

Application of the localized surface plasmon resonance of silver nanoparticles for the determination of ethion in water samples

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Abstract: Identification and measurement of pesticides in water is one of the significant public benefits of protecting humans from the effects of water pollution. In this study, a new method was presented for the determination of ethion (ET) as an organophosphate insecticide in water samples based on the use of localized surface plasmon resonance (LSPR) of citrate-capped silver nanoparticles (CC-Ag NPs). The LSPR band was scanned by UV-Visible spectrometer and transmission electron microscopy (TEM) was used to show the interaction, surface features and particle size. By placing CC-Ag NPs in the vicinity of ET, it was observed that the pollutant was adsorbed by silver nanoparticles in aqueous solution. As a result of the adsorption process, aggregation of silver nanoparticles was observed. On the other hand, it was also observed that the ET concentration had a direct effect on the color of the mixture from light yellow to brown. As a result of the aggregation of silver nanoparticles, the adsorption peak in the range of 393 nm was reduced and a new adsorption peak was observed in the wavelength of 540 nm. The absorbance ratio at the wavelength of 540 to 393 nm (A_{540}/A_{393}) was used as the analytical signal. The shift in wavelength and the change in adsorption intensity were the expression of the characteristics of the structure and concentration of the pesticide, respectively. The interaction between the sensor and the pesticide was considered to be the result of the binding of the soft metal surface with the soft sulfur atom of the pesticide. Factors affecting adsorption efficiency were investigated including pH, ionic strength, reaction time and sodium citrate concentration. The proposed method had good repeatability, reproducibility and stability. Finally, the applicability of the proposed method for real sample analysis was examined, which was consistent with the obtained experimental results.

Keywords: Ethion insecticide; colorimetric sensor; silver nanoparticles; localized surface plasmon resonance.

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1. Introduction

Organophosphate pesticides (OPPs) are the most widely used among the pesticides (~ 36%) used in agriculture, horticulture and household [1]. One of the first reports on the synthesis of OPPs dates back to the 1930s when Gerhard Schrader synthesized parathion as one of the OPP's used even today [2]. Structurally, the organophosphates are esters of phosphoric acid containing central phosphorus atom, a characteristic

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thiophosphoric (P=S) or phosphoric (P=O) bond, and a replaceable leaving group (X), through which nucleophilic substitution takes place [3]. Based on the literature, the global consumption of OPPs has increased significantly between 2011 and 2015 (from 5 to 8.6 million tons) [4].

However, the unreasonable use of OPPs has led to the severe pollution of environmental resources along with the production of OPPs residues and by-products beyond the permissible limit. Therefore, it is inevitable that OPPs residues enter the food chain and its negative effects on humans and living organisms. For example, organophosphates such as ethion inhibit the enzyme acetyl choline esterase (AChE), which mediates the breakdown of acetylcholine in the central and peripheral nervous systems [5].

As a member of the organophosphorus family, ethion is a non-systemic insecticide used to control leaf feeding insects, scales, mites and other crops. Figure S1 shows the schematic of the chemical structure of ethion. Its characteristics include insolubility in water and solubility in most organic solvents. Also, its boiling point is in the range of 165°C [6].

Among the capabilities of ethion, we can mention inhibition of AChE enzyme. Also, poisoning by OPPs leads to a decrease in the activity of AChE enzyme in nicotinic, muscarinic and central nervous system (CNS) receptors. Among the acute cholinergic symptoms of poisoning with OPPs, we can mention miosis, sweating, urination, bronchospasm, lacrimation, gastrointestinal upset, fasciculation's, hypertension, muscle weakness, bradycardia, and allergy [7-9]. With the occurrence of cholinergic problems, the disorder in the nervous system continues for 24 to 96 h, and the poisoned person will have symptoms such as paralysis of the proximal limbs, cranial nerves, respiratory muscles, and face [10].

OPPs usually have a short decomposition life. However, they have the ability to remain in the soil, the environment, and leached into groundwater, which limits their use and increases the importance of their identification and measurement. Various methods have been developed to detect OPPs, including gas chromatography (GC), mass spectrometry (MS), high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), HPLC tandem mass spectrometry (HPLC-MS-MS), immunoassay (ELISA), enzymatic inhibition (EM), and electrochemical sensing [11]. In addition to special application capabilities, the described methods are expensive instrumentation and require trained personnel [12]. Therefore, the development of fast, inexpensive, and portable methods for pesticides sensing has always been an essential requirement.

LSPR is a surface phenomenon in the nano scale that has been much attention in recent years. In this phenomenon, nanoparticles create strong adsorption spectra that are used to detect many chemical and biological samples. When a noble metal in the nanoscale is exposed to irradiation of light, the free electrons collectively oscillate at a resonant frequency in a phenomenon called LSPR, which results in sharp adsorption spectra [13]. Optical properties, especially plasmonic properties of noble metal nanostructures are used in the development of a new class of optical sensor in modern chemistry.

Metal nanoparticles (MNPs) are show intense bright colors and corresponding specific extinction bands in their UV-Vis spectrum. Dispersing metal nanoparticles in an aqueous solution leads to the creation of different colored solutions. It is well known that the color change of the solution is proportional to the size of the dispersed particles. For example, when the surface electrons of silver nanoparticles (Ag NPs), surface resonance bands of noble MNPs, are exposed to light radiation or electromagnetic radiation and absorb it, the color of the solution appears yellow [14]. The color changes of a solution in the presence of analyte can be investigated by spectrometry and used as an analytical signal for the detection of chemical species. Meanwhile, Au NPs are ideal candidates by providing stability and chemical inertness, however, the high price is the main limiting factor for their use. On the other hand, Ag NPs have become more popular among researchers due to their higher extinction penetration coefficient and lower price [15, 16]. In this regard, in several literatures, LSPR of Ag NPs have been studied for the determination of some pesticides, toxins, drugs and important biological compounds [17-20].

In this study, a sensitive and fast method was presented for the determination of ET in water using LSPR of silver nanoparticles. Adding ET to the solution containing citrate-capped silver nanoparticles (CC-Ag NPs) led to a change in the color of the solution from light yellow to brown. UV-Visible absorption spectroscopy was used to measure the adsorption spectrum of CC-Ag NPs solution before and after adding ET. The change in the wavelength of the plasmonic peak was investigated as a characteristic of structure and concentration of the ET. Thus, the aim of this work is to develop a simple and low-cost method to detect and

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determine the ET in water by silver nanoparticles with real-time identification with the naked eye and without pre-treatment of the sample. Affecting factors the assay were investigated and optimized, including reaction time, ionic strength, pH, and stabilizing agent concentration. The parameters affecting the interaction between plasmonic Ag NPs and ET insecticide pesticide were investigated. Finally, the real water sample was examined.

2. Experimental

2.1. Chemicals and Reagents

All reagents and solvents were of analytical grade. Silver nitrate (AgNO_3 99.9999%), sodium borohydride (NaBH_4 $\geq 99\%$), sodium citrate dehydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ $\geq 99\%$), sodium chloride (NaCl $\geq 99\%$), sodium hydroxide (NaOH $\geq 98\%$), hydrochloric acid (HCl 37%), Ethanol ($\text{C}_2\text{H}_5\text{OH}$ $\geq 99.8\%$), ET ($\text{C}_9\text{H}_{22}\text{O}_4\text{P}_2\text{S}_4$, analytical standard, quality level:100) were obtained from the Sigma Aldrich company and used without further purifications. MilliQ water was used in the synthesis process and solution preparation after deoxygenating by N_2 gas. Stock solution of ET was prepared in ethanol and standard solutions of ET with different concentrations were prepared by successive dilution of the stock solution.

2.2. Synthesis of CC-Ag NPs

Silver nanoparticles were synthesized by the reduction of AgNO_3 with NaBH_4 . Under vigorous magnetic stirring, 0.012 g NaBH_4 was added quickly to 100 mL aqueous solution of AgNO_3 with concentration of 0.1 mmol.L^{-1} , producing a light yellow colored solution. The colloidal solutions of Ag NPs were covered with aluminum foil and kept under 4°C [21]. A standard solution of 1000 mg.L^{-1} was prepared from ET insecticide pesticide. CC-Ag NPs were prepared following a procedure mentioned above and yellow color was achieved (Figure 2.a). Then defined volumes (3 to $120 \mu\text{L}$) of ET solution were added to 2.50 mL aliquot of CC-Ag NPs solution and after adjustment of pH and ionic strength, final volume reached to 5 mL. UV-Visible spectrometry was used to characterize the changes in the LSPR band with the addition of different concentrations of the pesticide.

2.3. Apparatus

Synthesized Ag nanoparticles were characterized using UV-Visible absorbance spectroscopy and TEM. Absorption spectra were recorded on an Agilent 8453 UV-Visible spectrophotometer. TEM images were acquired using Philips EM208 operating at 80 kV accelerating voltage. All pH measurements were made by using a Metrohm 744 pH-meter equipped with a combined pH glass electrode.

3. Results and discussion

3.1. Aggregation of Ag NPs

The characteristic LSPR band of Ag NPs was measured around at 393 nm (Figure 1. a). The stability of silver nanoparticles was reduced due to the interaction between ET and the surface of the CC-Ag NPs. As a result of this interaction, the color of the solution has changed from light yellow to brown, it was also found that the color of the solution was affected by the concentration of ET. Thus, as a result of the aggregation process, the LSPR peak of silver nanoparticles was reduced around 393 nm and a new characteristic peak was created around 540 nm (Figure 1. b). In other words, the aggregation of NPs has caused a change in the LSPR of plasmonic NPs, so that it could be recognized by the naked eye.

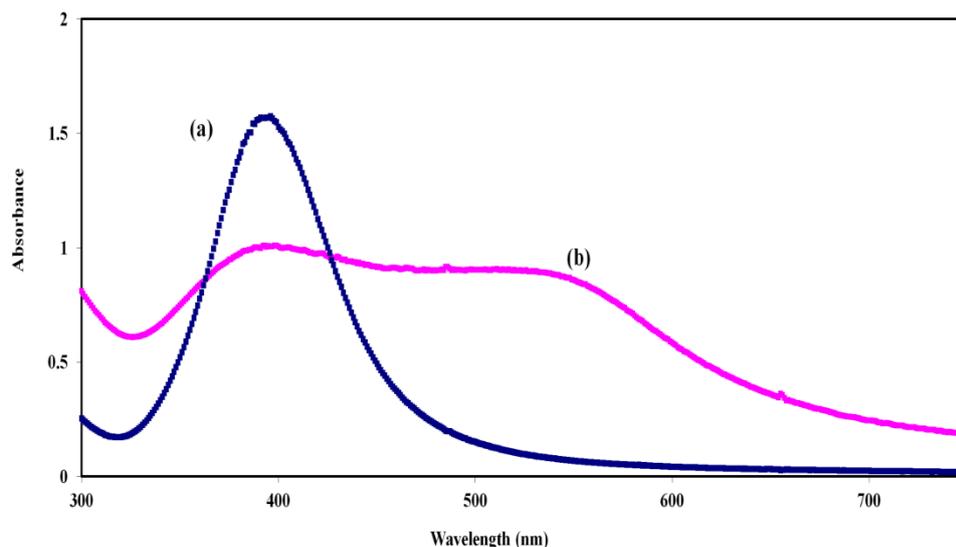


Figure 1. UV-Visible spectrum of the plasmonic nanoparticles and color of silver nanoparticles in the (a) absence and (b) presence of 12 μM ethion

According to the obtained TEM images, it can be stated that silver nanoparticles before and after adding ET to CC-Ag NPs solution have a spherical geometry (Figure 2). As mentioned earlier, the addition of ET caused the aggregation of silver nanoparticles, which can be clearly seen in the TEM image (Figure 2b). According to the figure, the size of the synthesized CC-Ag NPs was measured to be around 10-15 nm.

The sensitivity of ET toward the NPs was found to rely of its structure and can be explained based on hard-soft acid-base (HSAB) theory. In accordance with this theory, soft acids interact more strongly with soft bases while hard acids interact more strongly with hard bases. OP pesticides are esters of phosphoric acids and their derivatives. The general structure of an OP pesticide comprises the central phosphorus (P) atom and the characteristic thiophosphoryl (P=S) or phosphoryl (P=O) group. According to the structure of ET, it can be stated that the surface interaction between silver nanoparticles and ET was caused by silver atoms (as a soft acid) and sulfur atoms of ET (as a soft base). In other words, when ET was added to the Ag NPs solution, it acts as a ligand that binds via S atoms with the NP surface [22, 23].

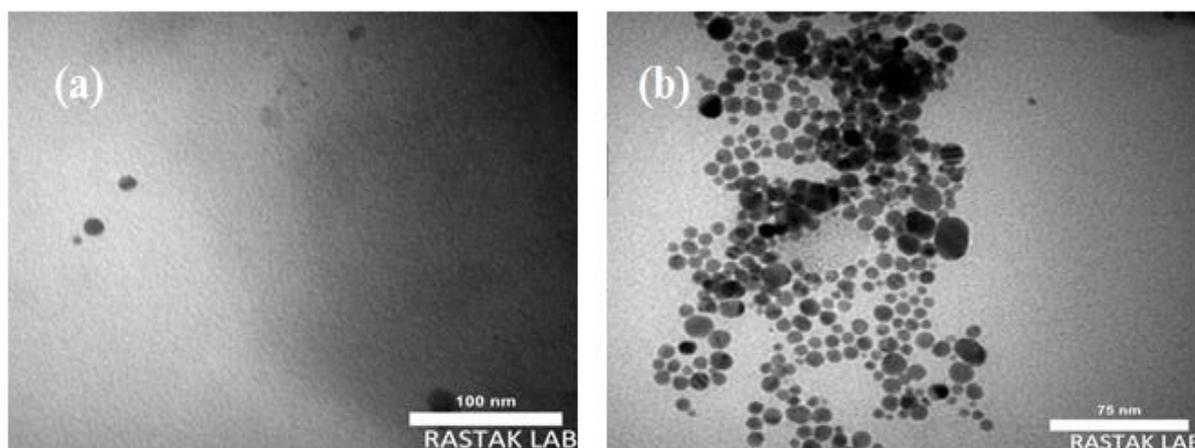


Figure 2. TEM mages of Ag NPs in the (a) absence and (b) presence of 12 μM ethion.

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3.2. Optimization of Assay Conditions

Early experiments have been done to determine optimized conditions in the assay. We have examined the effect of reaction time, ionic strength concentration and pH in the assay on the aggregation of citrate capped silver nanoparticles.

3.2.1 Effect of Contact Time

In this study, nanoparticle stability in the presence of ET was measured by monitoring changes in LSPR using UV-Visible spectrometry. The absorbance ratio of A_{540}/A_{393} was used as the analytical signal [24]. Contact time or reaction time between addition of ET to solution of CC-Ag NPs and absorbance measurement is as critical parameters in the assay that must be optimize to enhance the sensitivity of the method. Figure 3 was shown the changes of the absorbance ratio of CC-Ag NPs colloid after different times over the range of 20-160 seconds. With the start of the experiment, the aggregation of CC-Ag NPs increased rapidly during the first few seconds, and then a slow growth of the aggregation rate was observed until reaching equilibrium. The results showed that signal of plasmon intensity reaches a maximum in 100 s then remain constant. Thus, a 100 s time interval was selected for subsequent studies.

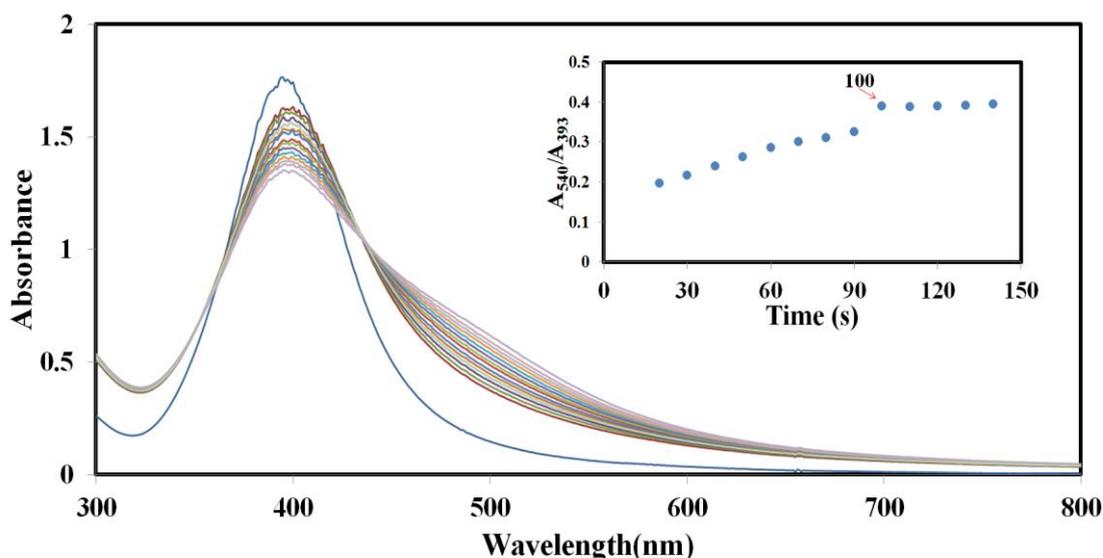


Figure 3. Effect of time on aggregation of silver nanoparticles in the presence 12 μ M ethion, 4 mM NaCl and pH=8

3.2.2. Effect of Ionic Strength

Due to the high surface-to-volume ratio in plasmonic NPs, the LSPR frequency is sensitive to the refractive index of the surrounding medium. Any change in the environment including surface adsorption and desorption of chemical agents, aggregation and the medium refractive index will shift the LSPR frequency and result in a colorimetric change that can be utilized as optical signals for detection. Effect of sodium chloride on aggregation of silver nanoparticles was explored because ionic strength of reaction media has a critical role on aggregation of Ag NPs. Figure 4 shows the changes of the absorbance ratio of the colloidal silver nanoparticles by various concentrations of NaCl over the range of 0-15 mM and under conditions where the concentration of sodium citrate was 20 mM and the concentration of ethion was 12 μ M and the reaction time was 100 s. It has been found with increasing NaCl concentration, the absorbance ratio (A_{540}/A_{393}) was increased, because sodium chloride can constrict the electrical double layer of capping citrate ions [25].

According to the results, 4 mM was selected as optimum electrolyte concentration for subsequent investigations.

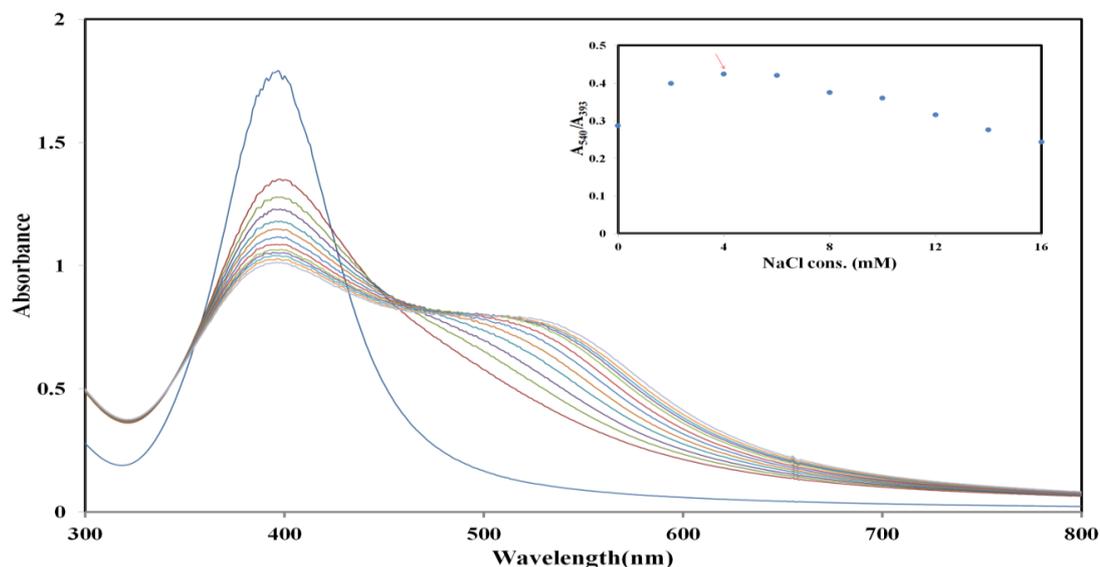


Figure 4. Effect of NaCl concentration on aggregation of silver nanoparticles in the presence 12 μM ethion in pH=8

3.2.3. Effect of pH

The pH of sensor solution is an important parameter affecting on aggregation of silver nanoparticles because of pH dependency of electrostatic interactions at the CC-Ag NPs surface [26]. The effect of pH on the CC-Ag NPs surface was investigated in the range of 3.0-10.0 and in conditions where the concentration of sodium chloride was 4 mM, the concentration of sodium citrate was 45 mM, and the concentration of ethion was 12 μM and the reaction time was 100 seconds. As shown in Figure 5 pH=8.0 was an optimum selection due to high stability of Ag-citrate complex. In $\text{pH} \leq 2.5$, Ag NPs were unstable and with raising pH from 3 to 8, stability of Ag NPs was increased by step by step de-protonation of citric acid. In basic solutions ($\text{pH} \geq 8.0$), citrate becomes more negative and electrostatic repulsion between CC-Ag NPs enhanced and aggregation was lowered [27].

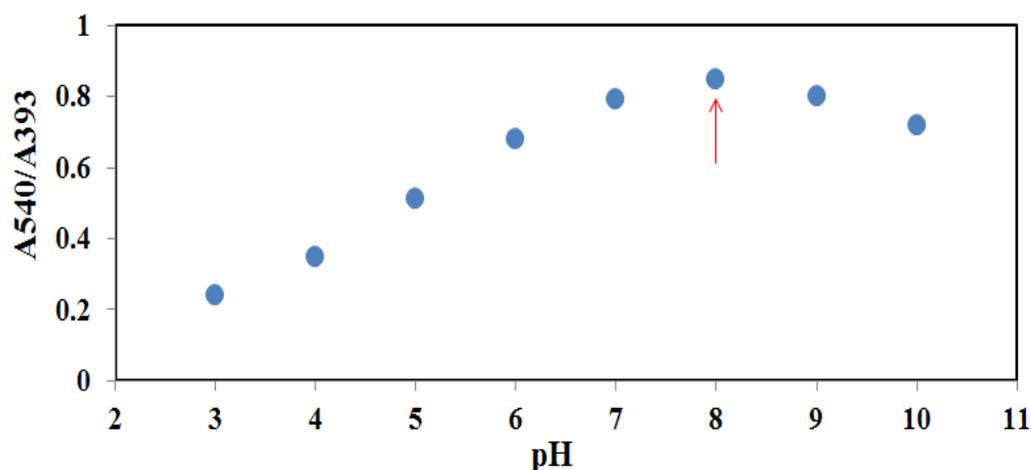


Figure 5. Effect of pH on aggregation of silver nanoparticles in the presence 12 μM diazinon and 4 mM NaCl

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3.2.4. Effect of the Sodium Citrate Concentration

It was investigated the effect of sodium citrate concentration on the plasmon absorbance intensity of the silver nanoparticles. Sodium citrate as the reductant and stabilizer contributes to synthesize and improve the monodisperse of Ag NPs. Besides, citrate ion was adsorbed on the surface of Ag nanoparticle through coordination effect with its two adjacent carboxyl groups etc. Meanwhile, the other carboxyl group extended outward, which made the surface of nanoparticle exhibit negative charges [28]. It was observed that the concentration of added sodium citrate within the range of 15 mM to 65 mM had significant effect on the plasmon absorbance and aggregation of silver nanoparticles. A concentration of 45 mM of sodium citrate was chosen as optimum (Figure 6).

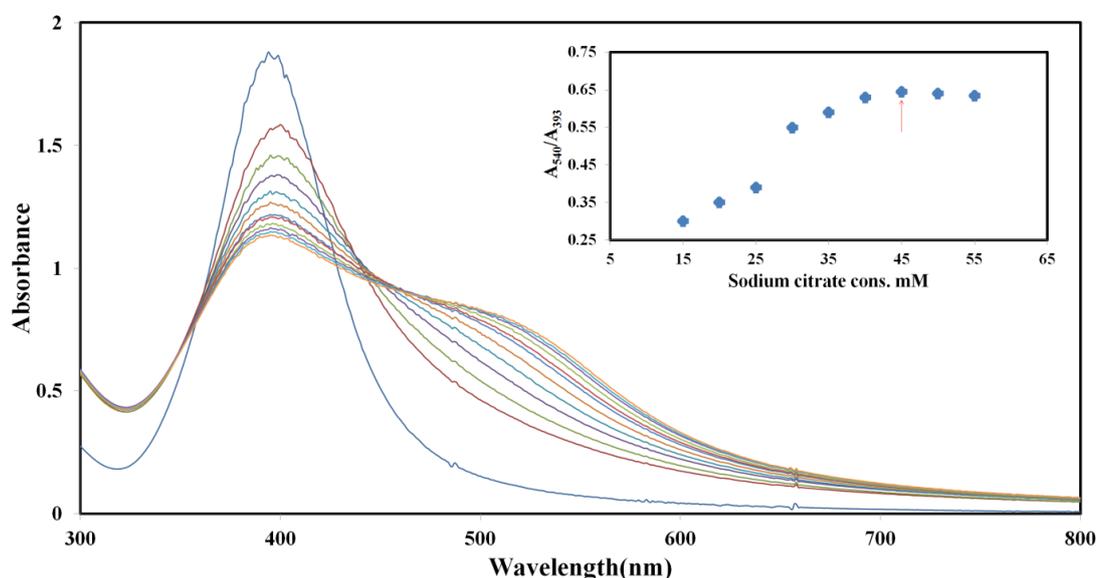


Figure 6. Effect of sodium citrate concentration on aggregation of silver nanoparticles in the presence 12 μM ethion and 4 mM NaCl

3.3. Analytical Figures of Merit

The limit of detection (LOD), limit of quantitation (LOQ), linear range (LDR), reproducibility, sensitivity and selectivity of the method were evaluated under the values optimized condition. The LOD for ET was calculated using the following Eq. 1:

$$\text{LOD} = 3s_b/m \quad (1)$$

where, s_b is the standard deviation of the absorbance ratio signal of blank solution used for the nanoparticle preparation (for ten successive measurements) and m is the slope of the calibration curve with various concentrations of ET [29]. Calibration plot was constructed using ET concentrations and the absorbance ratio (A_{540}/A_{393}) data of sensor (Figure S2a). As the concentration of the ET increases, the absorbance ratio (A_{540}/A_{393}), corresponding to the change in plasmon peak of the Ag NPs was increased. Under the specified experimental conditions, LOD and LOQ obtained for the determination of ET, was 0.06 and 0.2 μM , respectively. Also the calibration curve for ET was linear from 1.0–40.0 μM with a squared correlation coefficient (R^2) of 0.9998 and equation of (A_{540}/A_{393}) = 0.0132[ET] + 0.0262 (Figure S2b). Relative standard deviation for a 12 μM standard solution of ET was achieved 1.5% ($n=10$).

In order to check the selectivity of the proposed method toward ET, it was investigated in pH of 8, reaction time of 100 and the sodium chloride concentration, the sodium citrate concentration and ET

concentration were 4 mM, 45 mM and 50 μ M, respectively. The selectivity of the Ag NP sensor towards ET was evaluated using the unique wavelength shift in Ag NPs after the addition of other OP pesticides. The selectivity of the developed method was evaluated by determining ET in the presence of common OP pesticides including malathion(MT), fenthion(FT), diazinon(DZ) and parathion(PT). The concentration of ET was measured in the absence and in the presence of an equimolar concentration of each interfering pesticide at a concentration of 50 μ M. The relative error in ET content in the presence of interfering toxins was less than 1.5% (selectivity coefficients ≤ 0.015). This selectivity results from the distinct difference in their LSPR wavelength from the 540 nm signal (characteristic LSPR wavelength of ET)[32]. The performance of the developed method was compared with some spectrophotometric methods reported for the determination of ET. Main figures of merit of proposed method are compared with some recent reports in Table 1. As it can be concluded from the data presented in Table 1, the developed method provides comparable results with other previously reported method.

Table 1. Comparison of merit figures of the developed method with other reported spectrophotometric methods for determination of ET

Method	LOD(μ M)	RSD%	LDR(μ M)	Ref
Spectrophotometry	0.02	3.5	0.04-0.13	[30]
Spectrophotometry	0.60	2.0	0.26-2.10	[31]
Colorimetric	23.40	1.2	-	[32]
Localized surface plasmon peak	0.06	1.0	1-40	This work

3.4. Detection of ET in Real Environmental Water Samples

The practical applicability of the synthesized sample was investigated for three water samples collected from tap water, underground water and river water (Karaj city located in Alborz province, Iran). First, the collected water samples were passed through a membrane filter (0.22 μ m) and standard solutions of ET were added to it in different concentrations. Finally, the proposed method along with colorimetric evaluation was used to analyze real samples.

The analytical results were given in Table 2 in comparison with results obtained for the same samples analyzed by HPLC as a reference method [33]. It was obvious that the observed concentration of ET was close to that determined by HPLC and that the CC-Ag NPs detection system can be used for analysis of ET in different water samples drawn from real environments. The results indicate good recoveries in the range of 97 to 103% in well water and 97 to 102% in tap water and 96 to 97% in river water. Also the relative standards deviations (RSD) of the method were found to be Less than <4% in the real water samples. Repeatability and reproducibility of the proposed method were also evaluated as precision indicators. Three similar experiments were performed for the analysis of samples at the same day and at five consecutive days. The obtained RSD values for the intra-day and inter-day spiked samples were to be <2% and <3%, respectively. The accuracy of the method was further evaluated by comparing the results of tablets analysis with those of chromatographic reference method. According to the t-test at 95% confidence level, there was no significant difference between the colorimetric and chromatographic results. Thus, the procedure was suitable for the determination of the ET in the in the sample type examined.

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Table 2. Determination of ET in real water samples

Sample Type	Added (μM)	Found ^a (μM)	Recovery (%)	Reference Method (μM)
Tap water	-	ND ^b	-	ND
	15	14.6 \pm 0.6	97.3	14.8
	30	30.5 \pm 1.0	101.7	30.2
Well Water	-	ND	-	ND
	15	15.4 \pm 0.5	102.7	14.9
	30	29.3 \pm 0.6	97.7	30.4
River water	-	ND	-	ND
	15	14.5 \pm 0.5	96.7	15.1
	30	29.2 \pm 0.4	97.3	29.5

a: The results were mean of three measurements \pm standard deviation.

b: ND=not detected.

4. Conclusions

In this work, a colorimetric probe based on Ag NPs aggregation effect was developed for rapid and sensitive detection of ethion. The presence of ET induces the aggregation of Ag NPs through metal-ligand interaction and change in LSPR of Ag NPs, in an alkaline environment ($\text{pH} \geq 8$). To improve the sensitivity of ET detection, the experimental conditions were optimized including pH, NaCl concentration and reaction time. Under the optimal conditions, a linear relationship was obtained between absorbance ratio A_{540}/A_{393} and the ET concentration in the range of 1-40 μM with a regression coefficient R^2 of 0.9998. A LOD of 0.06 μM was also determined which was lower than maximum residue limit (MRL) in agricultural and environmental water samples, regulated by of four OP pesticides (malathion, fenthion, diazinon and parathion). The detection of ET based on the proposed colorimetric probe was also achieved in environmental water samples including tap water, well water and river water with a recovery of 96–103% and a RSD less than 3%. As a consequence, due to its advantages of low cost, rapid detection, good selectivity, and high sensitivity, the proposed colorimetric probe can provide enormous potential for on-site detection of ethion in environmental water samples.

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Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/journal-of-chemical-metrology>

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