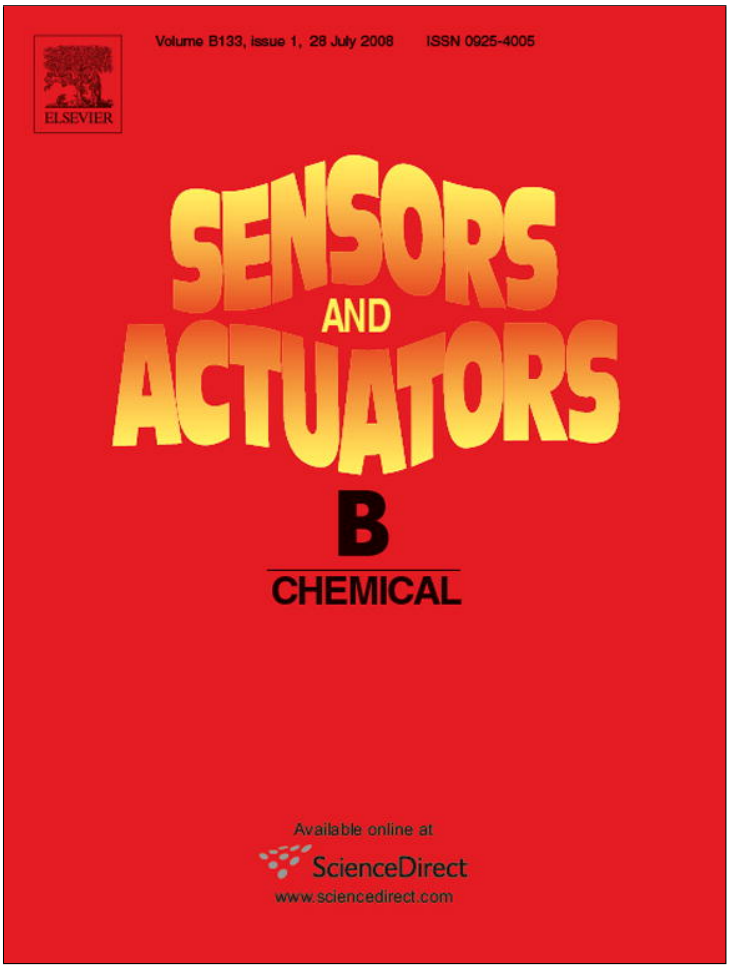

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An opto-fluidic ring resonator biosensor for the detection of organophosphorus pesticides

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Abstract

We developed a novel label-free opto-fluidic ring resonator (OFRR) biosensor for detection of an organophosphorus (OP) pesticide. The OFRR is based on a micro-sized glass capillary whose circular wall forms a ring resonator that supports the whispering gallery modes (WGMs). The WGMs has an evanescent field in the capillary core and interacts with the analyte flowing in the capillary. We used parathion-methyl as a model system to investigate the OFRR sensing performance in terms of bulk refractive index sensitivity, surface activation for affinity property, detection limit, and reproducibility. The performance of the OFRR was further compared with that of the Biacore 3000 SPR system. Our results show that the detection limit of 3.8×10^{-11} M for parathion-methyl was achieved with an analysis time of about 0.5 min, 10 times faster than the surface plasmon resonance (SPR) system. Furthermore, the OFRR biosensor demonstrated excellent reproducibility (R.S.D. = 3.5%, $n = 5$). The OFRR offers optical label-free detection mechanism with integrated microfluidics. It is a promising technology platform for development of portable multi-channel biosensors with high sensitivity, quick detection time, and sub-nanoliter detection volume.

Keywords: Ring resonator; Label-free; Refractive index; Microfluidics; Surface plasmon resonance; Pesticide detection; Parathion-methyl; Organophosphate

1. Introduction

Organophosphorus (OP) pesticides are a broad-spectrum insecticide used on a wide range of crops including vegetables, fruits, grains and ornamentals. They are designed to kill or repel pests but may be harmful and/or fatal to other organisms, including humans. Pesticides contribute significantly to overall cancer mortality [1]. They have the potential to cause adverse effects to the nervous system in humans at low concentrations and can be very ecotoxic to aquatic organisms, birds and bees. OP pesticides all act by inhibiting the nervous system enzyme acetylcholinesterase (AChE) and as such are termed anticholinesterase insecticides [2]. The phosphorylation of AChE and the resultant accumulation of acetylcholine are responsible for the typical symptoms of acute poisoning with OP compounds. In addition to acute health effects, OP compound exposure can result in chronic, long-term neurological effects [3].

According to the statistical data of the Pesticide Data Program (PDP) published by the United States Department of Agriculture (USDA) in 2005, overall, 73% of fresh fruit and vegetables and 61% of processed fruit and vegetables showed detectable residues. The tests screened for various insecticides, herbicides, fungicides, and growth regulators from 10 sampling states. Residues exceeding the tolerance were also detected in 0.2% of the 13,621 samples tested, which included fresh and processed fruit, vegetables, pork, bottled water, and drinking water for [4].

The most widely used methods for detection of organophosphate pesticides are high-pressure liquid chromatography (HPLC), and gas chromatography (GC) in combination with mass spectrometry (GC/MS) [2,5]. Although these methods offer quantitative analysis with sensitivity and selectivity, they are slow, expensive, and laborious. Therefore, they are not

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suitable for field applications. An alternative method is to use rather non-specific color reactions that result from changes in the activity of the enzyme AChE. While this is rapid, qualitative or semi-quantitative, it can give ambiguous results [6–8]. For food safety, quick and accurate tests are essential to allow detection of contaminated foods before they are distributed to consumers.

For these reasons, a portable biosensor that can be applied for on-site rapid detection of OP compounds and other pesticides is of great practical interest for overcoming such problems. Numerous biorecognition receptors have been studied for the detection of OP compounds, such as acetylcholinesterase [2,5], glutathione *s*-transferase [9], organophosphorus hydrolase [10], and whole cells [10,11]. Based on these biorecognition receptors, a number of different biosensors with different signal transduction mechanisms, including surface plasmon resonance (SPR) [12–16], fiber optic [17], photothermal [18], modified electrochemical [7,19–23], and biomarker [24–26] have been reported. Among them, optical label-free detection of analytes in their native forms has received great attention due to the real-time measurement of molecule–molecule interaction, high sensitivity, and excellent regeneration capability. A well-known label-free sensor is the SPR biosensor, which is commercialized in such instruments as the Biacore series, Spreeta, and IBIS sensor. However, these SPR systems are based on bulk optics and can measure only one channel or one analyte at a time.

The opto-fluidic ring resonator (OFRR) sensing platform is a recent advancement in opto-fluidic technology that integrates photonic sensing technology with microfluidics. It features a label-free sensing protocol, quick detection time, small sample volume, and accurate quantitative and kinetic results [27–31]. The most predominant advantage of the OFRR integrated with microfluidics is that we can potentially realize the multi-channel and portable biosensor that detects numerous analytes simultaneously, as shown in Fig. 1. Thus, it is useful to evaluate the OFRR and to establish a choice of models for detecting several hundreds of pesticide residues around the world. Simple fluidics design. Protein and DNA detection with the OFRR have been carried out previously, which showed a detection limit that rivals that of the SPR system [27,32]. In this paper, we developed a pesticide sensor based on the OFRR. The OFRR

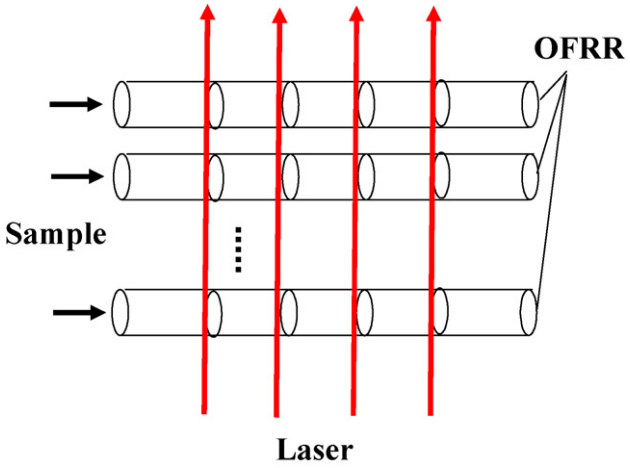

Fig. 1. A conceptual illustration of the multi-channel OFRR.

sensing performance was investigated in terms of bulk refractive index sensitivity, surface activation for affinity property, detection limit, and reproducibility by using parathion-methyl as a model system. The OFRR sensor was further compared with the standard Biacore SPR system to validate its sensing performance.

2. The OFRR biosensor

Fig. 2 displays a schematic of the OFRR sensing mechanism. The optical ring resonator is constituted in the circular cross-section of the micro-capillary fluidics. The capillary wall acts as a waveguide for repeatedly circulating light coupled in from a tapered fiber cable. Resonant frequencies, referred to as whispering gallery modes (WGMs), forms an integer number of wavelengths as indicated by the resonance condition, $2\pi Rn_{\text{eff}} = m\lambda$, where R is the capillary inner radius, n_{eff} is the effective refractive index (RI) experienced by the WGMs and is determined by the RI of the sample, capillary wall and the surrounding medium, and m is an integer number which represents the mode's angular momentum term [31]. The WGMs has an evanescent field that extends beyond the inner wall of the OFRR capillary. When analyte moves through the inside of the

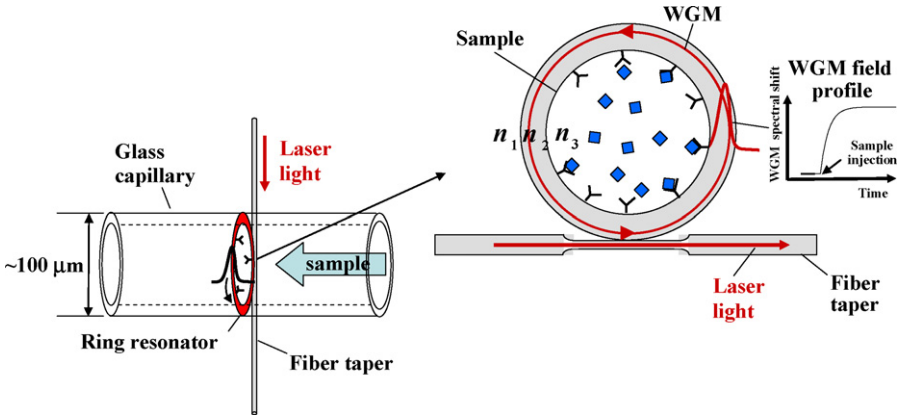

Fig. 2. The schematic diagram of the OFRR sensing platform.

capillary, it interacts with biorecognition receptors immobilized at the inner OFRR surface. Immobilization of the analyte causes the effective RI experienced by the mode to change, resulting in the resonant wavelength to shift spectrally, as shown by the above equation.

3. Experimental

3.1. Materials

Standard parathion-methyl pesticide, AChE from Electric Eel (EC 3.1.1.7), 98% ethanol, 10 mM NaOH (10 mM HEPES-NaOH (pH 7.4)), anhydrous methanol, 48% hydrofluoric acid (HF), 3-aminopropyltrimethoxysilane (3-APS), glutaraldehyde 70% (v/v), casein blocking buffer and phosphate-buffered saline (PBS), pH 7.4 were obtained from Sigma–Aldrich (St. Louis, MO, USA). The SPR device used to compare the OFRR performance was BIAcore 3000 (Biacore AB, Uppsala, Sweden). Common chemicals used in BIAcore 3000 were an amine coupling kit with 70% of glycerol, BIAdesorb solution 1 including 100 mM *N*-hydroxysuccinimide (NHS) and 400 mM *N*-ethyl-*N*′-(3-dimethylamino-propyl) carbodiimide hydrochloride (EDC), BIAdesorb solution 2 and 1 M ethanolamine pH 8.5. The sensor chip used was a commercial CM5 chip.

3.2. Experimental setup

The experimental setup is shown in Fig. 3. The OFRR was connected with tubing (#EW-06418-01, Cole-Parmer, Vernon Hills, IL, USA) for sample delivery using a peristaltic pump (Cole-Parmer, Masterflex #7562-10). The tapered fiber optic cable (#SMF28, Corning Inc., Corning, NY, USA) was connected on one end to a 1550 nm tunable laser (#QF939/251, Philips, Netherlands) and on the other end to a photodetector. The laser is scanned in wavelength. When the wavelength is resonant with the WGMs, the light is coupled into the OFRR, causing a decrease in the light intensity at the fiber output, as shown in the inset of Fig. 4. During the experiment, this intensity dip was used to indicate the WGM spectral position, which shifted

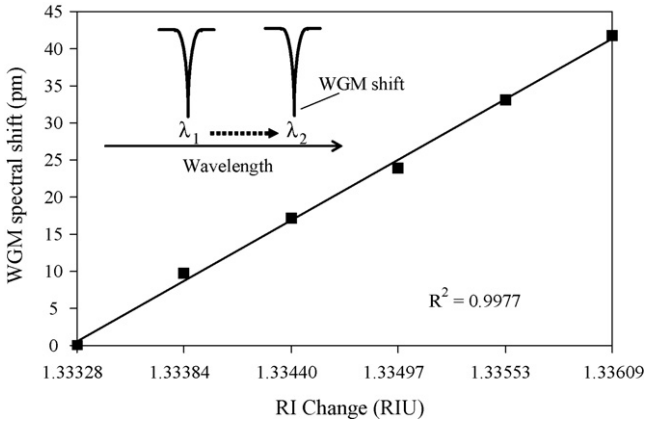

Fig. 4. OFRR bulk refractive index sensitivity is obtained by monitoring the WGM spectral shift when the OFRR core RI changes with various concentrations of ethanol in water.

when molecules bind to the OFRR inner surface. The microscope camera (GP-KR222, Panasonic Inc., Japan) was installed to observe the sample flow and a contact position between the OFRR and the tapered fiber optic cable. Tunable laser control and data collection were performed by a data acquisition (DAQ) card (#NI PCI-6221, National Instrument, Austin, TX, USA) in the PC, under the algorithms of code written in LabView program. After each scan, the sample values were stored in a file on the PC. The LabView program also displayed the measured voltage with a sensorgram graph in real-time.

To produce the OFRR with a thin wall, we stretched a commercially available glass capillary (#A120-85-10, Sutter Instrument, Novato, CA, USA) to thin its dimensions to expose an evanescent field at the inner surface [28]. The preform glass capillary was heated to the softening point while one end is pulled away from the heating zone and the other end is slowly fed into the heating zone. After pulling the OFRR capillary, the outer diameter is typically 100 μm and the wall thickness could be around 10 μm. We further thinned the wall via etching by passing diluted concentrations of HF while intermittently characterizing the sensitivity. The final wall thickness is approximately 3 μm.

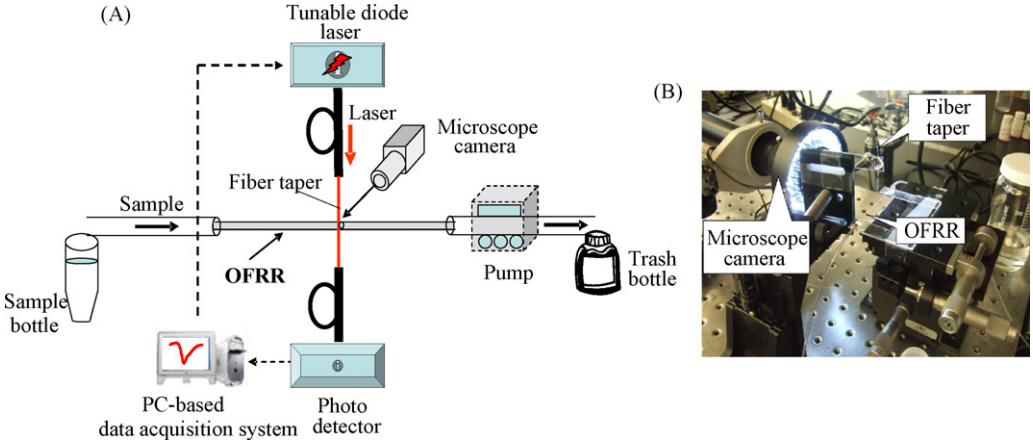

Fig. 3. (A) Schematic of the OFRR experimental setup and (B) a picture of the experimental setup.

3.3. Bulk refractive index sensitivity and preconcentration

After producing the OFRR, we performed a bulk refractive index sensitivity test. The sensitivity is characterized using solutions of known refractive indices, as the sensing mechanism of the OFRR is based on refractive index detection. We used solutions of ethanol in water [28], as the refractive index of the varying concentrations is known well. Establishing the WGM spectral shift for refractive index change of the sample indicates the refractive index sensitivity of the OFRR sensor.

Then we performed a preconcentration test to determine the optimal immobilization buffer and pH, as preconcentration is also the procedure to give a high local protein concentration at the sensor chip surface [12]. In this way, the immobilization of the protein to the sensor surface is more efficient. Therefore, to determine the effect of pH on enzyme activities, experiments were carried out using different sodium acetate buffer (pH 7.0–3.5). These studies are very useful in establishing conditions for handling the enzyme and in establishing methods for controlling enzyme activity in the OFRR [33].

3.4. Surface activation

The first step in surface activation was to rinse the inner surface of the OFRR using a 1:1 HCl:methanol mixture for 30 min, followed by the rinse with methanol. This produces a charged surface. Then, PBS running buffer, which was used throughout the entire experiment was pumped through the OFRR at a volumetric flow rate of 10 μL/min for 10 min. This created the baseline to measure WGM spectral shift. Then, 1% 3-APS in PBS buffer was run through the OFRR for 10 min at 10 μL/min. After this step, PBS rinse was applied to remove the non-specifically bound molecules. To crosslink aminated probes to the OFRR surface, 5% glutaraldehyde in PBS buffer was pumped through for 30 min and then the OFRR was again rinsed by PBS for 10 min. Next, 0.1 mg/mL AChE enzyme was mixed with 10 mM sodium acetate buffer certified by preconcentration test, and pumped through for 10 min or more so that it was strongly immobilized on the OFRR surface. The immobilization process concluded with the blocking of the modified surface using casein blocking buffer. Finally, the OFRR was filled with PBS and ready for the detection of parathion-methyl.

To activate the chip surface for SPR equipment, a mixture of NHS/EDC (0.1/0.4 M in water) was pumped over the gold-coated sensor surface with a quantity of 35 μL. EDC converts the carboxylic acid of the alkanethiol into reactive intermediates (NHS esters), which react with the free amine groups of the AChE protein conjugate (0.1 mg/mL in 10 mM sodium acetate buffer, pH 5.0 selected by preconcentration test) [9]. After AChE enzyme was immobilized, the chip surface was blocked by using 1 M ethanolamine, pH 8.5.

3.5. Detection of parathion-methyl

Serially diluted pesticides were introduced starting from the lowest concentration (10^{-14} M) to highest concentration (10^{-4} M) for the sensitivity test, linear regression, detection limit, and reproducibility. Parathion-methyl, which has been frequently detected in food and agricultural products, was used as the model OP pesticide. The serial dilutions were made in PBS buffer to reduce bulk effects. After detection, 10 mM NaOH washing buffer for surface regeneration was injected into the OFRR and the SPR system to remove bound pesticide analyte from immobilized AChE ligand.

4. Results and discussion

4.1. Bulk refractive index sensitivity characterization

The OFRR RI sensitivity was characterized by the change of WGM spectral shift as a function of the bulk RI of the ethanol–water mixture as shown in Fig. 4. A linear relationship between WGM spectral shift and RI was accomplished in the RI range of 1.333–1.336. The sensitivity of 14.9 nm/RIU was then obtained by measuring the slope of the WGM shift versus the RI change. This sensitivity is consistent to the OFRR wall thickness of approximately 2.6 μm, based on the theoretical model that we developed earlier [28].

4.2. Effect of different buffer pH on AChE immobilization

Fig. 5 shows the effect of different buffer pH on enzyme activities within the OFRR and the SPR system. The AChE enzymes diluted in different pH sodium acetate buffer were injected from pH 7.0 to 3.0 serially. Regardless of the change of pH, enzyme activities were not changed for the OFRR. Therefore, we could choose the buffer of any pH between pH 7.0 and 3.5.

On the other hand, in case of the SPR system, the binding interaction was minimal for pH higher than 5.0 or lower than 3.0. The binding affinity for the interaction is the highest at pH 4.0. This result indicates that the electrostatic attraction between negative charges on the surface and positive charges on the AChE enzyme at pH 4.0 was higher than the other pH values for covalent linking [34]. Based on the above results, we chose to operate at pH 4.0 to immobilize AChE enzyme on the surface for both the OFRR and the SPR system.

4.3. AChE immobilization

Fig. 6 shows the sensorgram for the surface activation and AChE immobilization described in the previous section. After the inner OFRR surface was rinsed using a 1:1 HCl:methanol mixture and methanol, the baseline was created by PBS buffer and WGM spectral shifts were measured and monitored as shown in Fig. 6A(a). Next, 1% 3-APS in PBS buffer was run through the OFRR. The WGM spectral position shifted and reached steady state at 11.5 pm during the deposition (Fig. 6A(b)). Then, the non-specifically bound molecules and bulk RI change were removed by PBS rinse, as indicated by the downward shift in the WGM spectral position (Fig. 6A(c)). For the surface activation, glutaraldehyde was used to activate aminated supports that could confer some ionic exchanger features

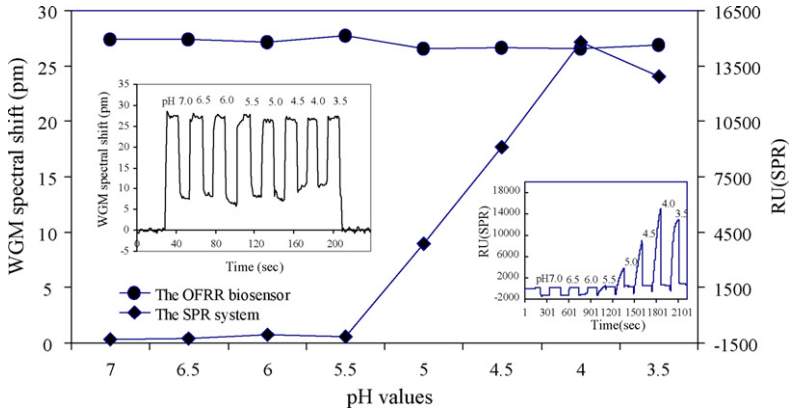

Fig. 5. Comparison of preconcentrations between the OFRR and the SPR system. Inset: sensor (left: OFRR; right: SPR) response to AChE immobilization at various buffer pH values.

to the support [35]. Its spectral position was shifted by 22.9 pm (Fig. 6A(d)). Hence, a physical adsorption could be possible before the covalent reaction. Next, AChE enzyme was injected and immobilized as much as 33.2 pm (Fig. 6A(e)). Eventually, the modified surface by AChE enzyme was blocked using casein blocking buffer (Fig. 6A(f)). The net WGM shift from the actual binding was 26.6 pm.

In case of enzyme immobilization for the SPR system, we used the general method offered by Biacore, also described in the previous section [9,13,15,16]. The measured amount of immobilized AChE protein was 12,046 RU, expressed as an arbitrary resonance unit for the SPR signal (Fig. 6B).

4.4. Pesticide detection

The OFRR was then applied to pesticide detection using parathion-methyl as the model OP analyte. Fig. 7(A) shows

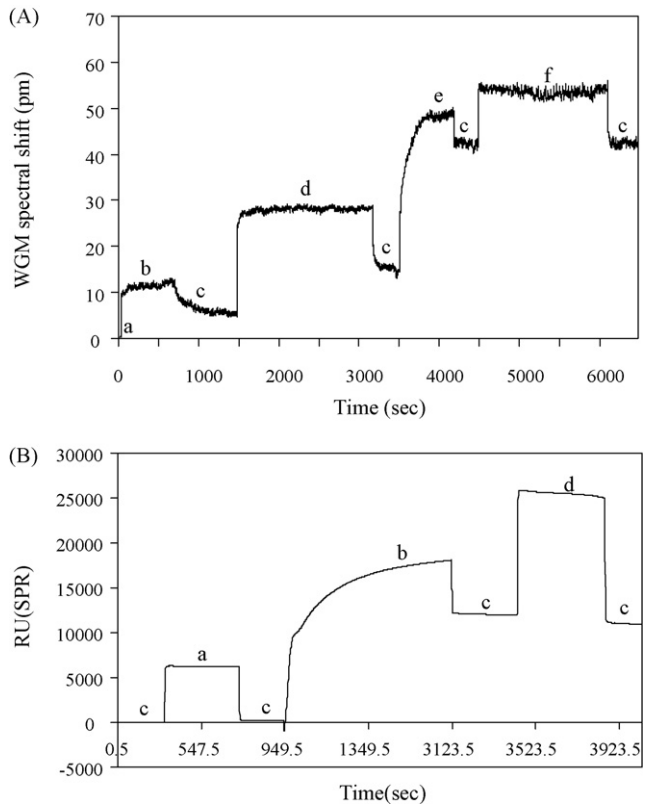

Fig. 6. Sensorgrams for surface activation and AChE enzyme immobilization. (A) The OFRR biosensor: (a) initial baseline, (b) 3-APS modification, (c) buffer rinses with PBS, (d) glutaraldehyde crosslinker, (e) AChE immobilization and (f) casein blocking buffer. (B) The SPR system: (a) EDC/NHS, (b) AChE immobilization, (c) PBS buffer and (d) ethanolamine blocking buffer.

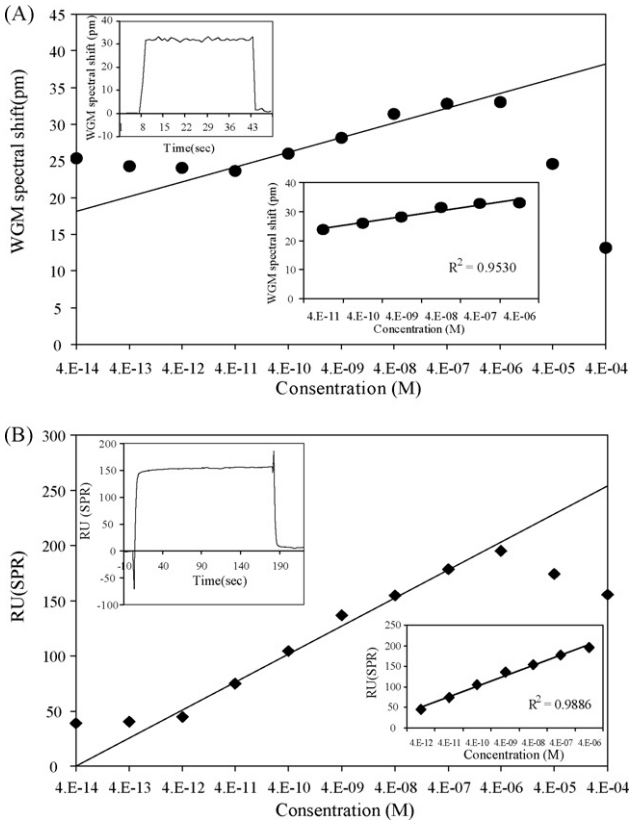

Fig. 7. Sensor responses to various concentrations of parathion-methyl. (A) The OFRR biosensor, regression equation about linear working range from 10^{-11} to 10^{-6} M: $y = 2.042x + 22.041$ ($R^2 = 0.9530$) and (B) the SPR system (Biacore 3000), regression equation about linear working range from 10^{-12} to 10^{-6} M: $y = 5.2018x + 43.711$ ($R^2 = 0.9886$). Insets on the left in (A) and (B) show the sensorgram for 38 nM parathion-methyl.

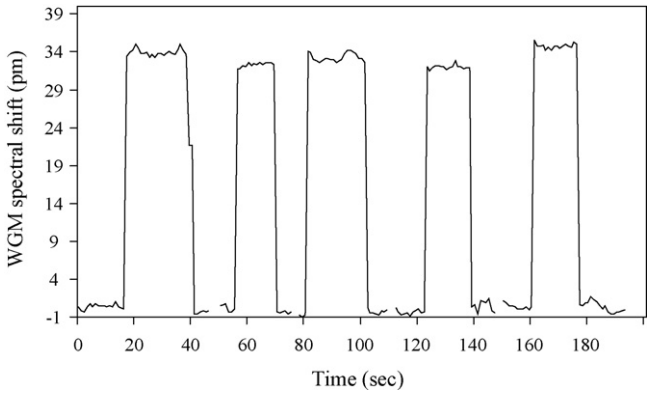

Fig. 8. OFRR sensing reproducibility for 3.8 μM parathion-methyl.

the response of the OFRR biosensor. This was obtained by plotting the WGM spectral shift versus the parathion-methyl concentration. The investigated pesticide concentration range was 10^{-14} to 10^{-4} M. The detection limit was decided by the linear working range, detected in the concentration range 10^{-11} to 10^{-6} M. The obtained value of the detection limit is 3.8×10^{-11} M. The WGM spectral shift of the samples increased with increasing parathion-methyl concentrations in the linear portion ($y = 2.042x + 22.041$, $R^2 = 0.9503$). Non-specific binding reactions came into existence with less than 10^{-12} M concentration and higher than 10^{-5} M concentration, which were excluded in the calculation of linear range. This negative errors and positive errors generated by a non-specific reaction do not always gave a plateau and can occur under any of the conditions [2,36], which is also reflected in the SPR system (Fig. 7(B)). In the SPR system, the linear working range was 10^{-12} to 10^{-6} M. The detection limit is 3.8×10^{-12} M ($y = 5.2018x + 43.711$, $R^2 = 0.9886$), as shown in Fig. 7(B).

We further evaluated the reproducibility of the OFRR biosensor in Fig. 8. The biosensor demonstrated excellent reproducibility (R.S.D. = 3.5%, $n = 5$) when periodically exposed to 3.8×10^{-6} M parathion-methyl. A rinsing and dilution buffer (10 mM HEPES-NaOH (pH 7.4)) was used after binding experiments for surface regeneration. Meanwhile, because the reproducibility of the SPR system was well known by many research results, we omitted the comparison each other.

4.5. Comparison of OFRR biosensor with SPR instrument

The main characteristics for a biosensor [37] between the OFRR and the SPR system (Biacore 3000), which vary with the different signal transduction mechanisms, are summarized in Table 1. The concentration range corresponds to the linear piece of the calibration curve in the plots of WGM spectral shift (the OFRR) and RU (the SPR system) against concentration (mol/L). The detection limit of the OFRR was 3.8×10^{-11} M, whereas the SPR system was 3.8×10^{-12} M. But both of them satisfy the tolerance for the maximum residue limits [4]. However, the response time of the OFRR went between 0.1 and 0.5 min, which was much faster than that of SPR system (5–10 min) [9,38], suggesting that the OFRR biosensor offers

Table 1
Comparison of characteristics between the OFRR biosensor and the SPR system for parathion-methyl detection

Type	Enzyme	Immobilization procedure	Concentration range (mol/l)	Detection limit (mol/l)	Linearity	Response time (min)
The OFRR	AChE (Sigma)	Modification with 3-APS, cross-linking with glutaraldehyde	3.8×10^{-11} to 3.8×10^{-6}	3.8×10^{-11}	$y = 2.042x + 22.041$ ($R^2 = 0.9530$)	0.1–0.5
SPR system (Biacore 3000)	AChE (Sigma)	Dextran-based CM5 chip, cross-linking with EDC/NHS	3.8×10^{-12} to 3.8×10^{-6}	3.8×10^{-12}	$y = 5.202x + 43.711$ ($R^2 = 0.9886$)	5–10

good opportunities for direct detection of toxic contamination in field.

5. Conclusion

The novel organophosphorus pesticide biosensor has been fabricated using the OFRR biosensor platform. Bulk refractive index sensitivity, linear working range and detection limit were tested by monitoring the change of WGM spectral shift for parathion-methyl pesticide, which was compared with the of SPR system (Biacore 3000). Experimental results showed that the OFRR biosensor was able to detect a concentration of 3.8×10^{-11} M, which was sensitive enough to detect trace amounts of parathion-methyl pesticides on the level of their admissible concentrations in environmental as well as food matrices. Additionally, the OFRR sensing performance can be improved with further optimization of the sensor platform. Consequently, the OFRR biosensor offers good opportunities for direct, highly sensitive, and inexpensive detection of harmful contaminants in the field.

Acknowledgments

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