Highly sensitive colorimetric determination of malathion using gold nanoparticles

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ABSTRACT

A highly sensitive method is presented for the colorimetric determination of malathion using gold nanoparticles (AuNPs). In this approach, the synthesized AuNPs solution was stabilized by the citrate anions as their repulsion protected the AuNPs from aggregation. The synthesized AuNPs were characterized morphologically by using transmission electron microscopy technique. Malathion caps the surface of AuNPs and induces the aggregation of AuNPs in Britton–Robinson buffer solution. The reaction was monitored spectrophotometrically by measuring the decrease in the plasmon resonance band of the AuNPs at 527 nm after 9 min. The effect of reaction variables on the reaction sensitivity was investigated and furthermore, the interference of common ions was effectively avoided. The calibration curve is linear over the concentration range $3.3 \times 10^{-7}$ to $3.3 \times 10^{-6}$ mol/L of malathion with good precision and accuracy and the detection limit was down to $1.5 \times 10^{-7}$ mol/L. The developed approach does not use complex and expensive instruments. The high sensitivity of the proposed method allowed its successful application to wheat and water samples. Thus, the proposed strategy can serve as a powerful method for the rapid diagnosis of malathion in agriculture products.

Key words | colorimetry, gold nanoparticles, malathion, plasmon resonance, spectrophotometry

INTRODUCTION

Pesticides are used on a large scale for agricultural purposes. Organophosphorus pesticides generally act as cholinesterase inhibitors and are used for the control of a broad range of pests on cotton, rice, tobacco, sorghum, sugarcane, and vegetables to improve the crop productivity (Xin et al. 2012). However, their extensive use also gives rise to pesticide residue on the plant, which has become a serious public health problem because of their toxicity and potential carcinogenicity in the food chain (Kwong 2002; Tang et al. 2014). Malathion, diethyl(dimethoxythiophosphorylthio)succinate, is a non-systemic, wide-spectrum organophosphorus insecticide. It is employed for the control of insects on fruits and vegetables, and is also used to control mosquitoes, flies, household insects, animal parasites (ectoparasites), and head and body lice (Quintás et al. 2004a, 2004b). It acts on the nervous system of the insects, by disrupting the function of neurons by interaction with the sodium channel (Tomlin 2000).

In recent years, increasing attention has been given to the development of reliable, fast and inexpensive analytical systems to monitor pesticides from environmental samples. In the last few years, many methods have been developed for the determination of malathion. The most widely used methods for its detection and determination are high performance liquid chromatography (Khuhawar et al. 1996; Abu-Qare & Abou-Donia 2001; Botero-Coy et al. 2012; Deme et al. 2015), capillary electrophoresis (García-Ruiz et al. 2005), ion mobility spectrometry (Jafari 2006), gas chromatography (Xin et al. 2012; Tang et al. 2014), Fourier transform infrared spectrometry (Quintás et al. 2004a, 2004b), solid-phase extraction (Baldim et al. 2012), micro-solid phase extraction (Bagheri et al. 2011), hollow-fiber
membrane extraction (Modin et al. 2008), voltammetry (Raghu et al. 2014; Gangadhar Reddy et al. 2014), luminescence (Azab et al. 2015), spectrophotometry (Pandey et al. 2014) and FT–Raman spectrometry (Quintás et al. 2004a, 2004b). However, these methods suffer from some limitations, such as long chromatography run times, low sensitivity and consumption of large sample volumes, and derivatization and time-consuming sample pretreatment stages prior to experiments. Among all these analytical techniques, the colorimetric method has received considerable attention owing to its simplicity, improved sensitivity and high selectivity. Moreover, the detection signal can be viewed by the naked eye (Xue et al. 2011) and this is a good advantage.

Nanoparticles have been the focus of research for many decades as a result of their intriguing optical properties (Sun & Xia 2005). Plasmonic nanoparticles, such as gold nanoparticles (AuNPs) and silver nanoparticles are a class of nanostructures whose optical properties are determined by their unique surface plasmon resonances (SPR) (Kelly et al. 2003). SPR is the resonant oscillation of conduction electrons at the interface between a negative and positive permittivity material stimulated by incident light. The resonance condition is established when the frequency of incident photons matches the natural frequency of surface electrons oscillating against the restoring force of positive nuclei. Thus, this process is resonant at a particular frequency of the light and termed SPR (Jain et al. 2008). This phenomenon arises from the interaction of the oscillating electromagnetic field of the light with the free electrons of the metal NPs. Upon this interaction, free electrons of NPs undergo a collective coherent oscillation with respect to the positive metallic lattice. As a result, the metal NPs display bright intense colors and corresponding specific extinction bands in their UV–vis spectra. Herein, we develop a rapid and sensitive approach for the colorimetric detection of malathion using AuNPs. The surface plasmon band of the generated AuNPs enabled the quantitative analysis of the malathion concentration. Malathion can combine with the AuNPs through an Au–S covalent bond. This phenomenon leads to the neutralization of nanoparticle surface charges and the loss of surface charge induced aggregation of AuNPs (Leesuttiphonchai et al. 2011).

**EXPERIMENTAL**

**Materials**

All the reagents of analytical grade were used without further purification. Doubly distilled water was used throughout. A $1.0 \times 10^{-3}$ mol/L solution of malathion (Aldrich, Milwaukee, USA) was prepared by dissolving the appropriate amount of malathion in water. All chemical reagents were purchased from Merck (Merck, Darmstadt, Germany). The wheat samples were harvested from a field in Ilam city. One of them was bread with the scientific name *Triticum aestivum* and type Chamran, and the other one was durum with the scientific name *Triticum durum* and type Yavarous. Some of the river water was randomly selected from Seimare river and Meymeh river in Ilam city as a real sample. The water of these rivers was colorless and tasteless. Preparation of AuNPs was performed as described previously (Roushani & Shahdost-fard 2015). Briefly, 500 mL tetrachlorauric acid (0.01% (w/v), H AuCl$_4$) was heated with stirring to reach boiling point. Quickly 7.5 mL of a 1% solution of sodium citrate (Na$_3$C$_6$H$_5$O$_7$·2H$_2$O) was added to this solution. After 25 s, the color of the solution was turned blue and finally after 70 s, the color was changed to red-violet. Boiling continued for an additional 10 min; the heating source was removed when the solution had turned deep red, and the colloid was stirred for another 15 min. A transparent red homogenous colloidal solution of AuNPs was obtained without any precipitate. The morphology of AuNPs was characterized by transmission electron microscopy (TEM) imaging of the solution. Britton–Robinson (BR) buffer solutions were used for fixing pH in the range of 5.0–10.0. These solutions were prepared using boric acid, o-phosphoric acid, acetic acid and sodium hydroxide.

**Apparatus**

Absorbance spectra were recorded using a double beam UV–vis spectrophotometer Perkin Elmer model 25 equipped with 30 mm quartz cell. The pH values were adjusted employing a Metrohm model 780 using a combined glass electrode. All glassware and storage bottles were
soaked in 10% HNO₃ overnight and thoroughly rinsed with deionized water prior to use.

**Colorimetric malathion measurements**

1.0 mL AuNP and 0.5 mL BR buffer were transferred into a 30 mm quartz cell. Then appropriate amounts of malathion (3.3 × 10⁻⁷ to 3.3 × 10⁻⁶) were transferred and the resulting mixtures were allowed to react for 9 min. The absorbance of this reaction was labeled as As and measured at 527 nm. The same procedure was repeated without addition of malathion to get the blank signal and the signal was labeled as Ab. The calibration graph was constructed by plotting ΔA = Ab – As vs. malathion concentration.

**Sample preparation and determination**

**Determination of malathion in wheat**

To investigate the performance of the developed method, some wheat seeds were planted in a sterilized environment and were irrigated with distilled water for 2 weeks. When the seeds grew and reached a length of several centimetres, the concentration of 10⁻⁶ mol/L malathion was prepared and sprayed on the wheat plant samples and kept for 24 h. After this time, the samples were harvested, collected and minced in a mortar. Afterwards, the resulting powder was rinsed with ethanol and filtered several times with ethanol. The obtained sample was collected and evaporated to dryness and the residue was dissolved in 0.1% acetic acid (Pandey et al. 2014). Finally, an aliquot of the obtained powder as a real sample was selected and treated under the developed method for measurement of malathion. This method was similar for both types of wheat.

**Determination of malathion in water**

The river water samples were collected, filtered and centrifuged at 2,000 rpm for 5 min.

The pHs of the samples were then adjusted by BR buffer to 7.0 and the samples were stored in a cool place and analyzed using the explained procedure.

**RESULTS AND DISCUSSION**

In our experiment, AuNPs were formed by the direct reduction of HAuCl₄ using sodium citrate at a certain pH and ambient temperature. The prepared AuNPs solution was stable and highly dispersed. The TEM images of AuNPs were obtained, as shown in Figure 1. Clearly, AuNPs were highly dispersed in the aqueous solution, with the size estimated as 12 nm (Figure 1(a)). The principle of malathion sensing and detection is based on aggregation of AuNPs in the presence of malathion. As can be seen in Figure 1(b), the AuNPs are aggregated after exposure to malathion and the shape of them is changed. The presence of malathion during AuNPs formation has effective influence on the plasmon resonance absorbance. This means that with concentration of malathion increasing, intensity of the plasmon resonance absorbance is decreased. On the other hand, the coordination of malathion on the surface of AuNPs through its sulfur atoms is inducing their aggregation (Kappi et al. 2014). The characteristic absorption peak of AuNPs in UV-vis spectroscopy is about 527 nm (Figure 2).

![Figure 1](https://iwaponline.com/ws/article-pdf/16/5/1214/411276/ws016051214.pdf)
As can be seen, when different concentrations of malathion were added, the absorption maxima at 527 nm decreased gradually. Therefore, by measuring the decrease in absorbance of AuNPs for 9 min from initiation of the reaction, the malathion contents in the sample can be measured. The effect of reaction variables such as reaction media, pH and time is studied by changing each variable in turn while keeping all others constant. Based on our investigations, each variable was studied and the optimum value of the variables was selected. The explanation of the optimal experimental conditions is presented as follows.

**Influence of reaction medium and pH**

Preliminary investigation showed that the inhibition effect can be observed in the reaction media, thus some efforts were made for choosing the best type of reaction media. So, the dependence of AuNPs aggregation on pH was investigated in the absence and in the presence of malathion. The effect of several buffers such as acetate, Britton–Robinson, phosphate, carbonate and ammonia buffer were tested. Based on the obtained experimental results, it could be seen that in the BR buffer, the ΔA value was strongly increased and reached its maximum. This means that the change maximum of the ΔA value is in the BR buffer as a medium. Therefore, the BR buffer was selected as an optimum reaction medium in all of the experiments.

In order to evaluate the effect of solution pH on aggregation of AuNPs, BR buffers were used for pH adjustment in the range of 5.0 to 10.0 pH unit (Figure 3). It can be seen that the optimum pH is 7.0 and at higher or lower pH a marked decrease in the ΔA values is observed. At low pH (pH < 7.0) protonated malathion could not displace sodium citrate located on the surface of the AuNPs. Meanwhile, the surface charges of the AuNPs cannot be partly neutralized and malathion cannot induce the agglomeration of the AuNPs (Qu et al. 2012). In addition, at relatively high pH (pH > 7.0), the AuNPs might partially agglomerate themselves and could not interact with malathion as well. Therefore, pH 7.0 was chosen as the optimum condition.

**Effect of time**

The reaction time is a key point that affects the aggregation of AuNPs. Absorption spectra of the reaction mixture were recorded at different times (Figure 4). It can be seen that the ΔA increased gradually from 3 to 9 min and kept steady from 9 to 30 min. This demonstrated that the aggregation of AuNPs was almost completed within 20 min. Thus, 9 min reaction time was chosen as the optimum.
Calibration graph and reproducibility

Using the optimum experimental conditions described above, the calibration graph (Figure 5) was linear in the range of $3.3 \times 10^{-7}$ to $3.3 \times 10^{-6}$ mol/L and is described by the equation:

$$\Delta A = 0.1008 \, C_{\text{malathion}} + 0.1379 \quad (R^2 = 0.9918; \, n = 6)$$

where $\Delta A$ is the absorbance ($\Delta A = A_b - A_s$), $C_{\text{malathion}}$ is the malathion concentration (mol/L), $R^2$ is the square correlation coefficient and $n$ represents the number of determinations. The limit of detection (LOD) was calculated according to the recommended formula by the International Union of Pure and Applied Chemistry as

$$\text{LOD} = 3 S_d/K$$

where $S_d$ is the standard deviation of the blank measurements and $K$ is the slope of the calibration curve. For the method reported here the LOD was $1.5 \times 10^{-7}$ mol/L on the basis of 10 blank measurements. Ten successive measurements of $3.3 \times 10^{-7}$ and $1.0 \times 10^{-6}$ mol/L malathion showed relative standard deviations of 3.11%, 2.69%, respectively. As we know, the standard deviation ($s$) is a statistical measure of the precision for a series of repetitive measurements. The advantage of using $s$ to quote uncertainty in a result is that it has the same units as the experimental data. Since the relative standard deviations of these measurements is low, we can conclude that the obtained values indicate that the proposed strategy is viable.

Selectivity study

The complexity of real samples presents a great challenge to the analytical methods for malathion detection, not only in the detection limit and sensitivity but, more importantly, in selectivity. The effects of various interfering species, which may accompany malathion in wheat and water samples, were studied, using $1.0 \times 10^{-6}$ mol/L malathion. The maximum tolerable concentrations of foreign species are shown in Table 1, where the tolerance limit was defined as the concentration of foreign species that produce a change in $\Delta A$ less than 5%. It was found that many of these ions did not interfere, even when present in excess of 10–1,000 fold. It is clear that the developed method has high selectivity toward malathion.

Real samples analysis

To evaluate the validity of the proposed method for real sample analysis, the proposed procedure was applied to real samples. As demonstrated above, the present colorimetric method for malathion detection with high selectivity

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of interfering species on determination of $1.0 \times 10^{-6}$ mol/L of malathion</th>
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<tbody>
<tr>
<td>Ions</td>
<td>Tolerance ratio ($w_{\text{ion}}/w_{\text{malathion}}$)</td>
</tr>
<tr>
<td>CO$_3$$^2$ $^-$, F$^-$, Cl$^-$, Br$^-$, K$^+$, Na$^+$, Mg$^{2+}$</td>
<td>1,000</td>
</tr>
<tr>
<td>Ba$^{2+}$, Zn$^{2+}$, Cu$^{2+}$</td>
<td>100</td>
</tr>
<tr>
<td>Al$^{3+}$, Fe$^{2+}$, Pb$^{2+}$</td>
<td>50</td>
</tr>
<tr>
<td>I$^-$, Fe$^{3+}$</td>
<td>10</td>
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</tbody>
</table>

| Table 2 | Determination of malathion in wheat and water samples ($n = 3$) |
|----------|-----------------|-----------------|-----------------|
| Sample   | Amount added (μmol/L) | Amount found (μmol/L) | RSD (%) | Recovery (%) |
| Bread wheat | 0.00 | 0.17 ± 0.03 | 3.21 | – |
| Durum wheat | 0.00 | 0.12 ± 0.04 | 2.68 | – |
| Seimare River water | 0.00 | Not found | 2.84 | – |
| Meymeh River water | 0.00 | 0.08 ± 0.03 | 2.84 | – |

Figure 5 | Calibration graph for the determination of malathion at optimized conditions.
provides a direct platform for assaying malathion in wheat and water samples. The malathion contents of wheat and water samples were determined by standard addition method using the developed procedure under optimum conditions. The results in Table 2 show that the method is accurate and gives good recoveries of added malathion.

**CONCLUSION**

A new approach for malathion detection is described based on a fast colorimetric method utilizing the AuNPs. In our research, it was found that the plasmon resonance intensity of AuNPs at 527 nm was significantly decreased in the presence of malathion. Based on this, a sensitive and simple approach was developed for the trace detection of malathion. So that, increasing the malathion concentration decreased the plasmon absorbance without any shift in the λmax, allowing for dimethoate measurement. Advantages such as simple instrumentation, good reproducibility and quick detection of malathion with high sensitivity has been achieved with this method. The method does not require any separation or pre-concentration steps and is directly applied to the determination of malathion ion in various real samples (such as wheat and water). The results show that the method is accurate and gives good recoveries of added malathion.

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**REFERENCES**


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