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Research Article

Hydrogen-Bonding Recognition-Induced Colorimetric Determination of Hydrazine Based on the Tryptophan Capped Gold Nanoparticles

Wenhua Gao,[1,2] Jing Xi,[1] Yunsheng Chen,[1] Song Xiao,[3] Xingqing Wang,[1] Jun Li,[1] Jincheng Xiao,[1] and Yaowen Chen[2]

[1] *Department of Chemistry, Shantou University, Guangdong, Shantou 515063, China*
[2] *Analysis & Testing Center, Shantou University, Guangdong, Shantou 515063, China*
[3] *Department of Chemistry, Medical College of Guiyang, Guizhou, Guiyang 550004, China*

Correspondence should be addressed to Wenhua Gao; whgao@stu.edu.cn

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A simple, cost-effective, and rapid colorimetric method for hydrazine detection using tryptophan-caped gold nanoparticles (Trp-AuNPs) has been developed. Tryptophan (Trp) is a protein with reducibility and amino group which can reduce chloroauric acid ($HAuCl_4$) to AuNPs and modify the surface of AuNPs simultaneously. The Trp-AuNPs could be used to quantitatively detect hydrazine and showed different responses to vary concentration of hydrazine in an aqueous solution based on the aggregation-induced color change of Trp-AuNPs. The real water sample analysis verified the conclusion. The sensitivity of the detection system was influenced by the size of AuNPs which is determined by the pH of the detection system, the concentration of Trp, and the react time. We found that higher temperature contributed to more rapidly results. The detection system can detect as low as 1 μM hydrazine. We expect our approach to have wide-ranging applications in the developing region for monitoring water quality in some areas.

1. Introduction

Hydrazine, a highly reactive base and a strong reducing agent, has been used as an important reactant in the preparation of pharmaceuticals, pesticides, photography chemicals, emulsifiers, and dyes in various chemical industries [1–3]. However, it is highly toxic and readily absorbed by oral, dermal, or inhalation routes of exposure, and long-term studies with laboratory animals indicate that hydrazine is mutagenic and carcinogenic [4]. Exposure to hydrazine at high levels (10 ppb, threshold limit value) can induce irritation of nose, temporary blindness, pulmonary edema, DNA damage, and even severe damage of the central nervous system [5–7]. In addition, contamination of rural drinking water supplies with hydrazine by livestock waste, organic wastes, and chemical fertilizers continues to be a problem. It has been a major concern throughout the world for several decades. Due to its widespread applications and toxic effects on humans, developing reliable and sensitive analytical methods for the selective detection of hydrazine is highly desirable.

So far, hydrazine can be routinely analyzed by a wide variety of techniques, such as gas chromatography [8], high performance liquid chromatography (HPLC) [9], ion chromatography [10], chemiluminescence (CL) [11], and electrochemical detection using a variety of chemically modified electrodes has also frequently been used [12]. Although these methods have high sensitivity, many of them are time-consuming and labor-intensive due to the complex pretreatment, require expensive instrumentation and high cost of personnel, and are not readily adaptable to on-site, detection. Undoubtedly, it is of great significance to develop the simple, on-site and sensitive method for hydrazine detection.

In recent years, colorimetric methods have attracted much attention due to its low cost, simplicity, and practicality. Since color changes can be read out by the naked eye, colorimetric sensor does not require expensive or sophisticated

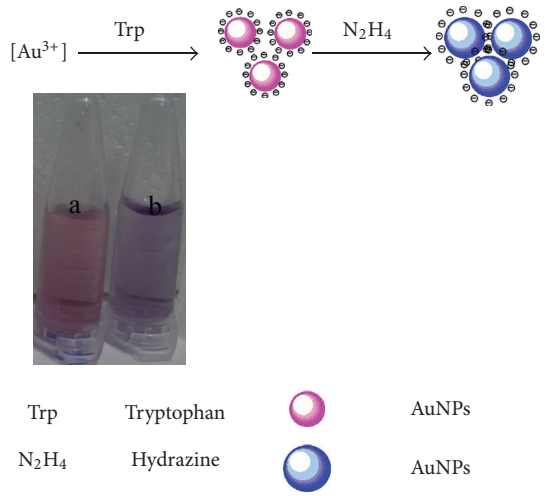


Figure 1: The schematic representation of the analytical process for detecting hydrazine. Insert: colorimetric visualization of the Trp-AuNPs generated in the absence of (a) and in the presence of hydrazine (b 2.0×10^{-4}) in pH 5.0 PBS.

instrumentation and may be applied to field analysis and point-of-care diagnosis. And these methods have shown great advantages over conventional assays, particularly in sensitivity, selectivity, and practicality.

For transformation of the detection events into color changes, a number of materials have been developed, including metal nanoparticles [13, 14], carbon nanotubes [15], grapheme oxide [16, 17], and conjugated polymers [18]. Among many materials, gold nanoparticle (AuNPs) has been regarded as the most promising candidate owing to their unique optical properties. Thus remarkable progress has been made on the design of AuNPs-based colorimetric biosensors owing to their intrinsically strong surface plasmon resonance (SPR) absorptions, with extremely high extinction coefficients, in the visible wavelength range. This color change effect is the result of the coupling of the SPR between particles in close proximity. Systems based on analyte induced aggregation of AuNPs have been employed for the colorimetric detection of heavy metal ions [19–21], melamine [22], acetamiprid [23], glucose [24], nitrate and nitrite [25], and some biological substances [26, 27].

These reported colorimetric assays generally require three steps: synthesis, modification of nanoparticles, and detection of targets. The aim of the former two steps is to fabricate a sensitive colorimetric probe for targets. And the functionalization of AuNPs is vital for triggering the colorimetric response of analytes, which also can be achieved during the synthesis of monodispersed AuNPs. Herein, we proposed a novel method to realize colorimetric detection of hydrazine based on tryptophan capped gold nanoparticles (Trp-AuNPs). In our study, there is only one step in the whole formation of Trp functionalized AuNPs without seeds. In this strategy, tryptophan served not only as a reductant but also a modifier that was capped AuNPs to avoid AuNPs aggregating autonomously to control the size of AuNPs. The colorimetric detection hydrazine was realized based on the visual color changing and ultraviolet visible (UV-Vis) absorption. In addition, the proposed method was successfully used for determination of the hydrazine in various environmental water samples. To the best of our knowledge, this is the first report to use this method for detecting hydrazine based on Trp caped AuNPs.

2. Experimental

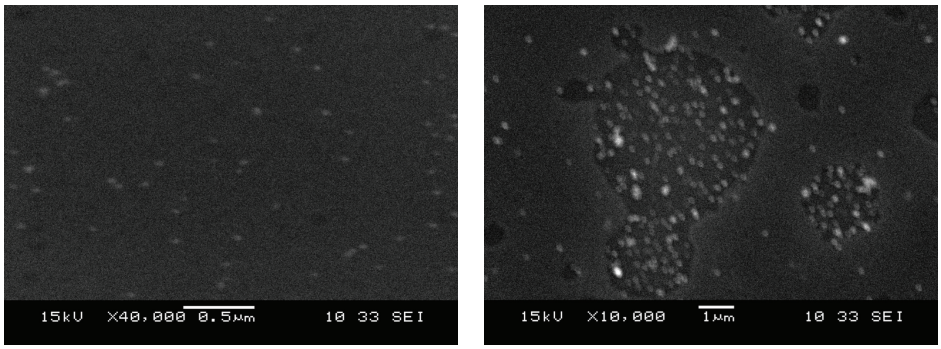
2.1. Chemical Reagents. Chloroauric acid ($HAuCl_4$) and hydrazine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tryptophan (Trp) was purchased from Shanghai Biochemical Reagents Co. (Shanghai, China). Sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other chemicals were of analytical reagent grade and used without further purification. And all stock solutions were prepared daily with double-deionized water obtained from a Milli-Q water purification system (Bedford, MA, USA).

2.2. Apparatus. UV-Vis spectra were obtained on a Lambda 950 spectrophotometer (Perkin Elmer, USA) at room temperature. The sample was thoroughly mixed with a shaker vortex (IKA Genius 3, Germany). The scanning electron microscope (SEM, JSM-6360LA, JEOL Ltd.) was also used to observe the aggregation and size distribution of Trp-AuNPs. A PHS-3CA precision pH meter (Dapu, China) was used in the experiment. The photo images of reaction solutions were recorded using a Coolpix 5400 digital color camera (Nikon, Tokyo, Japan).

2.3. Procedures. All glassware used in the following procedure were cleaned in a bath of freshly prepared aqua regia, rinsed thoroughly in double-deionized water, and dried in air prior to use. Before detecting hydrazine, 1.2 mL premixed solution in 1.5 mL centrifuge tube containing 600 μL 2.0×10^{-2} M phosphate buffer solution (PBS, pH 5.0), 200 μL 2.0×10^{-3} M $HAuCl_4$ and 400 μL 1×10^{-2} M Trp were mixed for by a shaker vortex and then incubated in a 40°C water bath for 30 min. Successively, 100 μL of diverse concentrations of hydrazine were added to the premixed solution, shaking 20 times at room temperature. And then the relative UV-Vis absorbance spectra and photographs of the reaction solutions were recorded.

3. Results and Discussion

3.1. Principle of the Colorimetric Detection. Figure 1 described the principle of the colorimetric determination of hydrazine. First, Trp was used to reduce $HAuCl_4$ to AuNPs. Due to electronic effect of the amino group of Trp, the formed AuNPs were capped by the Trp and could be monodisperse before the addition of hydrazine. Then, the Trp-AuNPs were unstable and tended to agglomerate gradually with addition of hydrazine, which is contributed to the increasing of inter-particles aggregation [28]. Meanwhile, the



(a) (b)

Figure 2: The SEM images of in the absence of (a) and in the presence of hydrazine (b). Condition: 1.2 mL premixed solution containing 1.0 $\times 10^{-2}$ M PBS (pH 5.0), 3.1×10^{-4} M $HAuCl_4$, 3.1×10^{-3} M Trp were mixed for by a shaker vortex and incubated in a 40°C water bath for 30 min. Successively, 2.0×10^{-4} of hydrazine were added to the premixed solution, shaking 20 times.

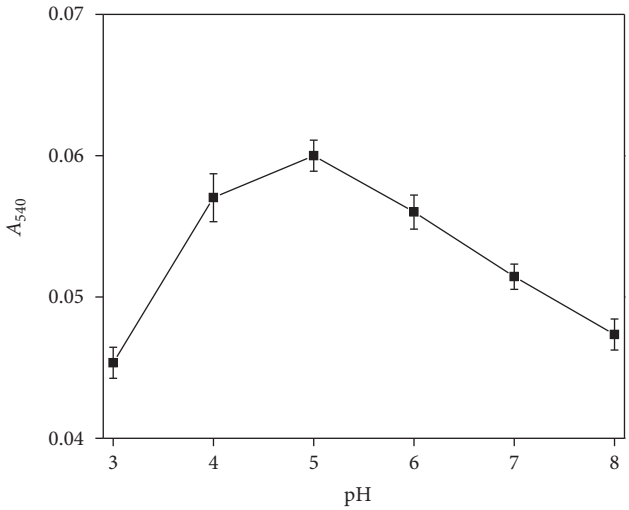


Figure 3: The effect of pH. For conditions see Figure 2. Influence of the conditions in the addition of 7.57×10^{-5} M hydrazine.

color of reaction solution was changed from light purple red to purple (showed in the inset of Figure 1), which is result to the absorbance of reaction solution around 540 nm increased. Therefore, the concentration of hydrazine could be quantified by the absorption of 540 nm (A_{540}). In addition, a hydrazine molecule contained two amino groups, and Trp also had one amino group which meant that two such units could easily form extended arrays of hydrogen bonding (NH⋯O and NH⋯N) which was particularly useful for sensing [29, 30]. Moreover, SEM was used to further verify the detection mechanism in Figure 2. Compared with monodisperse AuNPs (A), the interparticle distance of AuNPs decreased and resulted in obvious aggregations (B).

3.2. Optimum Conditions. In order to obtain the optimal condition, several impact factors were optimized. As well known, color change effect was the result of the coupling effect on the strong surface plasmon resonance (SPR) between particles in close proximity [31]. Thus we tested the pH of the reaction solution, the concentration of Trp, the temperature of the premixed solution (the premixed temperature) and the time of premixed solution (the premixed time).

3.2.1. Influence of pH. The pH not only influenced the interaction between Trp-AuNPs and hydrazine, but also affected the stability of Trp-AuNPs. To investigate the effect of pH on the detection sensitivity, the reaction solutions at diverse pH values (3.0 to 7.0 in intervals of 1.0) were tested. Figure 3 shows that the relative UV-Vis absorption spectra in the presence of different values of pH (3.0 to 7.0) of 1.0×10^{-2} M PBS. The best sensitivity was obtained at pH 5.0. This was probably due to the fact that the hydrogen-bonding was quite weak in strong acidic solution. However, the ionization of Trp was adverse to the stability of Trp-AuNPs when pH was higher than 5. Therefore, pH 5.0 was chosen for further experiments.

3.2.2. Influence of the Concentration of Trp. The selection of Trp both as a reductant and a modifier was due to its strong reducing capacity and amino group. The concentration of Trp was very important to the size and modification of Trp-AuNPs, while the size of the Trp-AuNPs could affect the color and absorbance. Therefore, the concentration of Trp played a dominant role in the detection. Various concentrations of Trp (from 0 to 4.6×10^{-3} M) were studied on the relevant optical absorption change. Figure 4 showed the UV-Vis absorption spectra in the presence of different concentrations of Trp (0 to 4.6×10^{-3} M) in 1×10^{-2} M PBS (pH 5.0). The experimental results showed that compared with others, 2.3×10^{-3} M Trp was more sensitive in the detection. Thus, 2.3×10^{-3} M Trp was selected for further experiments.

3.2.3. Influence of Operational Temperature. The operational temperature included the premixed temperature and binding temperature. Before adding the hydrazine, the Trp and $HAuCl_4$ should be well mixed. Therefore, the premixed temperature was a key factor for the reaction between them.

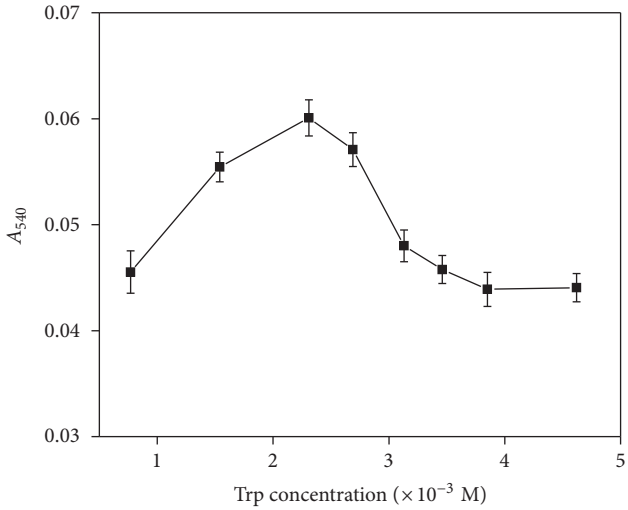


Figure 4: The effect of Trp concentration. For conditions see Figure 3.

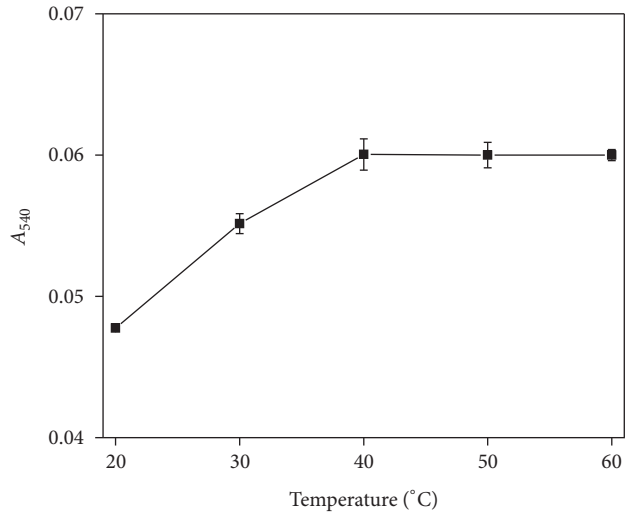


Figure 5: The effect of the premixed temperature. For conditions see Figure 3.

In particular, the mixture temperature also influenced the detection sensitivity. Thus, we tested in the range of 20–60°C at intervals of 10°C. As shown in Figure 5, the absorption was the highest at 40°C. The possible reasons were as follows: first, the high temperature was beneficial to the formation and modification of Trp-AuNPs. Second, the high temperature could induce self-aggregation of Trp-AuNPs, which was adverse to the assay [32]. Therefore, 40°C was chosen as the premixed temperature for all experiments.

When hydrazine added, the binding temperature affected hydrogen-bonding reaction. However, the self-aggregation of Trp-AuNPs attributing to high temperature should be avoided because the aggregation should be induced by hydrogen-bonding. Taking into account operational convenience, the room temperature was chosen as the binding temperature.

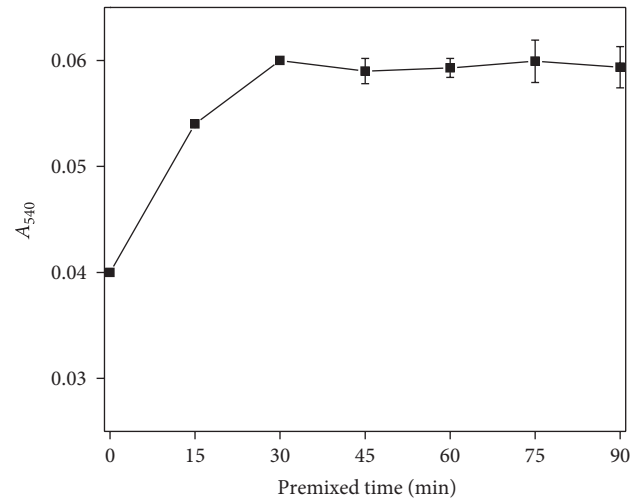


Figure 6: The effect of the premixed time. For conditions see Figure 3.

Table 1: The comparison of the LOD between different methods.

Sample	Methods	LOD ($\times 10^{-6}$ M)	Reference
Hydrazine	HPLC	62.5	[9]
Hydrazine	Chemiluminescence	0.5	[11]
Hydrazine	Kinetic spectrophotometric	3.1	[33]
Hydrazine	Cyclic voltammetry	11	[34]
Hydrazine	Amperometric assay	8.5	[35]
Hydrazine	Colorimetry	1.0	Current work

3.2.4. Influence of Premixed Time. The premixed time also impacted the detection which was an inconvenient factor. To obtain the optimum condition, the mixture time was investigated in the range of 0–90 min at intervals of 15 min. As shown in Figure 6, the value of A_{540} sharpy increased at first 30 min and was kept in the same level after 30 min. Therefore, 30 min was selected as the perfect premixed time.

3.3. Colorimetric Detection of Hydrazine

3.3.1. Sensitivity. Quantitative analysis was realized by the absorption of 540 nm (A_{540}). The corresponding UV-Vis absorption spectra were recorded. A linear correlation existed between the absorption ratio A_{540} and the hydrazine concentration in the range from 7.57×10^{-6} to 2.01×10^{-3} M (shown in Figure 7). The regression equation was $Y = (1.359 \pm 0.03982) \times 10^{-4} + (0.05166 \pm 0.0035)\ C$ ($n = 6$) with a correlation coefficient (r) of 0.9957, where Y was the absorption at 540 nm and C was hydrazine concentration ($\times 10^{-6}$ M). The limit of detection (LOD) calculated as 3 times the standard deviation for the blank solution was 1.0×10^{-6} M, which was significantly lower than the maximum hydrazine concentration allowed for water sources in China

Table 2: Application of the proposed method to determination of hydrazine in real water samples spiked with different amounts of hydrazine.

Sample	Concentration of hydrazine hydrate ($\times 10^{-6}$ M)		Recovery (%)
	Amount added	Amount found[a]	
Tap water	75.7	75.7 ± 3.32	100
	757	764 ± 35.54	101
Lake water	75.7	77.7 ± 4.76	103
	757	780 ± 46.83	103
Sea water	75.7	81.7 ± 6.42	108
	757	785 ± 50.88	104

[a]Average value of three determinations ± standard deviation.

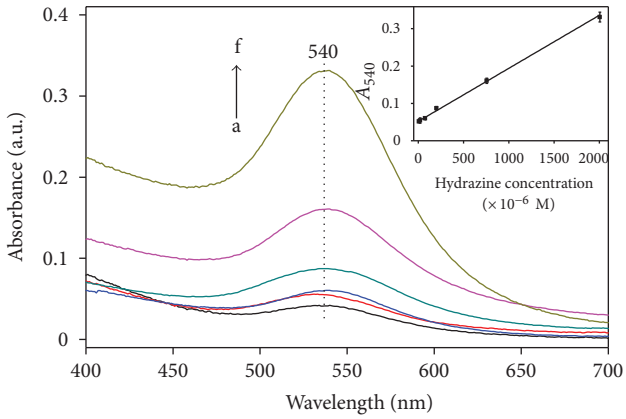


Figure 7: The relative UV-Vis absorbance spectra of reaction solution in the presence of different concentrations of hydrazine (the hydrazine concentration from a to f: 7.57×10^{-6}, 2.01×10^{-5}, 7.57×10^{-5}, 2.01×10^{-4}, 7.57×10^{-4}, and 2.01×10^{-3} M) under the optimum conditions. Insert: the liner correlation between the absorption ratio A_{540} and the hydrazine concentration.

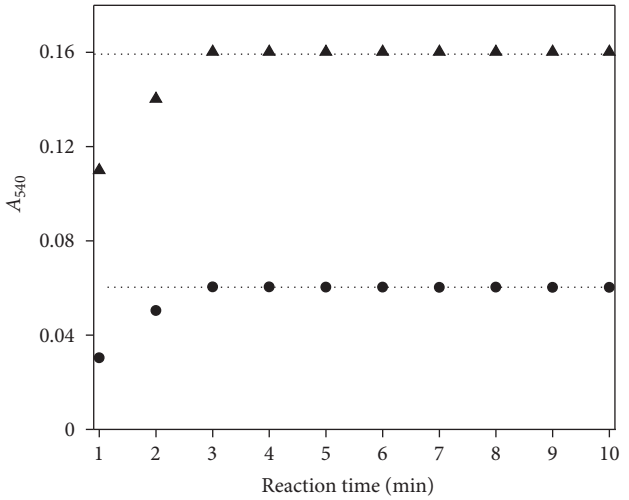


Figure 8: The reaction time in two different hydrazine concentration levels (a: 7.57×10^{-5}, b: 7.57×10^{-4}) under the optimum conditions.

(6.25×10^{-6} M, Chinese National Standard GB 18061-2000). As shown in Table 1, the comparison of the LOD in different methods indicated that our study provided a better LOD than most of them. Moreover, our work provides a simpler method without any sophisticated instruments and complicated experiment operations.

What is more, to verify the rapidity and reliability of the proposed method, the relative UV-Vis spectra of the reaction solution were recorded at a regular interval of one minute in 10 min after adding two different concentration of hydrazine. The reaction time-dependent response curves were shown in Figure 8. The absorption ratio kept constant after 3 min, indicating that the detection was extremely speedy and complete in three minute.

3.3.2. Selectivity. Various substances were most likely to be present in environmental water samples, such as metal ions, amino acids, and organics which could interfere with the determination of hydrazine. Therefore, the interferences in the 20 times concentration of hydrazine (7.57×10^{-5}) were used to carry out the experiment to investigate the selectivity of the proposed method. The values of A_{540} were shown in Figure 9. Hydrazine obtained the largest absorption of A_{540}, indicating that the determination of hydrazine had enough tolerable limit to the interferences.

3.4. Applications. In addition, we tested its colorimetric response to tap water samples, lake water samples, sea water samples, respectively, in order to evaluate the reliability of the proposed method. Samples of tap water were from our laboratory without any additional pretreatment. Lake water samples were from the artificial lake in our campus, and sea water samples were obtained from the shore of Shantou. The samples of lake water and sea water were diluted with double distilled water (1 : 1, v/v). These real water samples were supplemented with two different concentrations of hydrazine, and the collected data of analytical recoveries and RSDs were shown in Table 2.

4. Conclusions

Based on the experimental results above, hydrazine could be detected with this simple, rapid, direct, and sensitive method, and it was suitable for routine analysis. The concentration of hydrazine in water samples can be determined by monitoring

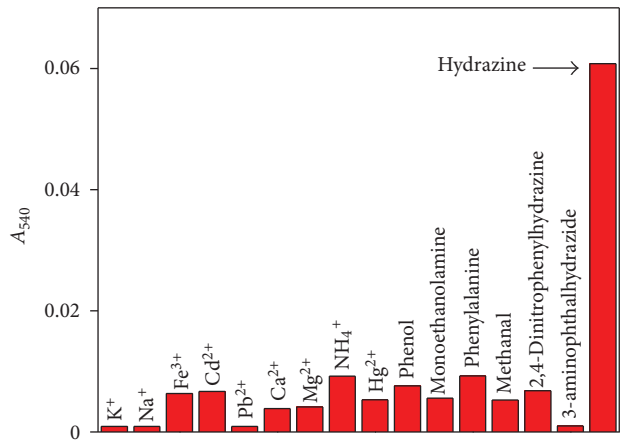


FIGURE 9: Absorption of reaction solution at 540 nm with the addition of hydrazine (7.57×10^{-5} M) or other interferences under the optimum conditions.

with the naked eye or a UV-Vis spectrometer. The method showed relatively good selectivity for hydrazine over other hydrazinium with the lowest detection concentration of 1 μM. Especially, the merits made the proposed method specially useful for on-site screening hydrazine levels well below the current safety limit in drinking water.

Acknowledgments

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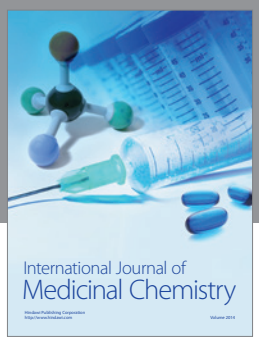

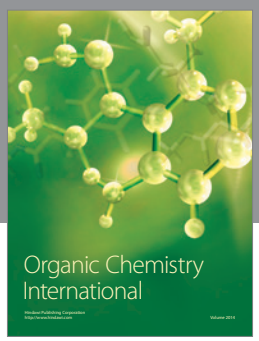

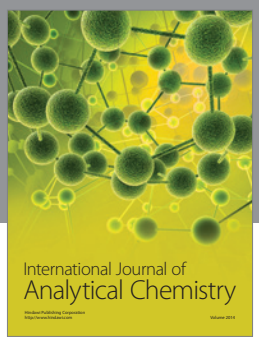

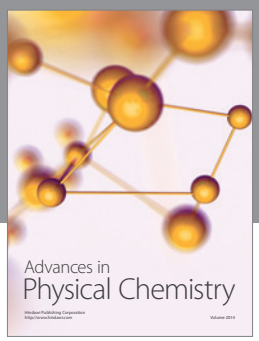

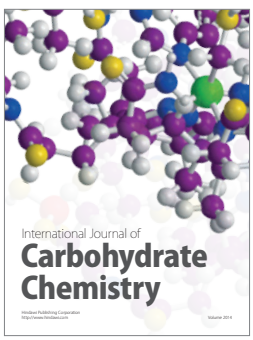

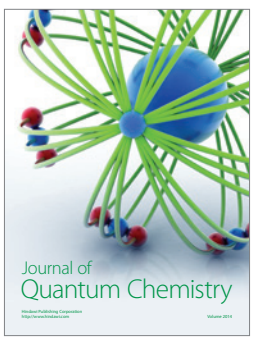

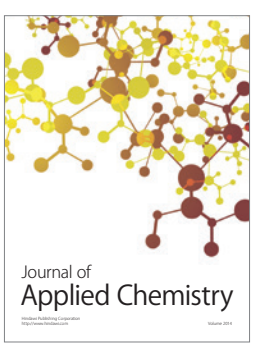

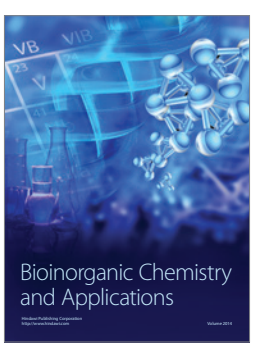

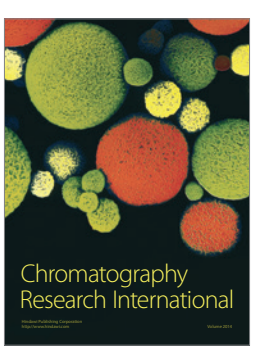

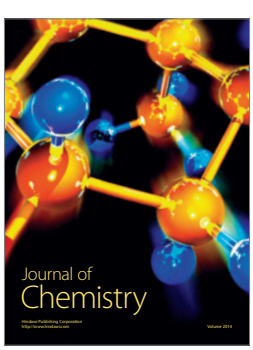

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