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Electrochemical behaviour of cross-linker glutaraldehyde as a receptor for carbaryl biosensor

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Glutaraldehyde (GA) is a famous cross-linker that is used for immobilization of enzyme or biologic material on electrochemical biosensor electrodes. It has an important role in pesticide biosensor studies. Because of the inhibitor effect of pesticides on acetylcholine esterase enzyme (AChE), it is generally immobilized on the electrode by cross-linking method. Glutaraldehyde is used as cross-linker agent to immobilize the enzyme on the electrode surface. Acetylcholine esterase activity is inhibited by pesticides or other neurotoxic compounds. This inhibition helps us to detect the pesticide residual. Although numerous electrochemical pesticide biosensors are based on generally the immobilization of the enzyme in the literature, an enzymeless biosensor based on GA has been developed in this study for the first time. Enzyme biosensors have high cost and needs special storage conditions.

In this study, glutaraldehyde detected the carbaryl pesticide as sensitive as an acetylcholine esterase enzyme biosensor. The biosensor electrode was constructed with coating GA on glassy carbon electrode (GCE). Thus, it is a simple, low cost and practical method for pesticide detection in the water samples with low detection limit of 5×10^{-9} mol L^{-1}.

Keywords: Carbaryl, pesticide biosensor, glutaraldehyde, acetylcholine esterase, acetylthiocholine chloride.

Introduction

Glutaraldehyde has been used for fixation of tissues since 1960s. Glutaraldehyde reacts reversibly with amino groups over a wide pH range (≥ pH 3.0). Between pH 7.0 to 9.0 only a little reversibility is observed[1].

High reactivity of glutaraldehyde toward proteins at around neutral pH is based on the presence of several reactive groups in proteins. Molecular forms of glutaraldehyde in aqueous solution, lead many different possible reaction mechanisms. Glutaraldehyde exists in multiple forms in aqueous solution. It has at least 13 different forms depending on solution conditions such as pH, concentration, temperature, etc.[2]. Different molecular forms of glutaraldehyde in aqueous solution, lead different reaction mechanisms.

Pesticides (herbicides, fungicides and insecticides) are used widely in agriculture and industry for their toxic effect on living organisms. Their toxicity arises mainly due to their inhibitory effect on acetylcholine esterase, a key enzyme for the nerve transmission[3]. Carbamates are carbamic acid derivatives and cholinesterase (ChE) inhibitors. A member of carbamate family which is called carbaryl is a chemical used in agricultural activities as an insecticide. Carbaryl (1-naphthyl-1-methylcarbamate) is not only a powerful inhibitor of acetylcholine esterase activity in the organism but also presents a potential teratogenic capability, thus requiring continuous monitoring in food and potable waters[4]. Carbaryl is reported as a human carcinogen and mutagens with serious risks on the blacklist released by the United States Environmental Protection Agency (EPA)[5,6]. Extensive usage of carbaryl in agricultural activities makes the detection of residual amount essential for public health and ecological health. There are many detection techniques including spectrophotometry, infrared spectroscopy, flow-injection chemiluminescence, fluorimetry, gas or liquid chromatography[3–8].

In recent years AChE biosensors were developed to overcome the need of these relatively more expensive instrumentation, time consumption and use of higher amounts of toxic organic reagents[9–19]. In many studies, glutaraldehyde

was used as cross-linker of the AChE enzyme for biosensor studies[9–13]. Glutaraldehyde binds the amine groups of organic molecules[2,10]. This property provides the cross-linker function of the glutaraldehyde. However, AChE biosensor has the features of hard preparation, high cost and low stability problems. Thus, mimetic enzyme sensor or enzyme-free (enzymeless) sensor studies came into prominence in recent years because of their advantages of easy preparation, low cost, long term stability and high sensitivity[20–24]. Enzymeless sensors were investigated by different electrochemical methods, including voltammetry, electrochemical impedance spectroscopy, chronocoulometry and chronoamperometry[25].

In this novel study, glutaraldehyde is used as receptor material of pesticide biosensor. It is not used as a well-known cross-linker. Some interesting results were obtained for detection of carbaryl pesticide. The biosensor electrode coated with glutaraldehyde detected the carbaryl almost as sensitive as an acetylcholine esterase enzyme biosensor.

Experimental

Materials and reagents:

A glassy carbon electrode (GCE) was polished with 5 μm sized alumina solution (Buehler, No. 40-6351-006) and cleaned with an ultrasonic bath (Bandelin Sonorex) at 40°C in ultra-pure water. The cleaned electrode was coated with 2% (v/v) glutaraldehyde (Sigma-Aldrich G6257, %25 in H_2O) which was diluted with ultra-pure water (18 MΩ). The coating was dried in a vacuum oven (nüve, EV018) and electrochemical measurements were applied with potentiostat (GAMRY Instruments, Reference 600 Potentiostat/Galvanostat/ZRA). In measurement studies phosphate buffer solution (PBS) (Sigma, P3619-1GA) was used as cell solvent and lithium perchlorate (Aldrich, 431567) was used as ion carrier reagent in the solution. In order to control the enzyme-glutaraldehyde interaction, acetylcholinesterase enzyme (from electric eel, Sigma C3389-500 UN) and acetylthiocholine chloride (Sigma, A5626-5G) substrate were used. Carbaryl (Fluka, 36856,100 ng/μL cyclohexane) was added to the electrochemical cell as enzyme inhibitor and analyte.

Electrode preparation:

Experimental design of sensor electrodes were exhibited in Fig. 1. A glassy carbon electrode (GCE) was polished with 5 μm sized alumina solution and immersed in an ultrasonic bath at 40°C including ultra-pure water. Finally it was rinsed with ultra-pure water and dried.

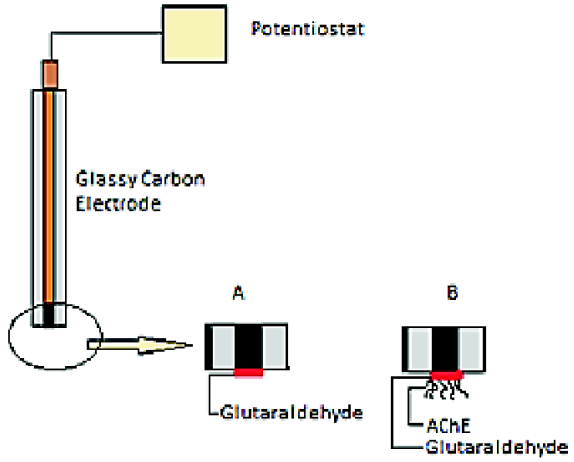

Fig. 1. Experimental design of glassy carbon electrode for (A) GA and (B) GA-AChE electrodes.

Group A electrodes: 10 μL volume of 1%, 2%, 3% and 25% concentrated solution of glutaraldehyde was dropped on the glassy carbon electrode surface and dried in a vacuum oven for 30 min to prepare A1, A2, A3 and A25 electrodes, respectively. After drying process, the electrodes were immersed in the lithium perchlorate salt containing phosphate buffer solution and electrical potential was applied for electropolymerization of immobilized GA on GCE.

Group B electrodes: 10 μL solution of AChE in 0.02 mol L^{-1} phosphate buffer solution was dropped on the surfaces of A1, A2, A3 electrodes prepared without electropolymerization of GA and dried in a vacuum oven at room temperature for 30 min to prepare B1, B2 and B3 electrodes, respectively. After the enzyme solution had been dried on the electrodes, they were exposure to electrochemical polymerization of GA to immobilize the AChE enzyme on GCE. In order to examine the cross-sectional area of Group B electrodes with a scanning electron microscope (SEM), an indium tin oxide-glass electrode was coated with the same procedure.

Measurement:

3 ml 0.02 mol L^{-1} phosphate buffer solution (pH 7.8) was loaded in an electrochemical cell and lithium perchlorate elec-

trolyte salt was added into this solution. An Ag/AgCl reference electrode, a modified GCE as working electrode and a Pt counter electrode were used for measurements. Scanning rate was 100 mV/s. Detection of carbaryl pesticide was studied with Group A electrodes. For Group B electrodes, acetylthiocholine chloride (ATCl) was added to the cell solution and after the reaction between ATCl and AChE enzyme had been occurred, carbaryl pesticide was added step by step with small amounts to the solution as an inhibitor. The inhibitory effect of carbaryl on AChE-ATCl reaction lead a decrease in the current intensity. The cyclic voltammetry (CV) and square wave voltammetry (SWV) techniques were applied on these systems to follow the interactions between GA carbaryl for Group A electrodes and AChE ATCl carbaryl for Group B electrodes.

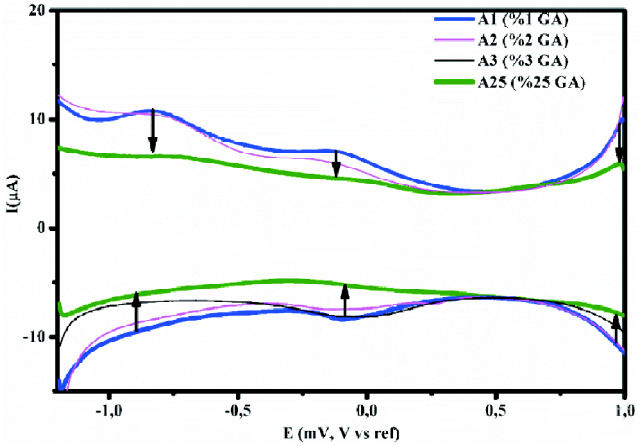

Fig. 2. A square wave voltammetry diagram of A1, A2, A3 and A25 electrodes.

Results and discussion

In Fig. 2, square wave voltammograms of Group A electrodes; A1, A2, A3 and A25 were exhibited. The current intensity of GA peaks of A25 electrode was lower than A1, A2, and A3. Because concentration of GA solution used for A25 electrode was higher than the other A group electrodes. Thus film thickness of the electrode was also higher. This thickness made the diffusion of the PBS solution and electrolytic ions through the receptor film harder. On the other hand, diluted GA solution deposition on the electrode surface formed thinner film and the peaks which belong to GA were observed more clearly. After these results 1%, 2% and 3% of GA solutions were used for further studies.

The polymerization reaction occurs when the electrical potential is applied to the electrode between –1.5 mV and 1.0 mV (Fig. 3). The reaction, given below, exhibits the polymerization reaction products as polyglutaraldehyde and water^2. The peak at –0.7 mV was observed after the peak at –0.2 mV had been achieved. These peaks show the polymerization of the GA by the reversible displacement of double bonds in the polymer.

When a small amount of carbaryl pesticide was added to the cell solution, the pesticide molecules bound to the polyglutaraldehyde and water formed as exhibited in Fig. 4. The bound pesticide molecules removed the double bound oxygens and the peak at –0.7 mV disappeared. This interaction lead the detection of carbaryl residuals. A specific peak of GA at about –0.7 mV potential and a reversible peak about –0.2 mV:0.0 mV were observed (Fig. 5).

After that result, 10 μL of 6×10^{-5} mol L^{-1} carbaryl was added to 3 mL 0.02 mol L^{-1} PBS in the electrochemical cell and 2×10^{-7} mol L^{-1} carbaryl concentration was obtained. Pure N_2 gas was stacked to the cell system and solution was mixed with N_2 purge to get a homogenous and oxygen free

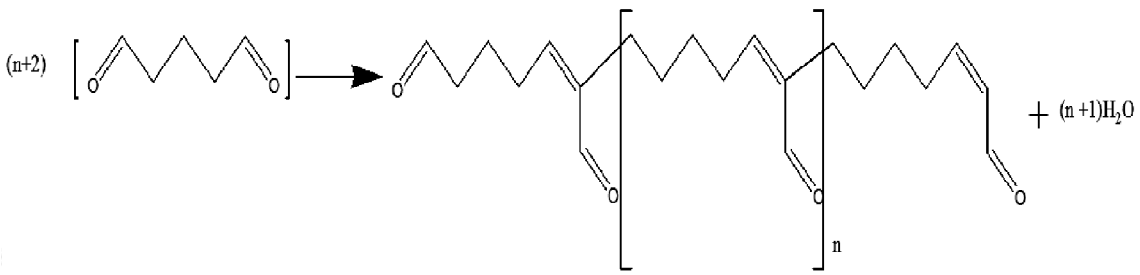

Fig. 3. Reaction mechanism of the polymerization of GA.

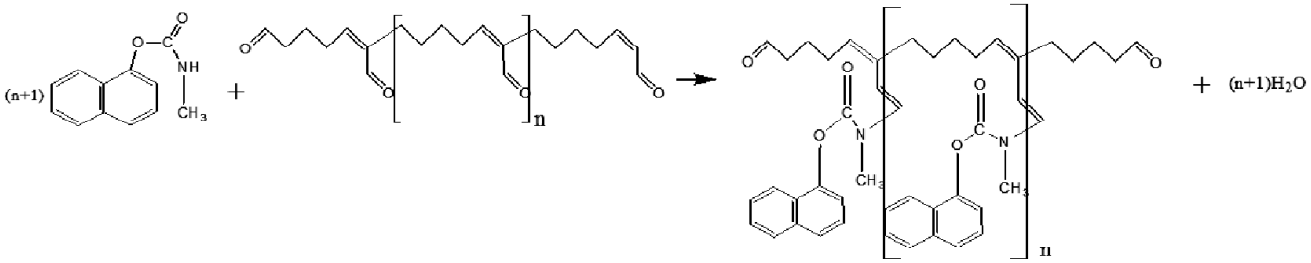

Fig. 4. Chemical interaction of polyglutaraldehyde and carbaryl.

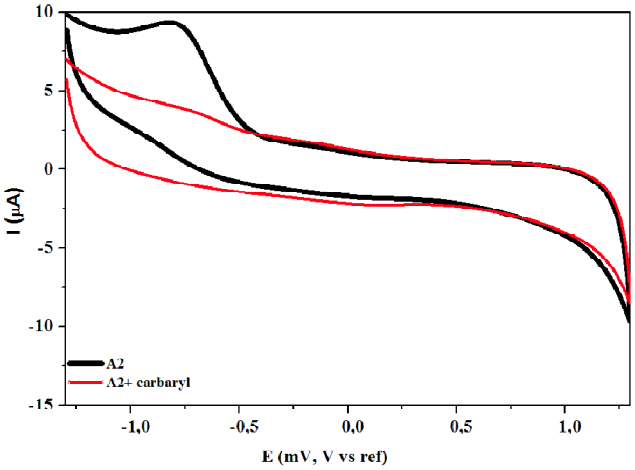

Fig. 5. Cyclic voltammetry analysis of A2 electrode and its interaction with carbaryl.

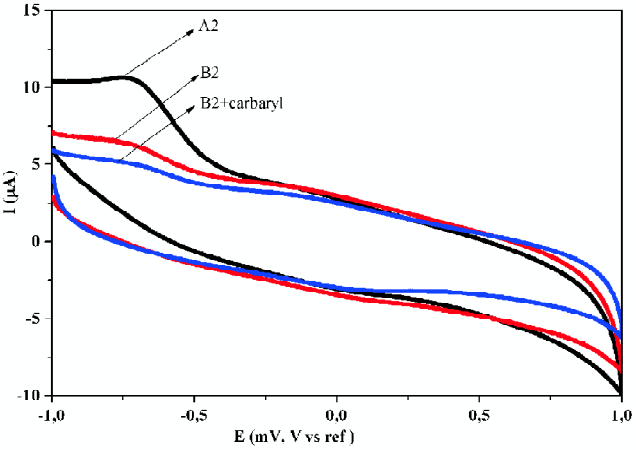

Fig. 6. Cyclic voltammogram of A2, B2 and B2-carbaryl.

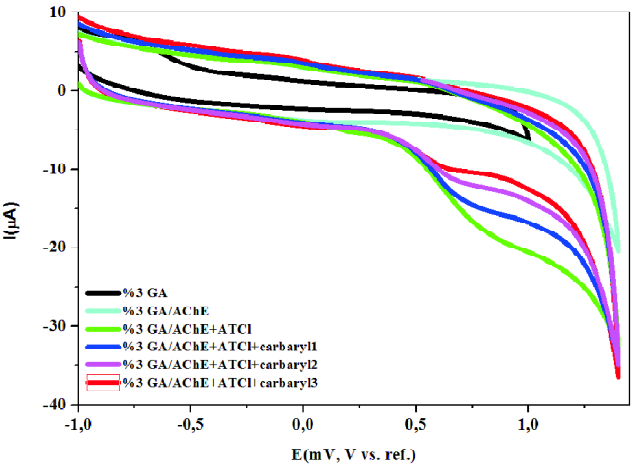

Fig. 7. CV diagrams of A3 and B3 interaction with carbaryl.

solution. In this homogenous solution a small amount of pesticide was already sufficient to inhibit the activation of GA film. The peak about –0.7 mV potential was disappeared while the other reversible peak remained as a stable peak.

In Fig. 6, the specific GA peak was observed in the cyclic voltammetry measurement of A2 electrode. B2 electrode was immersed in the same electrochemical cell which was filled with 3 mL 0.02 mol L^{-1} PBS and 0.05 mol L^{-1} lithium perchlorate reagent. Cyclic voltammogram of B2 electrode exhibits the effect of AChE layer on the GA peak amplitude clearly. AChE enzyme layer forms a resistance against the PBS diffusion to GA layer and the amplitude of GA peak at –0.7 V decreases. When the carbaryl was added, the peak amplitude was decreased.

In Fig. 7, A3 and B3 cyclic voltammograms are shown. First 3% of GA coated GCE was observed with a CV measurement. In this measurement, a specific GA peak was obtained at about –0.7 mV. Then a GCE electrode which was coated with 3% of GA solution and AChE enzyme respectively was investigated with cyclic voltammetry analysis. There was a slight GA peak because of the immobilized enzyme

on the GA layer. At first enzyme layer contacts with electrolytic solution in the cell, then it reaches to the GA layer by diffusion through the enzyme layer. After the addition of acetylthiocholine chloride substrate, a peak at about +0.6–0.7 mV was appeared. After addition of carbaryl insecticide (5×10^{-9} mol L^{-1} in the cell solution), the peak was extinguished by the inhibition of AChE enzyme. The amount of carbaryl was increased gradually. CV diagrams of 3% GA/AChE + ATCl + carbaryl 1–3 exhibited the enzyme inhibition with disappearing GA peak by carbaryl addition. When there is GA and AChE enzyme on the biosensor electrode, both of them interact with carbaryl at the same time[15,26].

Conclusions

Scientists who study on developing pesticide biosensor mostly use AChE enzyme and the inhibitor effect of pesticides on this enzyme. However, this is a high cost method and it is not convenient for practical use of pesticide biosensors. Biomolecules such as enzymes require special conditions especially for their instability during storage and long-term use.

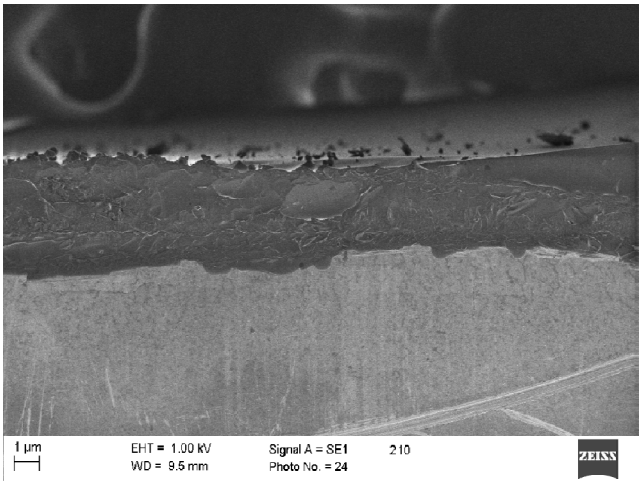

Fig. 8. SEM image of the cross section of the B2 electrode at 210 magnification.

In order to overcome stability problems of biomolecules, biomimetic binders and catalysts have been generated for biosensors[27,28]. Although numerous electrochemical pesticide biosensors, based on generally the immobilization of the enzyme and mediators with cross-linker glutaraldehyde were constructed in the literature, an enzymeless biosensor based on GA has been developed in this study for the first time. The biosensor prepared with GA coated GCE is an original, simple and low-cost method for pesticide detection. The cross section image of the indium tin oxide-glass electrode was obtained with the SEM technique at 210 magnification (Fig. 8). A GA film about 2 µm and AChE enzyme entrapped on the film were observed.

The detection limit of developed sensor was 5×10^{-9} mol L^{-1} for carbaryl pesticide. This sensitivity of the GA based biosensor indicates its possible application for the determination of carbaryl in water samples.

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