection of Fe(III) based on hydroxamic acid functionalized gold nanoparticles.

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**Experiment.** All compounds were obtained from Sigma-Aldrich and Merck. Milli-Q water was used in the experiments, and the solvents and reagents were used as received without further purification. All glassware used in the procedure was cleaned in a bath of freshly prepared 3:1 HCl-HNO₃ rinsed thoroughly in water and dried in the open air. UV-vis absorption spectra were acquired on a Cary 100 UV-vis spectrometer (Varian, USA) at room temperature (23–25°C). ¹H NMR spectra were recorded on a Bruker spectrometer operating at 200 MHz in CDCl₃ and DMSO-d. Transmission electron microscopy (TEM) was carried out on a Philips CM 200 FEG scanning transmission electron microscope at 200 keV. A pH meter (Metrohm AG, 827) was used for the pH adjustment.

The synthesis of C4-AuNPs (1) was carried out with a modified Brust method as described previously [29] using octanethiol (C4) (2.7 mmol) as ligands for nanoparticles preparation in the course of gold reduction with sodium borohydride. Nanoparticles were purified by precipitation and centrifugation using ethanol or a mixture of ethanol/acetone, and finally C4-AuNPs were dispersed in THF and stored in the dark.

In the next stage, 11-mercapto-AuNPs (2) was prepared by dissolving (50 mg, 0.06 mmol) of C4-AuNPs (1) in 10 mL of tetrahydrofuran and then 40 mg (0.18 mmol) of 11-mercaptoundecanoic acid was added, and the mixture was stirred at room temperature for 6 h [30].

Hydroxamic acid-AuNPs (3) was prepared by dissolving 40 mg (0.05 mmol) of 11-mercapto-AuNPs in 10 mL of dichloromethane, and then 70 mg (0.25 mmol) of triflic anhydride (Tf₂O) was added and the mixture was stirred at room temperature for 20 min. Then 0.3 mmol of acid hydroxylammonium chloride was added to the mixture and the mixture was stirred at room temperature for 90 min. The solvent was removed via rotary evaporation and washed copiously with water and acetone (3:7, v/v) to remove the unreacted starting materials.

A solution with a different concentration of Fe(III) ion in 2 mL acetonitrile was added to 1 mg of hydroxamic acid-AuNPs, and the obtained mixture was stirred at room temperature to offer enough time for reaction. The final concentrations of Fe(III) in each solution are 0–200 nM. After being stirred for another 30 min, the UV-vis absorption of these solutions was measured and the response curve of the ratio of absorbance of hydroxamic acid-AuNPs at A650/527 versus the concentration of Fe(III) ion was plotted.

Following the steps of Fe(III) determination above, a certain volume of Mg(II), Ca(II), Ag(I), Cu(II), Mn(II), Cr(II), Ni(II), Co(II), Fe(II), Fe(III), Hg(II), and Pb(II) (5 mL, 1 × 10⁻⁷ M) solution was added to 1 mg hydroxamic acid-AuNPs. After mixing, the UV absorbance A650/527 of these solutions was determined.

**Results and Discussion.** The synthesis of butanethiol gold nanoparticle (C4-AuNPs) with hydroxamic acid is planned as shown in Scheme 1. The C4-AuNPs (1) were synthesized following the Brust procedure [31], and we prepared 11-mercapto-AuNPs (2) following the Murray model for the place exchange reaction using an excess amount of 11-mercaptoundecanoic acid as an incoming ligand. Therefore, for preparation of hydroxamic acid-AuNPs, 11-mercapto-AuNPs was put into reaction with hydroxylammonium chloride in the presence of Tf₂O [17]. The success of the thiol exchange process can be proved by utilizing ¹H NMR spectroscopy. Figure 1A shows that the ¹H NMR spectrum of C4-AuNPs has only broad

![Scheme 1. Scheme of synthesis and assembly of hydroxamic acid-AuNPs.](image-url)
resonances at 0.88 and 1.25–1.75 ppm attributed to protons on the terminal methyl group and methylene protons of the alkanethiolate chain. Figure 1B represents the $^1$H NMR spectra of 11-mercapto-AuNPs and shows that the new signals appeared at 2.13 ppm in addition to the signals observed for C4-AuNPs. These new peaks are assigned to the protons of $\alpha$ methylene unit adjacent to the carboxylic acid groups. Figure 1C represents the $^1$H NMR spectra of hydroxamic acid-AuNPs (3) and shows that the new signals appeared at 5.2–5.4 ppm and 8.2–8.3 ppm in addition to the signals observed for 11-mercapto-AuNPs. These new peaks are assigned to the protons of the hydroxyl and the protons of the amine group. In addition, the UV-Vis absorption spectra of C4-AuNPs, 11-mercapto-AuNPs, and hydroxamic acid-AuNPs are shown in Fig. 2a.

**Principle of the proposed method for Fe(III) ion detection.** The aggregation of nanoparticles can be associated with the red shift of the SPR band according to the Mie theory [32]. This theory states that when the distance between the two nanoparticles is less than the sum of their radii, the SPR band displays a red shift, broadening and decreasing in intensity [33]. The transition of nanoparticles from dispersion to aggregation exhibits a distinct change in color, and this is due to the coupling of plasmon absorbance as a result of their proximity to each other [34]. However, the latter originates from surface plasmon bands related to collective oscillations of the electrons at the surface of NPs interacting with the electromagnetic field of the incoming light. For the gold surface, plasmon bands are located in the visible region of the electromagnetic spectrum and can be effectively controlled by the shape of NPs [35, 36]. On the other hand, the gold nanoparticle with $>$5 nm diameter is brown or brown red, so when the aggregation occurs, the surface plasmon resonance shifts to lower energies; thus, this low energy band results from the coupling of the plasmon resonance of equidistant neighboring hydroxamic acid-AuNPs [37, 38], causing the absorption and scattering peaks to occur at longer wavelengths. The aggregation of AuNPs was induced by the binding of hydroxamic acid-AuNPs N and O group to Fe$^{3+}$ ion as a result of bonding between the two or three molecules, leading to a SPR change, as shown in Scheme 2, prior to the addition of Fe(III) ion, and the color change is easily detected with the naked eye. It is obvious that the change in plasmon absorbance spectroscopy of the nanoparticles solution is associated with the increase in the size of aggregated nanoparticles [27]. The ability
of hydroxamic acid to form a complex with Fe(III) ion was previously reported [39]. This motivated us to fabricate a simple nanoparticles based UV-visible spectroscopy sensor for Fe(III) ion based on the SPR changes. Accordingly, the ability of the prepared nanoparticles to detect Fe(III) ions was investigated in detail using UV-Vis spectroscopy and TEM analyses.

It is necessary to examine the optimum conditions for a sensor. We investigated responsive conditions in our sensor including the pH of solutions. The value of $A_{650}/A_{525}$, which could characterize the ratio of the dispersed to the aggregated forms of AuNPs, was used as an indicator for performance evaluation. The response of $A_{650}/A_{530}$ was achieved over the pH range 4.24–11.51 in Fig. 2b. In addition, Fig. 2b presents the UV/Vis spectrum of hydroxamic acid-AuNPs; it shows its original SPR band at 537 nm. After adding a drop of an acid solution (pH 4.24), a significant redshift of the band from 537 to 546 nm was observed. Upon the addition of one drop of an alkaline solution (pH 9.32) to the resulting solution, the SPR band shifted from 546 nm back to 536 nm. To obtain better performance, the pH at 9.32 was chosen to avoid aggregation of particles. This is attributed to interparticle interactions: at a low pH the hydroxamic acid groups facilitate interparticle interactions through hydrogen bonding and an increase in hydrophobicity, resulting in the formation of particle aggregates; however, particle aggregation is disfavored due to the repulsive interactions between negatively charged carboxylate particles [40].

Scheme 2. The possible mechanism of the phenomenon of binding the free hydroxamic acid-AuNPs with Fe(III) ion.

Fig. 2. UV-vis absorption spectra (a) of buthanethiol-AuNPs (C4-AuNPs) (1), 11-mercapto-AuNPs (2), and hydroxamic acid-AuNPs (3), and influence of pH on the UV-vis spectra of hydroxamic acid-AuNPs (b).
To investigate the metal recognition ability of hydroxamic acid-AuNPs, we tested the selectivity of this method in the presence of various metal ions. The same concentration ($5 \times 10^{-7}$ M) of these metals was added into the solution of hydroxamic acid-AuNPs. The color of the solution containing Fe(III) was determined by the presence of surface plasmon resonance at 525 nm, while other metals had no effect on the surface plasmon resonance. With increasing concentrations of Fe(III) ion, the surface plasmon resonance shifted to red. The red shift clearly indicated the aggregation of hydroxamic acid-AuNPs (Fig. 3a). In other words, the morphology of the functionalized hydroxamic acid-AuNPs before and after addition of Fe(III) ions was studied by transmission electron microscopy (TEM) as shown in Fig. 4. The average particle diameter for hydroxamic acid-AuNPs, deduced from the TEM image (Fig. 4a), is $3 \pm 0.5$ nm, and the image shows the shape of these modified hydroxamic acid-AuNPs is regular and close to spherical with a reasonable degree of monodispersity. The TEM image of this nanoparticle acid in the presence of Fe(III) is shown in Fig. 4b. Upon adding Fe(III), the SPR of hydroxamic acid-AuNPs acid solution changes, and the TEM image of hydroxamic acid-AuNPs exhibited a significant aggregation driven by Fe(III) ions, and the average diameters are $15 \pm 3$ nm. These images clearly show the transition from dispersion.
to aggregation in response to Fe(III) recognition. The UV-visible spectroscopy response is thus attributed to aggregation induced by the complex between hydroxamic acid and Fe(III) ions (Scheme 2). The UV-Vis absorbance values ($A_{360}/A_{527}$) increase with increasing concentration of Fe(III) ions in the system and show a linear correlation for target analytics in the range 0–200 nM ($R^2 = 0.9776$) in Fig. 3b, and the detection limit is 45.8 nM on the basis of $S/N = 3$.

**Conclusions.** We synthesized a highly sensitive UV-visible spectroscopy sensor for the detection of Fe$^{3+}$ using gold nanoparticles modified with hydroxamic acid and characterized by UV-visible spectroscopy, TEM and $^1$H NMR techniques. This new method offered several advantages over other Fe$^{3+}$ detection techniques. First, the UV-vis spectrometer method did not require complicated and expensive instruments. Second, Fe$^{3+}$ was the only metal ion that induced aggregation of hydroxamic acid-AuNPs, resulting in a corresponding plasmonic absorption shift from 527 to 650 nm. The method allowed us to detect concentrations up to 45.8 nM. These advantages make this method quite promising for rapid detection of Fe$^{3+}$ in aqueous solutions.

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