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Detection of Pesticide Thiram in Plant Leafs Using Voltammetric at Nanotube Electrode

Chang-Hyun Lee*, Suw-Young Ly[1)]

Division of General Education, Pyeongtaek University, Gyeonggi-do 405-701, Korea
[1)]Biosensor Research Institute, Seoul National University of Technology, Seoul 139-743, Korea
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Abstract

Voltammetric diagnostics of pesticide thiram was studied in plant leafs in vivo fluid with DNA immobilized on a carbon nanotube electrode (DCE). Sensor properties of carbon nanotube (CE) and DNA immobilized nanotube were compared. DCE was more effective than CE in target detecting. The parameters such as pH strength, stripping accumulation, amplitude, and increment potential were examined to find the optimum condition for detection of pesticide thiram in a sesame leaf. The optimized conditions were as follows 550 Hz frequency, 0.15 V amplitude, 0.005 V increment potential, -1.2 V initial potential, 4.78 pH, 500 sec accumulation time. Under optimum condition, the detection limit of thiram was attained at 0.01ng/L.

Key Words : Stripping voltammetry, Pesticide, DNA nanotube, Sesami leaf, Thiram

1. Introduction

Recently, Korea is suffering from serious environmental problems caused by spraying pesticides. They are overused not only to increase agriculture production, but also to meet the demands of indiscreet country clubs. Today, about 1,300 kinds of pesticides are being manufactured as products and, out of these, about 430 kinds are used at farms. There are many ways to detect pesticides that cause serious problems in our environment.

The thiram(tetramethylthiuram disulfide, TMTD) is used mostly as a pesticide or a seed-sterilization for fruit trees and vegetables cabbages such as apples, peaches, melons, vegetables cabbages, spinages, and carrots etc. in our farms. Korean farms have been using a pesticide thiram (tetramethylthiuram disulfide) long before because it prevents or reduces agricultural losses caused by pests, resulting in improved yield and greater availability of food, vegetable crops and seeds, as well as a vulcanizing agent in the rubber industry at a reasonable price, all year round (Cooper and Dobson, 2007; Cereser. et al., 2001). However, it has been reported that the trace of Thiram can cause Parkinson's disease, Alzheimer's disease (Gauthier et al., 2001), Paget's disease of bone (Leve, 2002), prostate and breast cancers (Landau-Ossondo et al., 2009), if it were exposed to people. It can also damage DNA (Garaj-Vrhovac and Zeljezic, 2000). For this reason, detection of pesticide thiram is important for human health.

*Corresponding author : Chang-Hyun Lee, Division of General Education, Pyeongtaek University, Gyeonggi-do 405-701, Korea
Phone: +82-31-659-8384
E-mail: lch@ptu.ac.kr

Many researches have been developed the various analysis methods such as opto fluidic ring resonator biosensor (Yang et al., 2008), flame photometric and mass spectrometric detection (Amirav and Jing, 1998), surface plasma resonance based fiber-optic sensor (Chand and Gupta, 2007), an amperometric micro-biodetector (Wu et al., 2009), SPME/SnO_2 gas sensor (Huang et al., 2004), liquid matrices by ion mobility spectrometry (Tuovinen et al., 2000), high-performance liquid chromatography (Huang et al., 2004), optical waveguide immunochemical sensors (Tuovinen et al., 2004), and label free optical immunoprobes (Perez-Ruiiz et al., 2005). Also included are an enzyme-based detection methodology (Lechunga et al., 1995), capillary zone electrophoresis (Brecht and Ganglitz, 1997), solid-phase micro-extraction, and gas chromatography with atomic emission detection (Ayyagari et al., 1995). Moreover an automated (Cheng et al., 2007), microextraction (Campillo et al., 2007), gas chromatography (Khalili-Zanjani et al., 2008) and others have been developed as well.

The separation by spectrophotometric methods that have been developed are too complex and time consuming in the analysis. Several detecting methods are reported for chromatography, pollarography, NMR, atom adsorption spectrometery, X-ray diffraction spectrometery, Raman spectrometery, mass spectrometery, etc. However, these precision tools are extremely expensive and they are all imported from overseas. In addition, it is usually recommended to use 2-3 methods or sometimes 4 methods combined in order to get more accurate result, especially below the ppb~ ppt level.

Thus, voltammetric sensors such as polymeric enzyme electrodes (Dutta et al., 2008), gold nanoparticles at tyrosinase electrode (Kim et al., 2008), poly 3,4 ethylenedioxythiophene modified wall jet electrode (Manisankar et al., 2005), ferophthalocyanine chemically modified carbon paste electrode (Ciucu et al., 2003), polymer multiwalled carbon nanotubes modified glassy carbon electrode (Manisankar et al., 2008), and alumina sol gel sonogel carbon electrode (Zejli et al., 2008) have been searched, These electrodes are sensitive to detect pesticide, but the production of these electrodes are not easy and are time-consuming. However, an electrochemical analysis such as square wave anodic stripping voltammetry(SWASV) and cyclic voltammetry (CV) is more powerful technique that can measure both metallic and nonmetallic organic elements quickly and precisely. It can also be used to detect very small-amount harmfull elements whether they are metallic or nonmetallic, and whether they are organic or inorganic.

It is considered that the electrochemical method employing CV and SWASV in a stripping mode is relatively less expensive and more sensitive than other voltammetric methods. Also, it can be used for detecting any harmful matetrials (Radi, 2005). Therefore, electrochemical instruments of voltammetric measurements with DNA immobilized on a carbon nanotube sensor (DCE) was developed and it was applied to detect a pesticide thiram in a sesame leaf.

2. Materials and Methods

2.1. Apparatus, Reagents, and Procedure

Electrochemical instruments of voltammetric measurements were used with the new system of Bioelectronics-1, which was first constructed at the authors' institute. The new version is a computerized handheld voltammetry with a 2.4 V potential range, a 2 mA current range, and a 10^{-12}A measuring current. The instrument size is similar to that of a typical cellular phone and can be used for the bioassay and sensor techniques for individual and laboratory application.

Carbon nanotube paste electrode (CE) was prepared using a 70% single walled carbon nanotube graphite powder and 30% mineral oil. DNA

immobilized carbon nanotube paste electrode (DCE) was prepared using a 40% DNA (double-stranded calf thymus sigma reagent), 40% single-walled carbon nanotube graphite powder (Nanostructured & Amorphous Materials, Inc.) and 20% mineral oil. The mixture was homogenized in a mortar for 5 minutes. The small amount was inserted into a plastic needle type capillary tube (1.5 mm in diameter and 5 cm in length) using a 0.5 mm diameter copper wire connected to the electrochemical workstation. A platinum counter of 0.5 mm diameter Pt wire, and a Ag/AgCl reference electrode was used in experiment. Thiram was obtained from Aldrich (Steinheim, Germany). In living samples, 10.381 g of sesame leaf was grinded and got its juice. Juice was dissolved in 10 ml distilled water. First, 0.1 ml of sesame juice was spiked and 0.1, 0.2 and 0.3 ml standard was spiked thereafter.

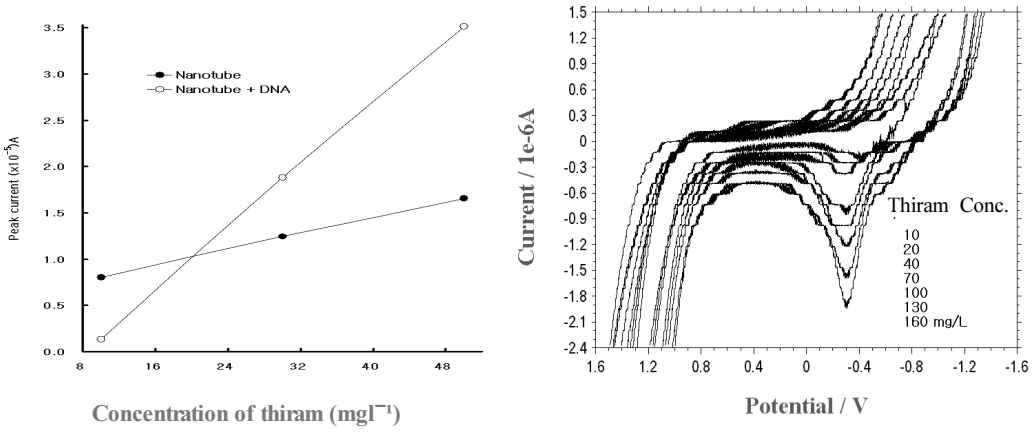
3. Results and Discussion

3.1. Comparison of cyclic voltammetric sensor

Fig. 1(a) shows the result of DNA immobilization effects. When a blank solution of 10 mg/L thiram was added, the peak currents of CE and DCE obtained 0.8015 x 10^{-5} A and 0.1352 x 10^{-5} A, respectively. When 30 and 50 mg/L were added, those of CE attained 1.2443 x 10^{-5}A and 1.656 x 10^{-5} A, respectively and those of DCE obtained 1.656 x 10^{-5} A and 3.515 x 10^{-5} A. respectively It is shown that the slope of DCE is more sensitive than CE. Thus, it was chosen as a working electrode. Fig. 1(b) shows the CV effect. The thiram concentration was varied from 10 mg/L to 160 mg/L. The peak current was observed at -0.3 V. Higher concentration caused a spike in the curves which were sensitive and sharp.

3.2. SW stripping voltammetric parameters

SW optimized conditions were examined using DCE. As shown in Fig. 2(a), the peak current obtained 10.98 x 10^{-7} A at pH 2.18. It decreased continuously and reached to 1.064 x 10^{-7} A at pH 3.67, then increased to 16.42 x 10^{-7} A at pH 4.78 and dropped again. Here maximum current was attained at pH 4.78 and it was chosen as an optimum pH strength. Fig. 2(b) shows that the peak current increased continually as much time was given. The peak current finally reached to 23.48 x 10^{-6} A at 500 sec and it was the maximum current. Accumulation time of 500 sec was chosen as an optimum condition.



(a) (b)

Fig. 1. (a) The comparison of two electrodes for 10, 30 and 50 mg/L variation by SW. (b) The CV scan using DCE ranges from 10 to 100 mg/L add in the 0.1 M phosphate at 4.0 pH electrolyte.

Fig. 2(c) shows that the peak current obtained 3.416 x 10^{-6} A 0.1V and increased to 4.184 x 10^{-6}A at 0.15V, then decreased. So 0.15 V was chosen as an optimum condition. Fig. 2(d) shows that the peak current obtained 5.065 x 10^{-6} A at 0.005 V. After that, it decreased quickly from 0.01 V to 0.025 A. It increased again from 0.03 V to 0.035 V, but it dropped again. Maximum current was attained at 0.005 V. The other optimum parameters were -1.2 V initial potential and 550 Hz frequency.

3.3. Statistics and applications

Under optimum conditions obtained from above section, low concentration of thiram was examined using CV and SW. Fig. 3(a) shows the CV voltammograms and their statistic equations in the range from 10 to 80 ng/L concentration. The peak current varied from 0.24 x 10^{-6} A to 2.358 x 10^{-6} A.

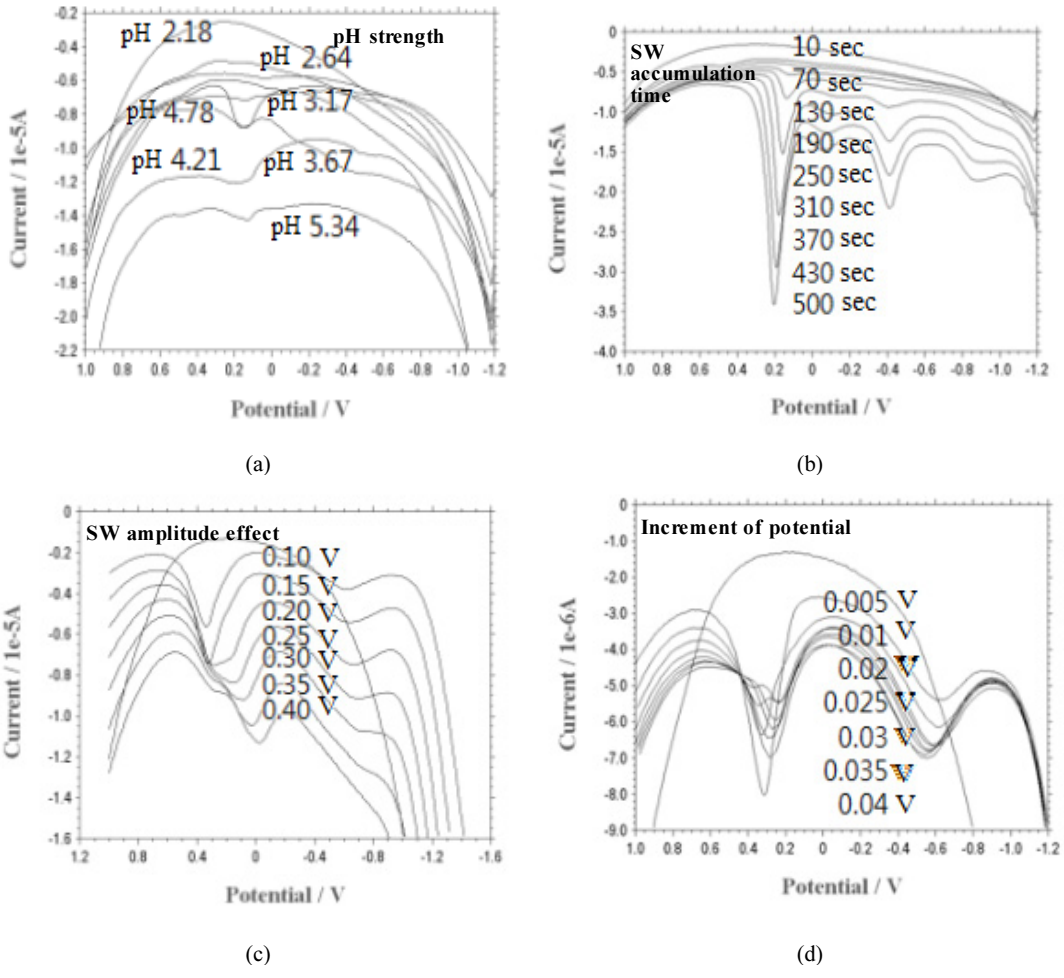
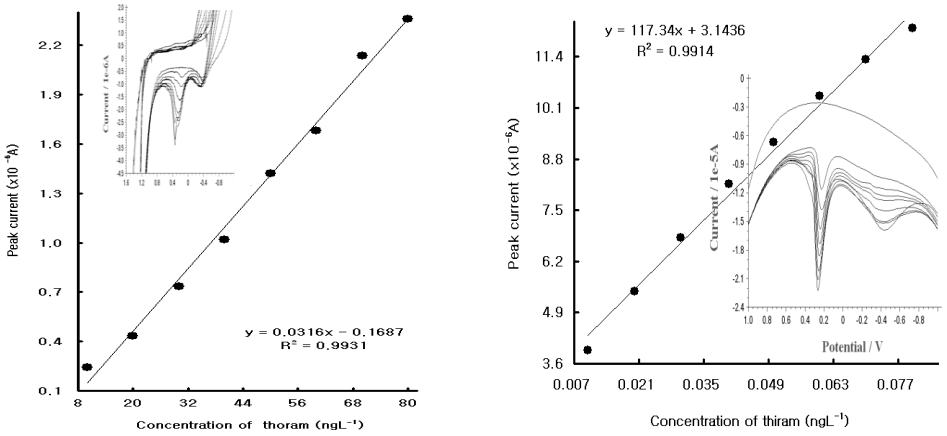
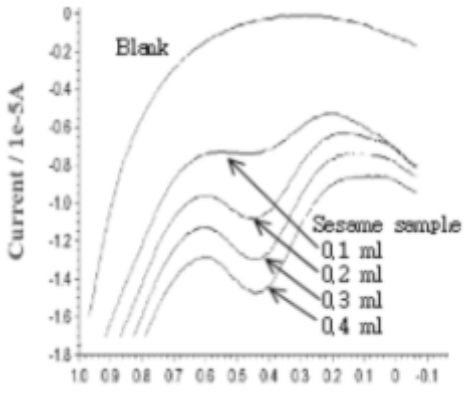


Fig. 2. The examination of various optimum conditions with SW using DCE (Initial potential: -1.2 V, frequency: 550 Hz). (a) pH strength was varied from 2.18 to 6. (b) SW accumulation time was varied from 10 to 500 sec. (c) SW amplitude was varied from 0.1 V to 0.4 V. (d) The increment potential was varied from 0.005 V to 0.04 V.

The peak were narrow and sharp and they spiked with higher concentration. Statistics equation of CV results was y=0.031X-0.168 and R^2=0.993. Moreover, SW results are better sensitive then CV, Fig. 3(b) illustrates the SW real voltammograms in the nano ranges of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07 and 0.08 ng/L concentration. When 0.01 ng/L was spiked, 3.95 x 10^{-6} A was obtained. The peak current increased quickly and reached to 12.12 x 10^{-6} A at 0.08 ng/L concentration. The obtained linear equation was y=117X+3.143 and precision was R^2=0.991. It is considered that the voltammograms are sharp and usable for trace detection in any field. Under this condition, in vivo vitro detection and analytical detection limit was examined using a sesame leaf. Fig. 3(c) SW shows the result obtained for the sesame leaf by standard addition method. 10 ml electrolyte blank, 0.1 ml sesame juice, and 0.1, 0.2 and 0.3 ml standard spike was added. The peak current was obtained of 0.6488, 1.717, 2.151 and 2.348 x 10^{-6} A.



(a) (b)



(c)

Fig. 3. (a) The CV effect using DCE in the nano ranges from 10 to 80 ng/L. (b) The SW working ranges from 0.01 to 0.08 ng/L variation using optimum parameters. (c) The result applied the developed sensor to a sesame leaf using standard addition method.

Linear equation was y=0.5532X+0.333 and precision was R^2=0.885. The pesticide thiram was detected in the sesame leaf, and its concentration calculated from this linear equation was 0.006 mg/L.

4. Conclusion

Electrochemical instruments of voltammetric measurements with DNA immobilized on a carbon nanotube sensor (DCE) was developed and it was applied to detect a pesticide thiram in a sesame leaf. The optimized conditions were as follows 550 Hz frequency, 0.15 V amplitude, 0.005 V increment potential, -1.2 V initial potential, 4.78 pH, and 500 s accumulation time. With these conditions, very high detection capacity was obtained. When this sensor was applied to the detection of pesticide in a sesame leaf with using standard addition method, this developed sensor could detect the pesticide thiram even in the low concentration. So, it could be considered that this analytical method can be expanded to the detection of the small amount of pesticide left in agricultural products and foods.

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