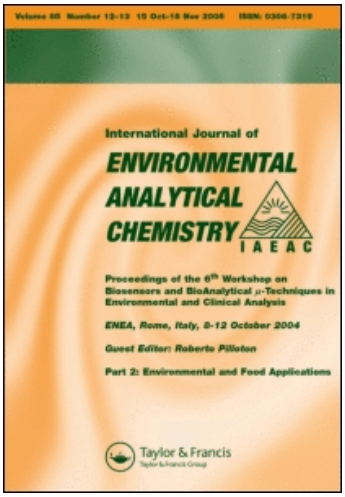

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Determination of Organophosphorus Pesticides Based on the Inhibition of Lipase with a Surface Acoustic Wave Sensor System

W. Wei[a]; Q. Cai[a]; R. Wang[a]; L. Nie[a]; S. Yao[a]
[a] Department of Chemistry and Chemical Engineering, Hunan University, Changsha, P. R. China

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DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES BASED ON THE INHIBITION OF LIPASE WITH A SURFACE ACOUSTIC WAVE SENSOR SYSTEM

W. WEI, Q. CAI, R. WANG, L. NIE and S. YAO*

Department of Chemistry and Chemical Engineering, Hunan University Changsha 410082, P. R. China

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A convenient and sensitive method based on the inhibition of the lipase-catalyzed hydrolysis by binding hydroxyl groups of lipase with organophosphorus pesticide is described for the enzymatic determination of organophosphorus pesticides. Employing the method proposed, the frequency response is proportional to organophosphorus pesticide concentration over the range of 250 ng/ml~1.25 μg/ml, the detection limit is 58 ng/ml. The effects of temperature, pH value, incubation time and solvent are also investigated.

KEY WORDS: SAW/impedance enzyme transducer, enzyme inhibition, methamidophos

INTRODUCTION

Organophosphorus pesticides represent a large number of agricultural chemicals in widespread use. Owing to their low persistence and high effectiveness, they are widely employed in agriculture. These pesticides exhibit potential mammalian toxicity, thus, sensitive, rapid and reliable detection and determination of these toxic substances in agricultural and food products and in environmental matrices has become very important for protection of the environment and human health.

The determination of these pesticides can be performed by various chemical methods. The most widely used techique is gas chromatography with nitrogen-phosphorus or flame-photometric detection[1] and spectroscopy[2-3] although liquid chromatography is also receiving attention for high detection sensitivity and selectivity[4]. However, in most cases, these techniques for the analysis of organophosphorus pesticides require tedious extraction and clean-up procedures prior to instrumental anaylsis which seems to be laborious, time-consuming and expensive, thus, are not very suitable for routine analysis.

The research of the effect of pesticides on enzyme activity has attracted considerable interest in analytical chemistry, especially in the detection of enzyme and inhibitors by the use of inhibited enzyme-catalyzed hydrolysis[5-6]. Because enzymes can be generally inhibited especially by low concentrations of a certain chemical, this kind of enzymatic method is considered to be an efficient way for the determination of inhibitors. A number of articles concerning organophosporus pesticide determinations utilizing enzymatic systems has been raised by employing cholinesterase[7-9] and lipase[5-6]. Since a great deal

* To whom correspondence should be addressed.

of work has been devoted to organophosphorus pesticides detection using cholinesterase/substrate system, to the best of our knowledge, no work on the analytical use of SAW device involving lipase/substrate system in the determination of organophosphorus pesticides has been proposed. In the new technique reported here, a sensitive, rapid and convenient method based on the inhibition of lipase activity is presented. The difference in enzymatic activity between the samples in the presence and absence of organophosphorus pesticides can be determined with a SAW/impedance enzyme transducer, which is composed of a SAW resonator oscillating at 61 MHz and a pair of parallel electrodes. There is a linear correlation between frequency shift (ΔF) of the SAW/impedance enzyme transducer and change of electrolyte conductivity (ΔK) of the liquid. i.e. $\Delta F = a\ \Delta K + b$, where a and b are constants which depend on the SAW device, amplifier circuit and experimental conditions. The proposed sensor system is advantageous in its high sensitivity, reliability, simplicity in construction and low cost[10].

In this work, we proposed an enzymatic method for the determination of organophosphorus pesticides based on its inhibitory effect on lipase which catalyzes the conversion of glycerol triacetate to glycerin and acetic acid. The influences of pH, temperature, incubation time and solvent are investigated.

MATERIALS AND METHODS

Apparatus

Experiments were performend with the SAW sensor system. The schematic diagram of the experimental set-up and the equivalent circuit is shown in Figure 1. The 61-MHz SAW resonator was manufactured with a Y,Z-cut lithium niobate piezoelectric crystal with aluminum and gold wire bonds. The nominal insertion loss of the device is 6.8 dB. The detection cell was stirred with a magnetic stirrer at a constant stirring rate. This apparatus was accommodated in a chamber thermostated at 25 ± 0.2°C.

Power (6.5 V dc) was delivered to the circuit board from an adjustable dual-track dc power supply. The oscillation signal was fed to an universal frequency counter (Model SC7210, Iwatsu), and the frequency changes due to the conductance fluctuation of the substrate solution were recorded.

Reagents

Lipase (EC 3.1.1.3, 3000 IU/mg), purchased from Biochemistry Research Institute (Shanghai, the Chinese Academy of Sciences) was used without further purification. Standard solution of enzyme (1 mg/ml) was prepared by the mixed solution of glycerin, carbon tetrachloride and double distilled water (7:2:1) and stored in a refrigerator before the experiment. Results show that the enzyme activity drops 10% after 2 weeks of storage. So the standard lipase solution was prepared every week in order to obtain constant reproducible results. The substrate (0.01M glycerol triacetate) was emulsified before use. Pesticides were obtained from Hunan Agricultural Institute. A stock solution of 0.9 mM methamidophos was prepared by dissolving 0.01 ml 50% methamidophos in 50 ml acetone. Working buffer was 1.0 mM Tris-HCl at pH 7.3. All other chemicals were of analytical reagent grade. Double distilled water was used throughout.

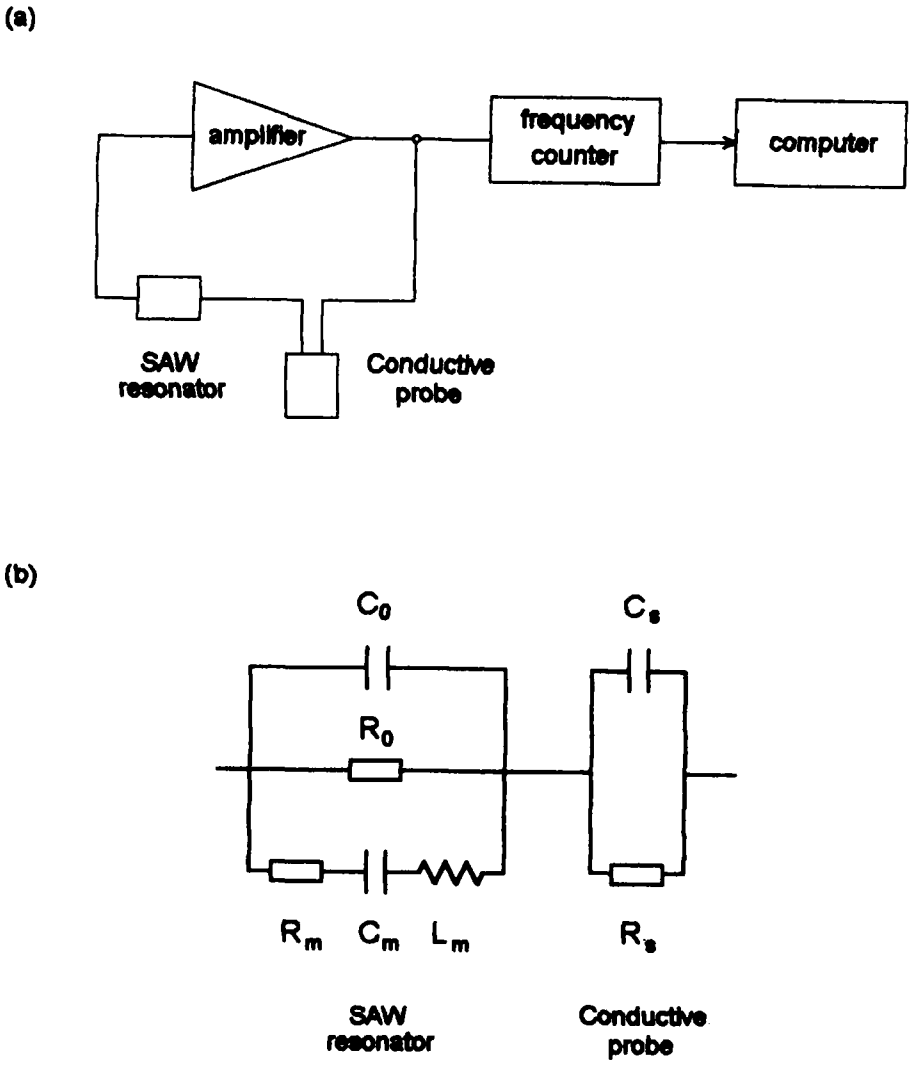

Figure 1 (a) Schematic diagram of the experimental set-up. (b) Equivalent circuit of the SAW sensor system.

Procedures

Thirty minutes pre-operation was needed to guarantee the stabilization of the whole setup including the frequency counter and power supply before the experiment. The air-bath chamber was thermostated at 25 ± 0.2°C. The solution in the detection cell was stirred by a magnetic stirrer. A typical experiment procedure was done as follows. 100 µl lipase and a different amount of methamidophos were mixed with 1 ml Tris-HCl working buffer and then was diluted with double distilled water to 9 ml exactly. Afterwards, a definite amount (1 ml) of glycerol triacetate solution was added. The enzymatic reaction was started by the addition of the substrate solution and the frequency

change was recorded with time. The rate of the reaction was characterized by the slope of the kinetic curves plotted as frequency shift vs. time. The blank solution was prepared and tested in the same way described above. In the determination of cucumber samples, 2 g cucumber paste was processed according to the same procedure.

RESULTS AND DISCUSSION

Inhibition effect of an organophosphorus pesticide on lipase activity

Organophosphorus pesticides exhibits an inhibitory effect on lipase activity. When an organophosphorus pesticide is introduced into a solution containing lipase glycerol triacetate, the enzymatic activity (in other words, the lipase-catalyzed reaction rate) decreases with the concentration of organophosphorus pesticide present in the solution. This fact presents a possibility for detection and analysis of low concentration of organophosphorus pesticides by alteration of the lipase-catalyzed reaction rate followed by the SAW/impedance transducer. As shown in Figure 2, the frequency response of the transducer decreases evidently with the organophosphorus pesticide amount present in the sample solution.

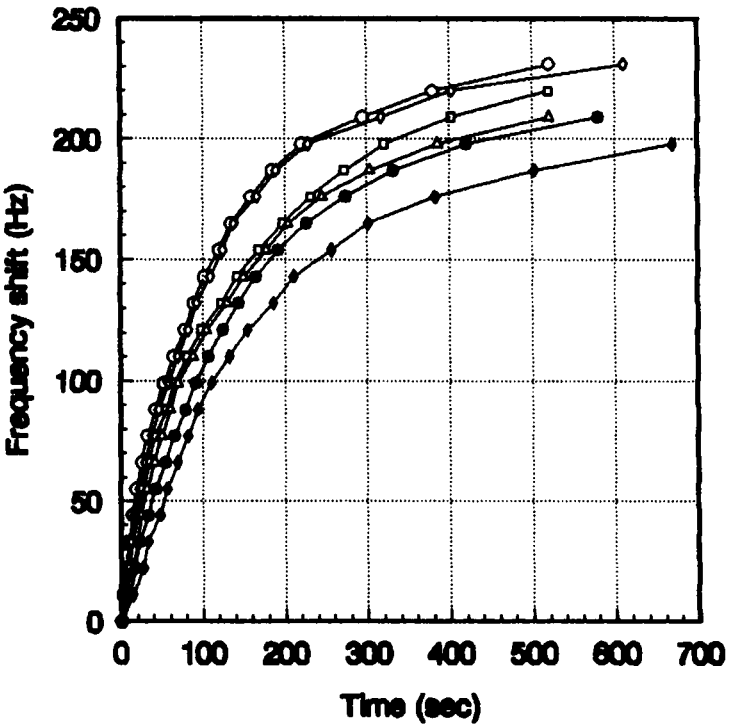

Figure 2 Influence of methamidophos on the frequency response curves. In the absence of methamidophos (o); In the presence of methamidophos 1.78 μM (◊); 3.56 μM (□); 5.35 μM (Δ); 7.13 μM (•); 8.91 μM (♦). glycerol triacetate concentration:1×10^{-3} M; lipase concentration: 0.01 mg/ml.

Influence of incubation time

Because the inhibition effect of the organophosphorus pesticide on lipase-catalyzed reaction is progressive, the inhibition effect of the pesticide depends on the incubation time. As shown in Figure 3, the inhibition effect was higher with longer incubation time, indication that better detection limit could be achieved with an extended incubation time. But with further increase in incubation time, no significant decrease in the initial rate of lipase-catalyzed reaction was observed. However, the experiments were conducted with an incubation time of 20 minutes, in order to maintain a short analysis time.

Influences of enzyme activity and substrate concentration of lipase-catalyzed reaction were investigated. Results indicate that a larger lipase amount and substrate concentration tend to yield a greater sensitivity, thus a shorter response time. Therefore 100 μl 1 μmg/ml lipase and 1 ml 0.01 M substrate were selected in our later experiments.

Influence of pH and temperature

The effect of pH of the test solution on the initial rate of lipase-catalyzed reaction in presence and absence of organophosphorus pesticide was examined in the range of pH 6.2~9.0 at 25°C. As shown in Figure 4 the activity of lipase in presence and absence of organophosphorus pesticide was maximal at pH 7.4 and 7.8, respectively. On the basis of

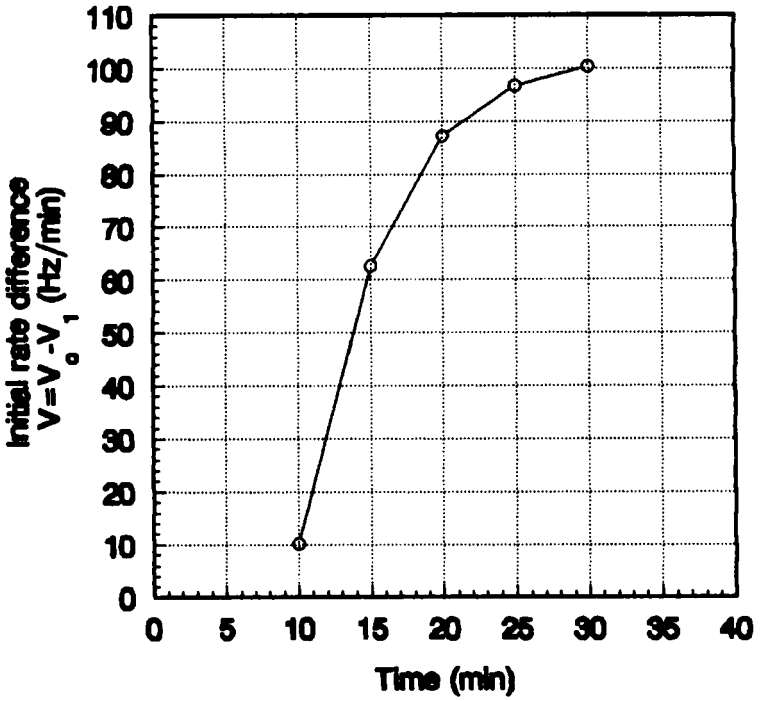

Figure 3 Influence of incubation time on the frequency response curves. V_0 is the initial rate in absence of solvent and V_1 is the initial rate in presence of solvent.

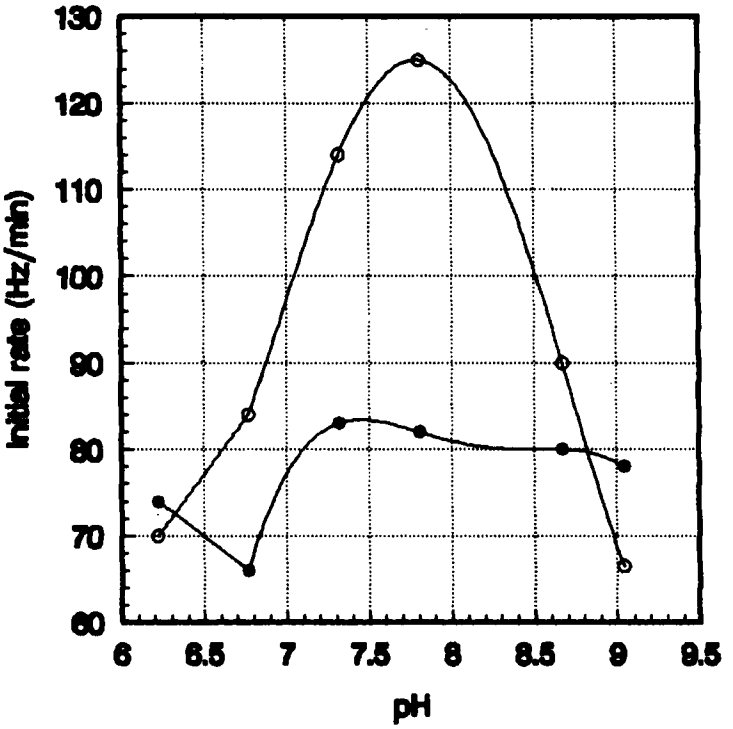

Figure 4 pH-dependence of initial reaction rate V_0 for lipase-catayzed reaction. In the absence of pesticide (o); in the presence of 7.13 μM methamidophos (•). Glycerol triacetate concentration: 1×10^{-3} M; lipase concentration: 0.01 mg/ml.

these results, the pH used in our determinations (pH 7.3), is a compromise between the optimum pH for the reaction to take place at an appropriate rate and the one where enzyme and organophosphorus pesticide is relatively stable.

The influence of temperature on lipase activity was investigated. The frequency changes, as a function of the temperature (Figure 5), shows that the temperature holds a considerable effect on the response. Since 25°C is the room temperature, it is easy to conduct the experiment under this ordinary situation. As far as the SAW sensor system is concerned, the noise level increased with the increase of temperature, that is to say, at 25°C the noise level is 2 Hz while at 40°C the noise level will go up to 8 Hz. On the other hand, the volatility of methamidophos becomes greater with higher temperature. Therefore, although the sensor system yields the greatest sensitivity at 34°C, all subsequent measurements were processed at 25°C by considering the above factors.

Influence of solvent

Since not all organophosphorus pesticides can be dissolved in double distilled water, a suitable solvent must be selected. The effect of solvent on lipase activity was studied. As can be seen from Table 1, there is no significant difference in frequency sensitivity in the

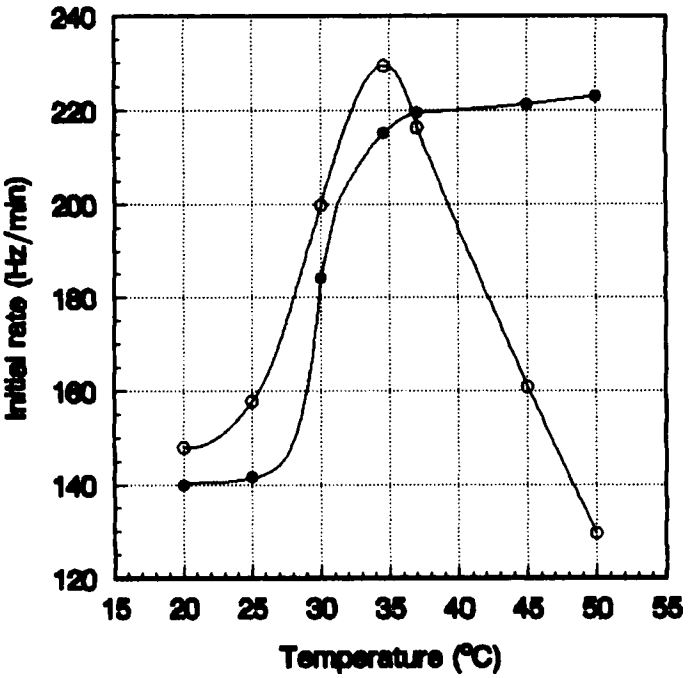

Figure 5 Temperature-dependence of initial reaction rate V_0 for lipase-catalyzed reaction. In the absence of pesticide (o); in the presence of 7.13 μM methamidophos (•). Experimental conditions are the same as in Figure 2.

Table 1 Influence of solvent.

Solvent	*Volume added (ml)*	*Initial rate (Hz/min)* $V^* = V_0 - V_1$
Acetone	0.05	8
	0.1	10
Ethanol	0.05	30
	0.1	50
Ethyl acetate	0.05	15
	0.1	26

* Average of three determinations
V_0 Initial rate in the absence of solvent
V_1 Initial rate in the presence of solvent

presence of these solvents. Therefore, acetone was selected as solvent in our experiment by considering the measurement condition.

Calibration graphs and recovery measurements

The calibration curve was constructed under the selected optimum conditions according to the above-mentioned investigations. A linear relationship was obtained over the range

of 250 ng/ml~1.25 μg/ml. The calibration curve can also be described by the following regression equation

$$V_0 = -16.84\ [\text{methamidophos}] + 228.29 \quad (1)$$

where V_0 is initial rate, in unit of Hz/min, the methamidophos concentration is in unit μM. The regression coefficient is 0.9991 (n = 6). The detection limit is 58 ng/ml, with the lowest noise level of 2 Hz and a signal-to-noise ratio of three.

The analytical precision and accuracy for an organophosphorus pesticide with the present assay method were calculated, the results are listed in Table 2. A comparison of the SAW-sensing method with previously reported techniques in detecting the concentration of this organophosphorus pesticide is shown in Table 3. It can be seen that the proposed method is comparative to the GC method, additionally the method is advantageous in its reliability, simplicity in construction and low cost.

Application

Fresh cucumber, added with a definite amount of methamidophos, was finely minced and shaken by a mechanical vacillator at 275 rpm for 30 min at room temperature.

Then 2 g cucumber paste, mixed with 1 ml Tris-HCl working buffer and 100 μl lipase, was diluted with double distilled water to 9 ml exactly. Afterwards, 1 ml glycerol triacetate solution was added. Results of the determination of methamidophos residue from cucumber samples are presented in Table 4.

Table 2 Precision and accuracy of the method.

Methamidophos (μM)		*R. S. D.*	*Recovery*
Added	*Found*[a]	*(%)*	*(%)*
1.780	1.854	3.0	104.2
3.560	3.530	2.8	99.2
5.350	5.080	1.6	95.0
7.130	7.173	3.2	100.6
8.910	9.029	1.9	101.3
			100.1[b]

[a] Average of five determinations [b] Mean of recovery

Table 3 Comparison of methods for analyzing organophosphorus pesticide.

Method	*Limit of detection*	*Recovery*	*Ref.*
SAW-sensing method	5.8×10^{-8} g/ml	95.0–104.2%	This paper
Single Sweep Oscilliopolarography	10^{-7}–10^{-4} g/g	93–95%	11
GC method	10^{-8} g/g	88.8–99.0%	12
SFE method	10^{-7}–2.2×10^{-6} g/g	70%	13
GLC method	10^{-10} g/g	86.3%	14

Table 4 Recoveries of methamidophos residue from cucumber samples.

Substrate	*Methamidophos (10^{-6} g/g)*		*R. S. D*	*Recovery*
	Added	*Found*[a]	*(%)*	*(%)*
Peel	0.080	0.077	2.1	96.7
	0.150	0.147	3.3	98.3
	0.650	0.644	1.6	99.0
	1.250	1.199	2.8	95.9
Flesh	0.080	0.078	2.6	97.8
	0.150	0.145	3.9	96.4
	0.650	0.648	2.2	99.7
	1.250	1.233	1.2	98.6

[a] Average of three determinations

CONCLUSIONS

In this work, the SAW impedance enzyme sensor system has been successfully applied in the quantitative determination of an organophosphorus pesticide. The proposed method possesses the characteristics of high sensitivity together with simplicity and continuous measurements, which makes it possible to be used extensively in agriculture, food industry, environmental protection and life sciences. Further applications of the enzyme SAW sensor impedance system are progressing in our laboratory.

Acknowledgements

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