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Colorimetric determination of thiram based on formation of gold nanoparticles using ascorbic acid

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ABSTRACT

A novel optical method for the determination of thiram has been developed using surface plasmon resonance peak of gold nanoparticles (AuNPs). The stable and dispersed AuNPs were directly synthesized by reduction of $HAuCl_4$ with ascorbic acid in micellar media according to a simple approach. The presence of thiram during formation of AuNPs results in the decrease of the intensity of plasmon resonance peak. The variation in the plasmon absorbance allows the colorimetric determination of thiram. The effect of different variables such as pH, ascorbic acid and CTAB concentrations was studied and optimized. The proposed method is capable of determining thiram over a range of $2.0\times10^{-7}-1.0\times10^{-5}$ mol L^{-1} with a limit of detection 1.7×10^{-7} mol L^{-1}. The relative standard deviation of the method was $<3.7\%$. The method was successfully applied to the determination of thiram in water and plant seed samples.

1. Introduction

In recent years, nanoscience and nanotechnology have attracted worldwide research interests which have become a rapidly expanding area in different branches of science [1]. In this field, the marvelous advances of metals nanoparticles have established numerous fundamental studies and applications in a various scientific research. Many of these applications are inspired by the unique optical properties of metal nanostructures, which derive from the localized surface plasmon resonance (LSPR), a collective oscillation of the conduction electrons that (for spheres) typically occurs in the visible to near-UV region of the spectrum [2,3]. Therefore, the preparation and characterization of these particles with nanometer-sized dimensions have become an important aspect of materials research [4].

Due to the considerable chemical and physical properties, gold nanoparticles (AuNPs) have attracted much attention as an advantageous platform for highly sensitive colorimetric detection of target analyte. One of the most interesting properties of AuNPs is their strong surface plasmon resonance (SPR) absorption in the visible wavelength range which depends on their size, shape and surrounding medium [5–7].

Recently, colorimetric sensing in aqueous solution using plasmon resonance band of AuNPs has been developed for sensitive and selective detection of various species such as fluoride ion [8], amino acids [9], Ag^+ [10], dopamine [11] and Hg^{2+} [12]. In many reported methods, AuNPs were prepared before use and then utilized to detection systems, such as citrate reduction procedure.

The appropriate synthetic methods using different reducing agents have been reported for preparation of AuNPs [13–15]. Furthermore, the previous studies revealed that the AuNPs could be enlarged in a solution containing $HAuCl_4$ in the presence of some active molecules such as H_2O_2 [16], flavonoids [17], cholesterol along with cholesterol oxidase [18] nicotinamide adenine dinucleotide [19] and glucose together with glucose oxidase [20]. These researches have been extensively used in design of different kinds of biosensors for detection of such active molecules.

However, the application of ascorbic acid as a reducing reagent for formation of AuNPs has many advantageous including its water solubility, biodegradability and low toxicity. Up to our knowledge, there is no report of colorimetric detection methods using AuNPs formed by ascorbic acid in aqueous solutions. Thus according to capabilities of ascorbic acid, it can be applied as reducer for developing fast, eco-friendly and simple detection methods based on plasmon resonance band of AuNPs.

Thiram (tetramethylthiuram disulfide) is a dimethyl dithiocarbamate compound (Fig. 1) which belongs to the group of *N*,*N*-dialkyldithiocarbamate pesticides [21]. Thiram has long been known as a fungicide for preservation of fruits, vegetables, ornamentals and turf crops from deterioration in storage or transport and it has been widely applied for the seed treatment of small grains. Thiram is also used in rubber industry as a vulcanization accelerator, in the treatment of human scabies

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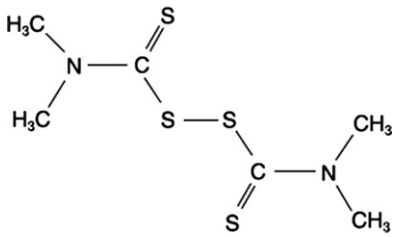

Fig. 1. Chemical structure of thiram (tetramethylthiuram disulfide).

and as a bactericide into soaps. Although the usability of this dithiocarbamate compound is undisputable, but its increasing use, results in being released into the environment leading to contamination of food and water which finally lead to hazardous effect in the living organisms through diverse pathways. The toxicity of thiram to the liver is well recognized due to the formation of carbon disulfide from the breakdown of thiram in the body [22,23].

Therefore, the use of thiram in different area requires the control of the concentration of this compound in underground and surface waters, soils and agricultural products.

In this study, a colorimetric assay method for the determination of thiram has been developed. The method is based on reduction of $HAuCl_4$ to AuNPs by ascorbic acid in the presence of thiram and CTAB. The quantitative analysis of thiram was achieved using plasmon resonance absorption peak of AuNPs.

2. Experimental

2.1. Apparatus

A GBC UV–vis spectrophotometer model Cintra 101 (Australia) was used for recording the spectra, and the absorbance measurements were made using a Perkin Elmer UV–vis spectrophotometer model 550S by 1 cm glass cells. Measurement of pH was performed using a Metrohm 632 (Switzerland) pH-meter with a combined glass electrode. Transmission electron microscopy (TEM) image of AuNPs was recorded by a Zeiss Em10C instrument (Germany) operated at 80 kV.

2.2. Reagents and solutions

All reagents were of analytical grade and double distilled water was used throughout the experiments.

A 1.0×10^{-2} mol L^{-1} of thiram stock solution was prepared by dissolving 0.121 g of thiram (Merck) in ethanol and diluting to 50 mL in a volumetric flask. Working solutions were prepared by adequate dilution of the stock solution. A hydrogen tetrachloroaurate(III) (chloroauric acid) stock solution, 1.8×10^{-2} mol L^{-1}, was prepared by dissolving 0.350 g $HAuCl_4\cdot3\,H_2O$ (Merck) in deionized water and diluting to 50 mL. An ascorbic acid solution, 1.0×10^{-2} mol L^{-1}, was prepared by dissolving 0.088 g of ascorbic acid (Merck) in water and diluting to 50 mL in a volumetric flask. This solution was kept in a dark cold place and working solutions were prepared daily. A 0.10 mol L^{-1} solution of CTAB (Cetyltrimethylammonium bromide) was prepared by dissolving 3.644 g of CTAB (Merck) in water and diluting to 100 mL. Britton–Robinson buffers [24] in the pH range of 2–13 were used for adjusting pH of solutions.

2.3. Analytical procedure

Under optimum condition, the appropriate amounts of Britton–Robinson buffer solution at pH 11.5, CTAB, $HAuCl_4$, thiram as analyte and ascorbic acid were added to a 10 mL volumetric flask, respectively. Then the solution was diluted to mark immediately and mixed slowly. After 6 min, the absorbance was measured at 527 nm which is λ_{max} of surface plasmon resonance peak of AuNPs. A blank solution was also run under the same procedure.

2.4. Preparation of plant seed samples

3.0 g of the plant seed sample (tomato, cucumber or watermelon) preserved by thiram, was weighed and placed into a 100 mL beaker, then 30 mL of ethanol was added, covered by a lid and stirred about 5 h. The solution was then filtered and diluted to 50 mL in a volumetric flask. An aliquot of the above solution was treated under the general procedure for determination of thiram.

3. Results and discussion

In this paper, AuNPs were formed by the direct reduction of $HAuCl_4$ using ascorbic acid in the presence of CTAB as stabilizer, at a certain pH and ambient temperature. The microscopic characterization of the prepared AuNPs showed that by the control of the experimental conditions, it was possible to synthesize highly dispersed AuNPs with an average size of 10 nm, as illustrated in the TEM image in Fig. 2. On the other hand, the plasmon absorption spectrum of AuNPs exhibits only a single peak at 527 nm. The presence of thiram during AuNPs formation has effective influence on the plasmon resonance absorbance which leads to decrease in its intensity. This effect can be due to interaction of sulfur atoms of thiram with gold.

The UV–vis spectra of the AuNPs plasmon in the absence and presence of different concentrations of thiram are shown in Fig. 3. As can be seen in the figure the absorbance at maximum wavelength, 527 nm, decreased with increasing of thiram concentration. Thus, the difference in plasmon absorbance of AuNPs in the absence and presence of thiram at 527 nm, ΔA, was used as analytical parameter for determination of this pesticide. The influence of different variables on ΔA was investigated and optimized.

3.1. Effect of pH

The formation and stability of AuNPs depends strongly on the pH of the solution. Therefore the effect of pH on the plasmon

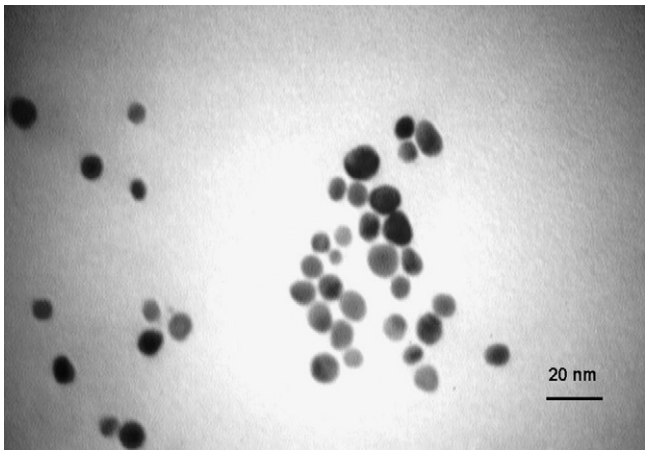

Fig. 2. TEM image of the obtained AuNPs. *Conditions:* $HAuCl_4$ concentration: 1.8×10^{-4} mol L^{-1}; CTAB concentration: 1.1×10^{-2} mol L^{-1}; Ascorbic acid concentration: 8.0×10^{-4} mol L^{-1}; pH=11.5.

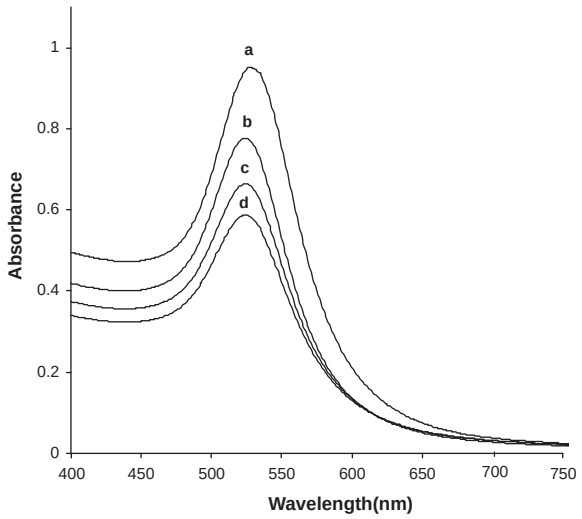

Fig. 3. UV–visible absorbance spectra of obtained AuNPs (a) in the absence and (b)–(d) in the presence of 1.0×10^{-6}, 7.0×10^{-6} and 1.0×10^{-5} mol L^{-1} of thiram, respectively. *Conditions:* $HAuCl_4$ concentration: 1.8×10^{-4} mol L^{-1}; CTAB concentration: 1.1×10^{-2} mol L^{-1}; Ascorbic acid concentration: 8.0×10^{-4} mol L^{-1}; pH=11.5.

resonance peak of AuNPs in the absence and presence of thiram was studied over the range of 2–13. The pH was adjusted by the addition of Britton–Robinson buffer solutions. The results indicated that the AuNPs were not formed at pH range of 2 to 6 and the solution was colorless. This could be due to protonation of ascorbic acid at low pH and consequently decreasing its reducing power [25].

At pH 9 to 10, a broad plasmone resonance absorption peak with low intensity appeared. However the stable and dispersed AuNPs with intensified plasmon absorbance bands was observed at pH 11–13. The ΔA was measured in this pH range and a maximum value of ΔA was obtained at pH range of 11–12. Thus, the pH 11.5 was used throughout the experiment.

3.2. Effect of CTAB concentration

In order to obtain stable and dispersed AuNPs with a distinctive particle size, it is important to use a capping agent. For this purpose, the utilization of surface-active agents (surfactants) as stabilizing agents and/or capping agents effectively influence the synthesis of AuNPs [26]. Cetyltrimethylammonium bromide (CTAB) is known as a structure-directing molecule with conferring a positive charge to the nano particles which is most commonly employed as capping agent [27]. For that reason, the effect of CTAB concentration in the range of 0.2×10^{-2}–1.4×10^{-2} mol L^{-1} on AuNPs plasmon peak and ΔA as analytical parameter was investigated. At concentration values less than 0.2×10^{-2} mol L^{-1} of CTAB, the stable and dispersed AuNPs were not formed and the solution became dark. The results revealed that the maximum ΔA was obtained in the concentration range of 1.0×10^{-2}–1.2×10^{-2} mol L^{-1} CTAB. Therefore, the concentration of 1.1×10^{-2} mol L^{-1} was chosen for further studies.

3.3. Effect of ascorbic acid concentration

The influence of ascorbic acid concentration as reducing agent on formation of AuNPs and consequently on ΔA was studied. The obtained results showed that the plasmon resonance peak of AuNPs appeared at ascorbic acid concentration higher than 2.0×10^{-4} mol L^{-1}. Also, the results indicated that the ΔA increased by increasing of ascorbic acid concentration up to 6.0×10^{-4} mol L^{-1} and above this value it became nearly constant. Thus, 8.0×10^{-4} mol L^{-1} was chosen as optimum concentration of ascorbic acid.

3.4. Effect of time

The plasmon absorbance changes of AuNPs were recorded versus time at 527 nm in the absence and presence of different concentrations of thiram using selected experimental conditions. The obtained results denoted that the reduction of $HAuCl_4$ was nearly complete within the first few minutes of the reaction. However, the intensity of the surface plasmon resonance increased slowly with time up to 5 min., which indicated the continued reduction of the $HAuCl_4$.

Since the ΔA reaches a constant value within 6 min the absorbance was measured after this time throughout the study.

3.5. Analytical figures of merit

Under the optimum conditions calibration graph was constructed by plotting ΔA values as a function of the thiram concentration. The calibration graph was linear in the range of 2.0×10^{-7}–1.0×10^{-5} mol L^{-1} (0.048–2.404 μg mL^{-1}) of thiram with regression equation $\Delta A=2.550\times10^{4}$ C (mol L^{-1})+0.038 and correlation coefficient (r) of 0.9990. The detection limit, based on three times the standard deviation of blank ($n=8$) was found to be 1.7×10^{-7} mol L^{-1} (0.041 μg mL^{-1}).

The relative standard deviations (RSD) resulted from the analysis of eight replicates measurements of solutions containing 8.0×10^{-7} and 9.0×10^{-6} mol L^{-1} thiram were 3.6 and 1.2%, respectively.

3.6. Effect of divers ions

A study of the effect of various ions on the determination of 0.240 μg mL^{-1} (1.0×10^{-6} mol L^{-1}) of thiram was examined under the optimum working conditions. The tolerance limit was defined as the maximum concentration of potentially interfering ions causing ±5% error in the determination of thiram. As it is obvious from Table 1, most of the anions do not interfere even at high concentrations, except I^-. The interference of Ca^{2+} and Mg^{2+} up to 1000 times, Cu^{2+}, Cd^{2+} and Zn^{2+} up to 100 times was eliminated using EDTA. The main interfering species in the determination of thiram were Hg^{2+} and Ag^+ which their interference up to 10 times was eliminated by using EDTA and Cl^-, respectively.

3.7. Analytical application

The application and validation of the proposed method was verified by employing the method to water and plant seed samples. Three water samples were chosen from tap water (Ahvaz, Iran), Maroon and Kheyrabad rivers (Khuzestan Province, Iran). However, in these water samples, the thiram was not detected according to the optimized procedure. These samples were spiked with standard solution of thiram and determined by the suggested method. The results reported in Table 2 show that

Table 1
Effect of interfering ions on determination of 0.240 μg mL^{-1} of thiram.

Ions	Tolerance ratio (w/w)
CO_3^{2-}, NO_3^-, NO_2^-, SO_4^{2-}, F^-, Cl^-, Br^-, K^+, Ca^{2+}, Mg^{2+}	1000
Ba^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}	100
Mn^{2+}, Co^{2+}, Al^{3+}, Fe^{2+}, Pb^{2+}, Cr^{3+}, Ni^{2+}	50
I^-, Fe^{3+}	20
Hg^{2+}, Ag^+	10

Table 2
Determination of thiram in water samples.

Sample	Amount of thiram (mol L^{-1})		Recovery
	Added	Found[a]	(%)
River water (Maroon)	–	N.D[b]	–
	4.00×10^{-7}	$4.21(\pm0.16)\times10^{-7}$	105
	6.00×10^{-7}	$6.00(\pm0.20)\times10^{-7}$	100
	8.00×10^{-7}	$7.78(\pm0.24)\times10^{-7}$	97
River water (Kheyrabad)	–	N.D	–
	4.00×10^{-7}	$3.98(\pm0.15)\times10^{-7}$	99
	6.00×10^{-7}	$5.78(\pm0.20)\times10^{-7}$	96
	8.00×10^{-7}	$7.78(\pm0.22)\times10^{-7}$	97
Tap water	–	N.D	–
	4.00×10^{-7}	$4.00(\pm0.17)\times10^{-7}$	100
	6.00×10^{-7}	$6.00(\pm0.21)\times10^{-7}$	100
	8.00×10^{-7}	$8.22(\pm0.25)\times10^{-7}$	103

[a] Mean ± standard deviation ($n=4$).
[b] Not detected.

Table 3
Determination of thiram in plant seed samples by proposed method and comparison with HPLC.

Seed Sample	Thiram found (mg g^{-1})[a]		
	Proposed method	HPLC method	Relative error (%)
Tomato	0.055 ± 0.001	0.056 ± 0.001	−1.78
Cucumber	1.100 ± 0.019	1.059 ± 0.020	3.87
Water melon	0.767 ± 0.011	0.790 ± 0.010	−2.91

[a] Mean ± standard deviation ($n=3$).

good recoveries are obtained for the determination of thiram in spiked samples. In addition, the thiram existing in three plant seed samples preserved by this fungicide was determined using the present method. For evaluating the accuracy of this work, a comparison between results obtained by proposed method and HPLC [28] was performed. As can be seen in Table 3, the results were in good agreement with those obtained from HPLC. There is no significant difference between the obtained results of both methods by the performing t-test at 95% confidence level.

4. Conclusions

In this paper, a new optical method for the sensitive spectrophotometric detection of thiram based on surface plasmon resonance absorption peak of AuNPs was reported. The stable and dispersed AuNPs were prepared using a simple, rapid and eco-friendly procedure by applying ascorbic acid and CTAB. The presence of thiram decreases plasmon intensity of AuNPs, and the absorbance changing at λ_{max}, 527 nm, was used for determination of thiram. In the proposed method the AuNPs are formed in situ by using ascorbic acid which has the advantage of saving time. This is different from conventional AuNPs-based methods such as citrate reduction procedure that AuNPs are prepared before use and then applied to the detection of analyte. The present approach can be used for the determination of thiram in the range of 2.0×10^{-7}–1.0×10^{-5} mol L^{-1} with a limit of detection 1.7×10^{-7} mol L^{-1} (0.041 μg mL^{-1}). The proposed method was successfully applied for the determination of thiram in water and plant seed samples.

A comparison of the proposed method with reported techniques in the literature for determination of thiram is shown in Table 4. As can be seen, the limit of detection of this work is better than reported techniques based on spectrophotometric detection [32–34] and some methods which require more complex analytical instrumentations [21,30,31]. In addition, the present work has a simple and fast procedure in comparison with the other reported methods for determination of thiram.

Table 4
Comparison of the proposed method with some of the previously reported methods for the determination of thiram.

Method	Linear range (μg mL^{-1})	LOD (μg mL^{-1})	RSD (%)	Ref.
Voltammetry	0.24–144.26	0.10	1.6	[21]
Chemiluminescence	0.0075–2.5000	0.0075	2.5	[22]
SPME[a]-HPLC	0.005–0.600	0.001	2.4–2.8	[28]
Voltammetry	0.048–2.4	0.013	2-5.8	[29]
HPLC-ECD[b]	1–10	0.14	3.5	[30]
Capillary electrophoresis	0.5–240	0.5	2.8	[31]
Prec.[c]–spectrophotometry	Up to 20	0.161	–	[32]
Derivative spectrophotometry	0–24	0.3	1.9	[33]
SPE-spectrophotometry	0.5–25	0.33	–	[34]
FI-chemiluminescence	0.01–1.0	0.005	1.0–2.2	[35]
AuNPs-SPR	0.048–2.404	0.041	1.2–3.6	This work

[a] Solid phase microextraction.
[b] Electrochemical detector.
[c] Preconcentration.

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