The Research of photonic-crystal fiber sensor

Yong Peng *[a], Yi Cheng [a]
Department of Physics, Dalian Maritime University, No.1 Linghai Road, Dalian, China 116026

ABSTRACT

To study the photonic-crystal fiber applied in the chemical sensor, the photonic-crystal fiber was used as transmission medium. With Sol-Gel method, we selective coated thin film containing fluorescent probe in the photonic-crystal fiber core, then attained an excellent photonic-crystal fiber acetylcholinesterase sensor. The sensor could be applied in biological / chemical research, clinical medicine, environmental protection, food inspection, biochemical preventive war field and so on. In organophosphorus pesticide residue testing, the experimental results indicated that the linear measurement range could arrive to $1\times10^{-9}\sim1\times10^{-3}$ mol/L, moreover the detection limit is 1×10^{-10} mol/L.

Keywords: Photonic crystal fiber, Sol – gel, Acetylcholinesterase biosensor, Organophosphorus pesticide

1. INTRODUCTION

Fiber chemical / biological sensor is a special sensor, which is relative to chemistry, physics, information science and other related technology. These sensors have become one of the warmest research subjects at home and abroad. Specially, it is the birth of photonic crystal fiber (PCF-Photonic Crystal Fiber) that promotes chemical / biological sensor developing. Compared with conventional fiber, the PCF has the following features: flexible design structure, various hole structure, great refractive index difference between core and cladding, many kinds of core forms, and cladding refractive index changing with wavelength. PCF had two characteristics, which prompted a wide application in sensor field. One was the light transmitting in the optical fiber which reacted with small volume gas or liquid in the air hole could form a long interaction; the other was the optical fiber and number of waveguide flexibility design. In recent reports, it shows that PCF has become the outstanding choice direction on a new generation of optical fiber chemical / biological sensors[1-5]. PCF sensors applying research have made successive progress.

We took PBG fiber as transmission medium, with Sol-Gel method, selective coated containing fluorescent probes thin film in PBG fiber. Thus we attained a superior PBG fiber acetylcholinesterase biosensor. Sensor could be applies in biological / chemical research, clinical medicine, environmental protection, food inspection, biochemical war prevention filed and so on. Organic phosphorus, amino acid lipid pesticides are widely used in China, however these types pesticides are usual high harmful pesticides. PBG fiber optic biosensor used acetylcholinesterase extracted from sensitive sources enzyme as detection reagent. We based on acetylcholinesterase activity change to detect residues in fruits and vegetables.

2. THE THEORY

Acetylcholinesterase (AChE - Acetylcholinesterase) could selective catalyse hydrolysis substrate, moreover its catalytic activity could be inhibited by organophosphate or carbamate pesticide. We used the characteristic to produce biosensors which could test organic phosphorus pesticides. With acetylcholine (ACh - Acetylcholine) as substrate, in the AChE catalysis function, the following reactions could occur:

$$\text{Acetylcholine} + H_2O \xrightarrow{AChE} \text{choline} + CH_3COOH \text{ (acetic acid)}$$

Because of substrate (ACh) hydrolysis produced Proton, Proton can quenching pH-sensitive fluorescence reagents and generated fluorescent. According fluorescence intensity changes we could monitor the substrate hydrolysis extent [6]. Organic phosphate and carbamate insecticides on lipid pesticides could inhibit acetylcholinesterase, which would lead to substrate hydrolysis level decreasing. Then proton which generated in hydrolysis reduced, thereby, it reduced fluorescence quenching. Though detecting fluorescence intensity changes, we could indirectly obtain organic pesticide

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*pengting25@163.com; phone 13841127315; fax 0411-84729331

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content. Inhibition rate is used to describe pesticides inhibiting enzyme degree, the formula is

$$I = (A_0 - A_t)/A_t \times 100\% \quad (1)$$

I ：Inhibition rate, A_0 ：normal values, A_t ： sample solution value.

3. THE RESEARCH OF AFFECTING BIOFILM SENSITIVITY FACTORS

3.1 Reagents, equipment and producing sensitive films

Reagent and instrument: choosing pure silicate B lipid (TEOS), anhydrous ethanol, hydrochloric acid (HCl); Luciferase - acetylcholinesterase (AChE) and acetylcholine (ACh) which were purchased in Shandong Peng Jing biological medicine company and Zibo Hertz biotechnology company respectively.

Silica Solution fabricating: B lipid silicate (TEOS), anhydrous ethanol, acidified deionized water (100ml deionized water droplets and 3-5 HCl) were mixed and stirred according the volume ratio 10:2:5. After reflux 1.5 hours at 60 ° C, solution was absolutely clear, then solution SiO2 could be got. Hold A solution 5ml, mixed different FITC-AChE solution B and A solution basing on a certain volume. At room temperature, after uniformly mixed and kept 24 hours aging, sol C of FITC- AChE doped silica could be gained. sol C of different doped ratio was coated on silica glass, FITC - AchE-doped different radio silica gel biological sensitive film. These biological sensitive film was rinsed one minute in the deionized water, consequently, rising out the bioactive molecules on sensitive film surface in order to using later.

3.2 The radio of enzyme and Sol affecting biofilm sensitivity

A certain amount of substrate solution were coated evenly on the biofilm, and with the fluorescence spectrometer (USB2000 type, Ocean optics Inc), detecting biofilm sensitivity to substrate. The measurement results were shown in figure 1.When fixing less FITC-AchE on the film, because luciferase (FITC)under the excitation source exciting produce weak fluorescence intensity, less AChE couldn't play strong role on the substrate hydrolysis catalyst. As a result, the fluorescence intensity did not change significantly. With the increase of doping FITC-AChE, sensitivity improved and response time became short, then fluorescence intensity corresponding increased.

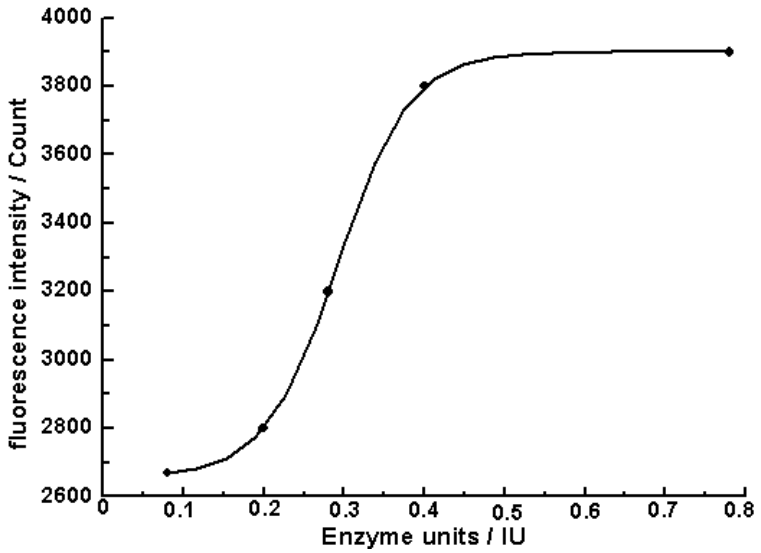

Fig. 1. The radio of enzyme and Sol affecting biofilm sensitivity

When AChE amount was over 0.4IU, substrate hydrolysis tended to the maximum, the response output changed flat. Used appropriate FITC-AChE loading, it would help to reduce barriers in the membrane and improve substrate and the product diffusion rate in the membrane. To low concentration pesticides detection of high sensitivity and short response time could be achieved.

3.3 Buffer concentration affecting biofilm sensitivity

When FITC-AChE amount was 0.4IU and substrate (ACh) concentration was 5×10^{-4} mol / L, we changed phosphate buffer pH value. Then inspecting phosphate buffer pH value affected sensitive biofilm response properties, and the results were given in figure 2. PH value was in the range of 7.0-9.0, the respondence of biofilm to the substrate (ACh) was mostly sensitive. Because organophosphorus pesticides in PH equal to 8.0 was prone to hydrolysis, in order to maintain a higher sensitivity in the When FITC-AChE amount was 0.4IU and substrate (ACh) concentration was 5×10^{-4}

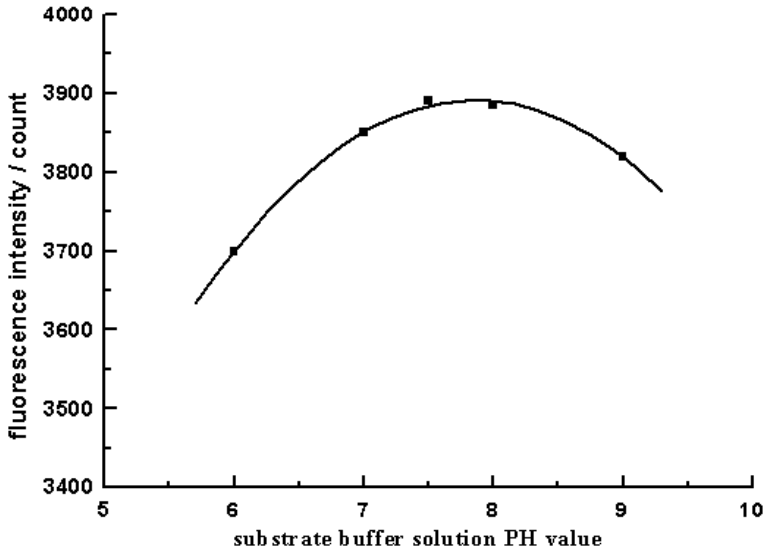

Fig. 2. Buffer pH value affecting biofilm sensitivity

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3.4 Substrate (ACh) concentration affecting the biofilm sensitivity

FITC-AChE in the amount 0.4IU, substrate (ACh) concentration 5×10^{-4} mol / L, we changed the concentration of phosphate buffer, the buffer on the inspection of sensitive film sensitivity of its results in fig.3 indicated. When FITC-AChE amount was 0.4IU, phosphate buffer pH value was 8, substrate (ACh) concentration was 5×10^{-4} mol/L,we changed phosphate buffer concentration.Then inspected buffer concentration effect on sensitive film sensitivity, and the results was given in figure 3. As seen from the diagram, the biofilm sensitive was contrary function of the buffer concentration.

When buffer concentration was low, sensitive biofilm had stong response, low measurement limit and short response time. This phenomenon can be explained as follow: when buffer concentration was high, excessive alkaline buffer ion doped in the enzyme sensitive layer, which suppressed the enzyme hydrolysis function. As a result, substrate hydrolysis rate slowed down, the hydrogen ion hydrolysis reduced, biofilm response speed reduced.

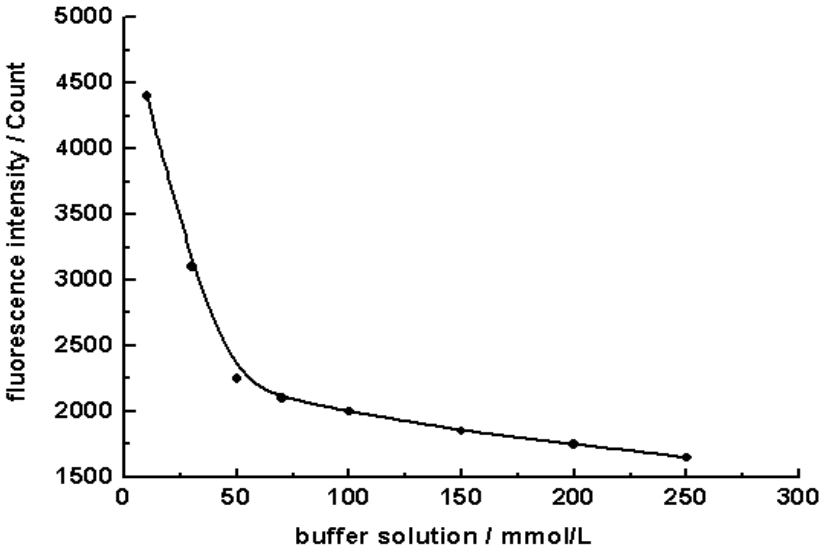

Fig. 3. Buffer concentration affecting biofilm sensitivity

3.5 Substrate (ACh) concentration affecting the biofilm sensitivity

When phosphate buffer concentration was 25mmol/L, pH value was equal to 7.5, ITC-AChE amount was 0.4IU, we measured that different substrate concentration had effect on the biofilm sensitivity. Experimental results indicated that substrate concentration was in the range of $4\times10^{-6}\sim10^{-3}\,\mathrm{mol/L}$ and the fluorescence intensity was linear to the substrate concentration.

4. SENSOR PROBE FABRICATING AND SYSTEM COMPONENTS

When two ends of 30 centimeters long PBG fiber (HC-850-01 Blazephotonics)were polished, put them in ultrasonic cleaning instrument and placed half an hour, then got out of ultrasonic treatment, put PBG fiber core liquid out, at last put them inside dryer, dried half an hour at 80 ° C. We used UV curable adhesive to locate PBG fiber center hole. Based on capillary principle, the optimal ratio AChE – FITC silica sol was selective injected in PBG fiber core (Sol height was 4cm). Then the coating sensitive membrane in fiber core, after coating it was preserved at 4□reserve. PBG fiber acetylcholinesterase biosensor consists of three parts: excited light source, coated PBG fiber and signal detection unit. Argon ion laser (488nm wavelength) sent light and input in PBG through 1×2 optical fiber coupler. Because of the excitation wavelength light, the PBG core inner FITC fluorescence produced fluorescence (520 nm wavelength), the emission light input into fluorescence spectrometer detection analysis through fiber coupling.

5. EXPERIMENTAL RESULTS

Prepared standard solution, which was 25mol / L phosphate buffer (pH = 7.5), substrate (ACh) concentration was 5×10^{-4} mol / L, then let a certain amount organic pesticide dissolved in standard solution, after uniformly mixed then got solution sample. According capillary principle the sample solution was input in PBG fiber sensitive area, then continued to the comparing detection. Organophosphate pesticides acted on inhibition AChE with time change shown in figure 4, the residual activity of enzyme began rapidly reduced after 10 minutes, 10 minutes inhibition completed 95%. In the pesticides determination process, incubating time was 10 minutes.

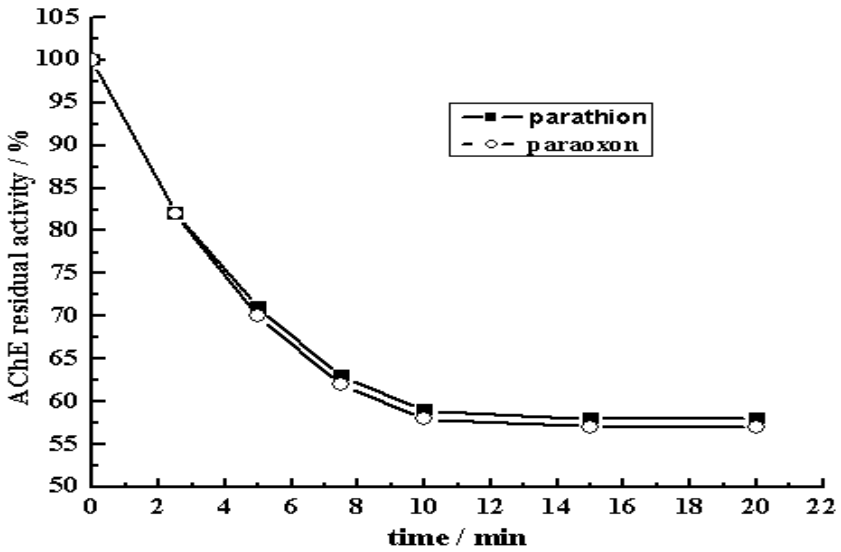

Fig. 4. The changes of organophosphorus pesticides actingon AChE with time

Because of organophosphorus pesticides inhibit AChE, which caused substrate (ACh) hydrolysis and proton concentration decreasing, the fluorescent sensitive to pH value producing fluorescence intensity was changing too. Experimental results show that organic pesticides percentage of inhibition and the pesticide concentration logarithm is a linear relationship in some area. Theoretically detection limit defined the 10% inhibition rate corresponding concentration of inhibitor value. Linear regression equation and correlation coefficient and detection limit were shown as the table:

Table 1. The changes of organophosphorus pesticides actingon AChE with time

pesticide	linear range（mol/L）	linear regression equation	correlation coefficient（γ）	detection limit（mol/L）
Parathion	$1\times10^{-9}\sim1\times10^{-3}$	$I=12.05\lg C+120.2$	0.992	1.2×10^{-10}
Paraoxon	$1\times10^{-9}\sim1\times10^{-3}$	$I=11.23\lg C+120.2$	0.995	1.5×10^{-10}

6. CONCLUSIONS

In summary, we used Sol - gel method to coating doped FITC-AChE SiO2 films in the PBG fiber core wall in this paper. The PBG fiber acetylcholinesterase biosensor we had got had high sensitivity to the substrate (ACh). In organic pesticide parathion (PIC) and paraoxonase (Paraoxon) determination, the linear measurement ranges could arrive to $1\times10^{-9}\sim1\times10^{-3}$ mol / L , and the detection limit was up to 10^{-10}mol/L . Comparing to the existing electrochemical biosensors and optical biosensor, it was higher than 1-2 grade [7 -9]. Experimental results shows that PBG fiber chemical / biological sensors contrasting with conventional fiber biosensor it has higher sensitivity, in the biological / chemical research, clinical medicine, environmental protection, food inspection and preventive war biochemical fields it also have broad prospects.

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