Chemistry Central Journal

RESEARCH ARTICLE

A novel chemiluminescence assay of organophosphorous pesticide quinalphos residue in vegetable with luminol detection

Haoyu Hu, Xiaoyu Liu*, Feng Jiang, Xin Yao and Xiaocheng Cui

Abstract

Background: Organophosphorous pesticides are the most popular pesticides used in agriculture. As acetylcholinesterase inhibitors, organophosphorous pesticides are toxic organic chemicals. The control and detection of organophosphorous pesticide residue in food, water, and environment therefore plays a very important role in maintaining physical health. A sensitive, rapid, simple chemiluminescence(CL) method has been developed for the determination of quinalphos based on the reaction of quinalphos with luminol-H_2O_2 in an alkaline medium. The method has been applied to detection of quinalphos in vegetable samples with satisfactory results.

Results: The CL method for the determination of organophosphorous pesticide quinalphos is based on the phenomenon that quinalphos can apparently enhance the CL intensity of the luminol-H_2O_2 system. The optimal conditions were: luminol concentration 5.0×10^{-4} mol/L, H_2O_2 concentration 0.05 mol/L.pH value 13. In order to restrain the interference from metal ions, 1.0×10^{-3} mol/L of EDTA was added to the luminol solution. The possible mechanism was proposed.

Conclusion: Under the optimum reaction conditions, CL was linear with the concentration of quinalphos in the range of 0.02 μg/mL -1.0 μg/mL and the detection limit was 0.0055 μg/mL (3σ). This method has been successfully applied to the detection of quinalphos in vegetable samples. According to the experimental data, the average recoveries for quinalphos in cherry tomato and green pepper 97.20% and 90.13%. Meanwhile, the possible mechanism was proposed.

Background

Organophosphorus pesticides are widely used in agriculture due to their high insecticidal activity[1]. They are toxic organic chemicals which can irreversibly inhibit acetylcholinesterase (AChE) which is essential for the function of the central nervous system [2,3]. As the pesticide residue is a potentially serious hazard to human health, the control and detection of pesticide residue plays a very important role in minimising risk[4]. Many methods have been developed in the last few years for the detection of organophosphorus pesticides. The most widely used methods are gas chromatography (GC) [5-7], high-performance liquid chromatography (HPLC) [8,9], gas chromatography-mass spectrometry(GC-MS) [10], immune assay and fluorescence [11,12]. These methods are accurate and selective, but they require relatively expensive instrumentation and skilled technicians.

Chemiluminescence (CL) is defined as the production of electromagnetic radiation (ultraviolet, visible or infrared) observed when a chemical reaction yields an electronically excited intermediate or end product, which either luminesces or donates its energy to another molecule responsible for the emission. The CL phenomenon can be applied as detection technique for the monitoring of a wide variety of compounds in diverse fields, such as clinical, pharmaceutical, biomedical, environmental and food analysis [13-15]. Compared with those methods mentioned above, the chemiluminescence(CL) method has been growing in popularity and acceptance because of its advantages such as high sensitivity, rapid assay speed and simple instrumentation. CL method has been

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applied to the determination of organophosphorus pesticides residues during recent years[16-19].

Quinalphos (*O,O*-diethyl-*O*-quinoxalinyl phosphorothioate) is one of the most widely used organophosphorus insecticides in agriculture, and is applied to control of incidence of pests over crops such as cotton, tea, citrus and rice. At present, most of the analytical methods employed for the detection of quinalphos residues are based on chromatographic techniques [20], chromatographic techniques-mass spectrometry [21] and fluorescence. Until now, to our knowledge, there have been no reports of detecting quinalphos using a direct CL method. In our study, we found that the CL signal could be enhanced on the reaction of quinalphos with luminol-H_2O_2 in alkaline medium. Based on these findings, a sensitive, simple CL method for the detection of quinalphos was developed. The method has been applied to the detection of quinalphos in vegetable samples with satisfactory results. Further study was focused on the mechanism of quinalphos and the possible mechanism was proposed.

Results and Discussion

Effect of luminol concentration

As the CL reagent, luminol concentration was an important factor which affected the CL intensity. The effect of luminol concentration on the CL intensity was examined in the range of 1.0×10^{-5} mol/L-1.0×10^{-3} mol/L, with 3 replicates. ΔI (the increasing amount of CL intensity) reached the maximum value when the concentration of luminol solution was 5.0×10^{-4}mol/L. Therefore, for the experiments, the concentration of luminol was maintained at the optimal value of 5.0×10^{-4}mol/L.

Effect of hydrogen peroxide concentration

The effect of H_2O_2 concentration on the CL intensity was tested in the range of 0.05 mol/L-1 mol/L, with 3 replicates. The result (Figure 1) showed that ΔI reached the maximum value when the concentration of H_2O_2 was 0.05 mol/L. Therefore 0.05 mol/L was chosen as the optimal H_2O_2 concentration for further experiment.

Effect of pH of luminal

The pH of the reaction of quinalphos with luminol-H_2O_2 in alkaline medium is an important parameter. The effects of a medium with pH in the range 12.5-13.5 were investigated, with 3 replicates. ΔI reached the maximum value when the pH was 13. Therefore pH 13 of luminol solution was selected as optimum for consequent research [22].

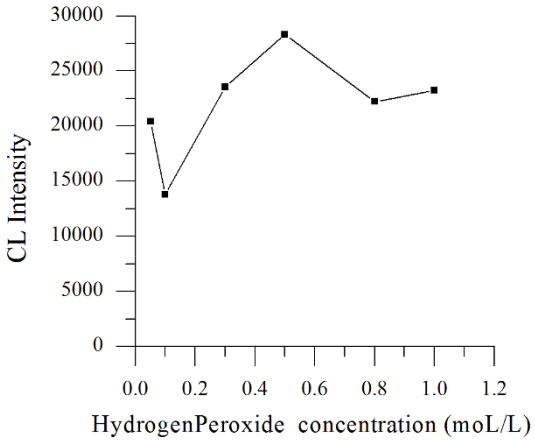

Figure 1 Effect of H_2O_2 concentration on the CL intensity.

Effect of enhancers

Our preliminary experiment showed that the CL reaction of luminol-H_2O_2- organophosphorus could be sensitized by several surfactants and inorganic salts. Surfactants can form micelles which can change the physical characteristics of molecular guests and their reactivity in aqueous solutions. Thus, two non-ionic surfactants (Tween-80, Polythylene Glucol-400(PEG-400)), two anionic surfactants (sodium dodecyl sulplhate (SDS), sodium dodecyl benzene sulplhate(SDBS)), one cationic surfactants (Cetyltrimethylammonium bromide (CTMAB)) and five inorganic salts (KBr, NaBr, NaCl, KI, KCl) were tested. However, the results (Table 1) showed that the effect of enhancers were not obvious under strong alkaline condition and the CL intensity was even inhibited by some surfactants. It indicated that quinalphos can apparently enhance the CL intensity of the luminol-H_2O_2 system without sensitizers.

Influence of coexisting foreign species

The effect of some common inorganic ions and organic compounds on the CL reaction was tested by analyzing a standard solution of quinalphos (1.0×10^{-7} g/mL) to which increasing amounts of foreign ions were added. The tolerance limit was taken as the amount which caused a relative error of ± 5% in the peak height. Because some metal ions such as Cu (II), Co (II) may interfere the CL system in the luminol-hydrogen peroxide method. The tolerable ratio for foreign species was 1000-fold for glucose, CO_3^{2-}, NO_3^-, Cl^-, Na^+; 100-fold for Mg^{2+}; 1-fold for Pb^{2+}; 0.01-fold for Co^{2+}, Fe^{3+}, Cu^{2+}[23-26]. It can be seen that obvious interference was caused by Co^{2+}, Fe^{3+} and Cu^{2+}. Thus, 1.0×10^{-3} mol/L of EDTA was added to

Table 1: Selection of enhancers

Enhancers	Concentration	The CL intensity of quinalphos
CTMAB	0.5%	2012.8
SDBS	0.5%	7671
SDS	0.5%	3095
Tween-80	0.5%	-
PEG-400	0.5%	-
NaBr	1 mol·L^{-1}	-
KBr	1 mol·L^{-1}	9189
KCl	1 mol·L^{-1}	8658
NaCl	1 mol·L^{-1}	7447
KI	1 mol·L^{-1}	10170
Without Enhancers		8424

the luminol solution to restrain the interference from metal ions.

The analytical characteristic of the CL method

Under the optimal conditions, the calibration curve of ΔI against quinalphos concentration was linear in the range of 2 × 10^{-8}g/mL-1 × 10^{-6}g/mL and the calibration curves was y = 1081 × -2650.4 (where × is the concentration of quinalphos, 10^{-8}g/mL), with the correlation coefficient of 0.9921. The detection limit of quinalphos was 0.0055 μg/mL, calculated from the International Union of Pure and Applied Chemistry (IUPAC) recommendations (3σ). The relative standard deviations (RSD) for 7 injections with 1 × 10^{-7}g/mL quinalphos was 5.6%.

Sample analysis

The proposed method was applied to the assay of quinalphos in vegetable sample. The eluent of quinalphos which was sprayed on the surface of vegetables was analyzed. According to the experimental data, the average recoveries for quinalphos in cherry tomato and green pepper 97.20% and 90.13% (Table 2). The relative standard deviations (RSD) for recoveries of quinalphos on the surface of cherry tomato and green pepper were 5.6% and 4.9%.

Kinetics curve of the CL reaction

In this experiment, the kinetic characteristics of the proposed CL reaction were studied. The response curve of luminol (5 × 10^{-4} mol/L) CL reaction in the present quinalphos (1 × 10^{-7} g/mL) was recorded to study the kinetic characteristic of the CL reaction. Figure 2 demonstrated that the CL intensity peak appeared within 1 s of the injection of the quinalphos. The CL signals would decrease to a very low level within 20 s. The kinetic curve indicated the CL method was rapid and sensitive enough to be suitable for the detection of quinalphos.

The CL reaction mechanism

Many research works have focused on the CL reaction mechanism of the luminol system. The research results have confirmed that the 3-aminophthalate anion was the emitter irrespective of medium and oxidant used. The maximum emission wavelength of luminol was about 420 nm. The resulting fluorescence spectrum (Figure 3) indicated that the maximum emssion wavelength of three systems (Luminol, Lumino-H_2O_2 and quinalphos-Lumino-H_2O_2) was also near 420 nm and the signal of fluorescence emission spectrum of quinalphos-lumino-H_2O_2 system is higher than lumino-H_2O_2 system. This means that the emitter of quinalphos-lumino-H_2O_2 system was also 3-aminophthalate anion.

Based on the analysis of the fluorescence spectrum combined with the kinetic curve, the possible mechanism of the present reaction was proposed as following: [1] Quinalphos was oxidized by H_2O_2 to peroxophosphonate;

Table 2: Analytical results of quinalphos in vegetable

Sample	Content of quinalphos	quinalphos Added	Found	Recovery (%)	RSD n = 7(%)
Cherry tomato	None	1 × 10^{-7}g	9.720 × 10^{-8}g	97.20	5.6
green pepper	None	1 × 10^{-7}g	9.013 × 10^{-8}g	90.13	4.9

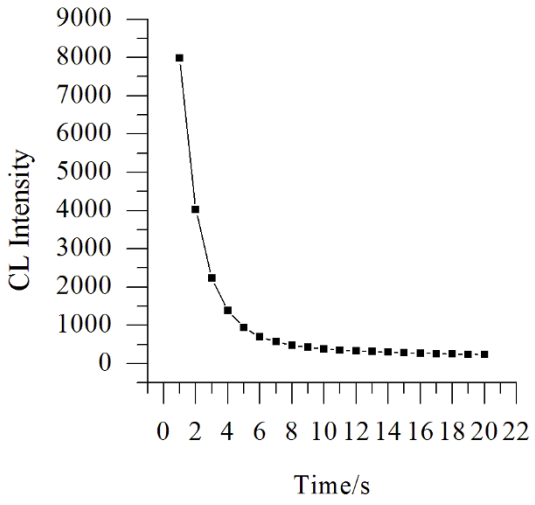

Figure 2 The kinetics curve of quinalphos -luminol -H_2O_2.

[2] Peroxophosphonate oxidized luminol to produce an excited state 3-aminophthalate anion; [3] The excited state 3-aminophthalate anion relaxed to the ground state, producing the observed emission [27,28] (Figure 4).

Conclusion

In this work, a direct CL method for determination of quinalphos is presented based on the reaction with luminol-H_2O_2 in an alkaline medium. CL reaction could occur with lower concentration of quinalphos without enhancers such as organized surfactant. The method was applied to the detection of quinalphos in vegetable samples. In the following, the mechanism was also investigated in detail through examination of the fluorescence spectrum and kinetic curve.

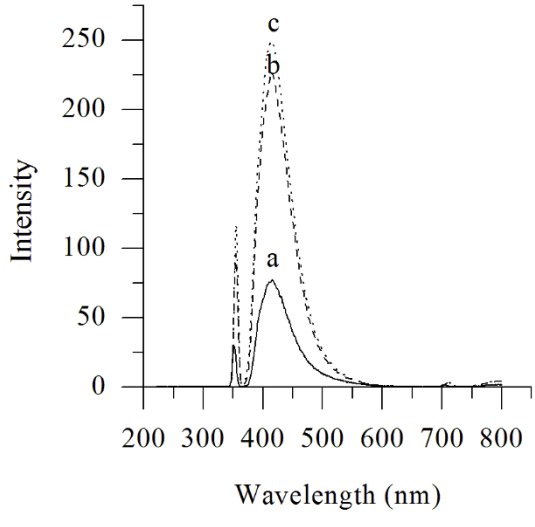

Figure 3 The fluorescence spectrum of the solutions. a. luminol solution b.luminol and H_2O_2 c. luminol- H_2O_2- quinalphos

Experimental

Reagents and solutions

All reagents were analytical reagent grade and distilled water was used throughout these experiments. Luminol (5-amino-2,3-dihydro-1, 4-phthalazinedione, 99%) was purchased from the Corporation of Sigma(USA). Quinalphos standard solution (0.1 mg/mL in acetone) was purchased from the Corporation of Standard Material (Beijing, China). Tween-80(Amresco), PEG-400(Guangdong, China), SDS(Guangdong, China), SDBS(Shanghai, China), CTMAB(Wuhan, China), KBr(Guangdong, China), NaBr(Tianjin, China), NaCl(Jiaozuo, China), KI(Wuhan, China), KCl(Wuhan, China) were analytical reagent grade.

The 0.01 mol/L luminol stock solution was prepared by dissolving 0.1772 g luminol with 2 mL 1 mol/L NaOH solution and diluted with distilled water to 100 ml. The luminol solution was stable for at least 1 week when stored in refrigerator at 4°C. Working standard solutions of luminol were freshly prepared from the stock solution by appropriate dilutions before use and adjusted its pH with 0.1 mol/L NaOH. The 0.01 mg/mL quinalphos stock solution was prepared by diluting 0.1 mg/mL standard solution of quinalphos with 1 mL acetone and then diluting to 10 mL with distilled water. The obtained stock solution was stored in refrigerator at 4°C [29-31].

Apparatus

The CL signal was measured by a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) as shown in Figure 5. The CL intensity, amplified by a sensitive photomultiplier tube (PMT) operated at -400 V, was measured with a detector under the control of a computer. The CL intensity ΔI was calculated by $\Delta I = I_s - I_0$, where I_s and I_0 are the CL signals in presence and absence of quinalphos respectively. The determination method was based on the relationship between ΔI and corresponding concentration of quinalphos. The fluorescence spectrum was obtained by RF-5301PC fluorescence recording spectrophotometer (Shimadzu, Japan).

Sample preparation

The proposed method was utilized for the determination of quinalphos in vegetable sample. Cherry tomato and green pepper were bought from the market in our campus. The weight of cherry tomato was about 15 g. The weight of green pepper was about 10 g. They were

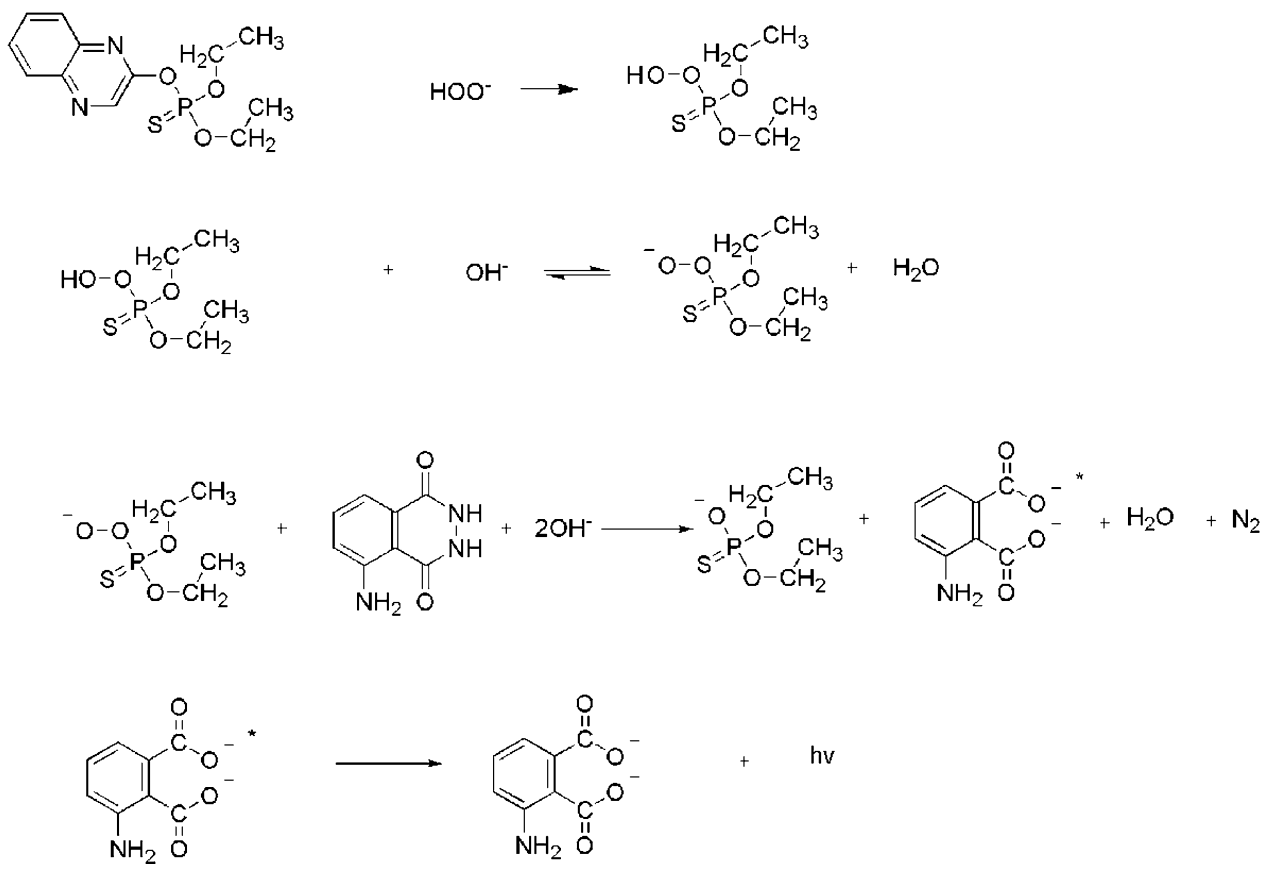

Figure 4 The possible mechanism of the reaction.

cleaned with distilled water. In order to perform the recovery test, a known amount of quinalphos standard solution was added to the sample and then washed with distilled water. The washings were diluted to suitable concentration with distilled water for analysis.

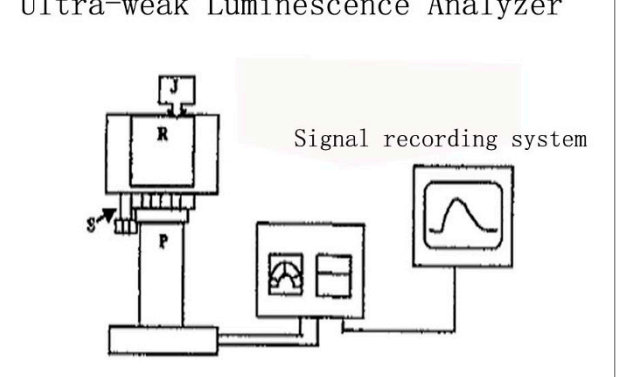

Figure 5 The detectable devices of static chemiluminescence. S: Switch P: Photomultiplier Tube J: Sample Injection R: Reaction Cell

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
All authors contributed to the experimental design. Xiaoyu Liu focused on the experiment design and contributed to drafting the manuscript. Haoyu Hu and Feng Jiang carried out nearly all of the laboratory work. Xin Yao and Xiaocheng Cui deal with the data analysis. All authors read and approved the final manuscript.

Acknowledgements
This study was supported by the Tackle Key Project Foundation of Hubei Province of China (Grant No.2006AA201C40).

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Received: 12 February 2010 Accepted: 24 June 2010
Published: 24 June 2010

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doi: 10.1186/1752-153X-4-13
Cite this article as: Hu *et al.*, A novel chemiluminescence assay of organophosphorous pesticide quinalphos residue in vegetable with luminol detection *Chemistry Central Journal* 2010, **4**:13