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An optical surface plasmon resonance biosensor for determination of tetanus toxin

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Abstract

Surface plasmon resonance (SPR) has been successfully applied for the simple, rapid, and label-free assay of various biomolecules. This assay evaluates a novel wavelength modulation SPR biosensor for the detection of tetanus toxin. The wavelength modulation SPR biosensor is designed based on fixing the incident angle of light and measuring the reflected intensities in the resonance wavelength range spanning 400–800 nm simultaneously. Tetanus toxin (TeNT), one of the most potent toxins known, is synthesized as a 150 kDa single polypeptide chain. The SPR biosensor has been shown to be capable of directly detecting concentration of tetanus toxin as low as 0.028 Lf ml^{-1}. Under selected experimental conditions, the SPR biosensor has a good reproducibility, sensitivity and reversibility. The results illustrate how wavelength modulation SPR biosensor can be used to detect biomolecular interactions.

Keywords: SPR; Tetanus antitoxin; Wavelength modulation; Angle modulation

1. Introduction

Tetanus is a fatal disease caused by the toxin of clostridium tetani, an anaerobic spore-forming Grampositive bacillus. Although tetanus is not infrequent in clinic, it sometime has short latent period, acute onset, severity of ill condition, and high fatality rate.

Tetanus toxin (TeNT), one of the most potent toxins known [1–3], is synthesized as a 150 kDa single polypeptide chain. This polypeptide is composed of a heavy chain of 100 kDa and a light chain of 50 kDa which are held together by a disulfide bond [4]. Digestion of the holotoxin with papain results in fragment B which comprises the TeNT light chain and the amino terminal half of the TeNT heavy chain (HN), and fragment C (HC) which contains the carboxy terminal half of the heavy chain. TeNT is specific for intoxication of neurons. The mechanism of TeNT binding to neurons is not completely understood, as many cell types express gangliosides but are not susceptible to tetanus intoxication. Here TeNT blocks neurotransmitter release [5].

Tetanus antitoxin, which is an immune horse's serum and is a direct antibody, can counteract exotoxin released in the process of the tetanus bacillus's propagator so that it may prevent tetanus. Therefore, tetanus-specific antibody (or antigen) measurements play an important role in serological surveillance of immunity for human. Traditionally, such measurements have been performed, such as: haemagglutination assay [6], enzyme-linked immunosorbent assay (ELISA) [7,8], the toxin binding inhibition test (ToBi) [9], and Vero cell assay [10] and others. Although these traditional assay methods can yield valuable information about the binding affinity between antibody and antigen, they need labeled immunoglobulin for the detection of antibodies bound to antigen and can not measure toxin neutralization in real time.

Surface plasmon resonance (SPR) biosensor based on the phenomenon of surface plasmon resonance is a powerful optical tool for monitoring of binding event between biomoleculars in real time without the need of intrinsic or extrinsic labels [11–15]. SPR biosensors can be used to provide both qualitative information and quantitative

Abbreviations: EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; ELISA, enzyme linked immunosorbent assay; LF, limit of flocculation; MPA, 3-mercaptopropionic acid; NHS, *N*-hydroxysuccinimide; PBS, phosphate-buffered saline; SPR, surface plasmon resonance; TM, transverse magnetic; ToBi, toxin binding inhibition test

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information on biomolecular interactions. Qualitative applications range from ligand and antigen epitope recognition to small-molecule screening and complex assembly studies. Therefore, they are rapidly becoming an essential component of both academic and commercial laboratories. To date, the tetanus toxin has not yet been characterized by SPR biosensor.

SPR is an optical phenomenon, in which surface plasmon waves are excited at the interface between a metal and a dielectric under specific conditions of total internal reflection of plane-polarized light. Surface plasmon waves are extremely sensitive to small changes in the refractive index near the biosensor surface and the changes in the refractive index are proportional to the sample mass [16,17]. So it is a surface-sensitive technique where the biomolecular recognition ligand is immobilized onto the biosensor surface and the analyte is injected into the flow cell which gives rise to a local refractive index change and binding event can be readily detected and analyzed. Antibodies are the most frequently used recognition ligand because they are highly specific, stable and relatively easy to use from a practical perspective. Optical biosensors have been applied to determine interaction between antibody and antigen such as anti-thyroxine monoclonal antibody [18], hepatitis B surface antigen [19] and B factor [20].

In this paper, we applied a novel SPR biosensor instrument installed in our laboratory for simultaneous multi-wavelength detection of the tetanus toxin. Recently, Homola et al. [21] describe a newly developed wavelength modulation-based SPR sensor for the detection of staphylococcal enterotoxin B in milk. We have already succeeded to determinate B factor [22], basic fibroblast growth factor [23] and to detect affinities and antigenic epitopes of bovine cardiac troponin I [24] with this wavelength modulation SPR biosensor in our laboratory. The biosensor was shown to be relatively simple to use, selective, rapid to respond, and cost-effective.

2. Experimental

2.1. Reagents and equipment

3-Mercaptopropionic acid (MPA) was purchased from Sigma. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were obtain from Shanghai Lizhu Dongfeng Biotechnology Co. The tetanus antitoxin (1500 IU) and the tetanus toxin were obtained from Changchun Biology Product Research Institute. All other chemicals were of analytical reagent grade. All the biological reagents were stored at 4 °C. All solutions were prepared with ultrapure water (>18.3 MΩ cm^{-1}). KQ118 ultrasonic clean up device was purchased from Kunshan Ultrasonic Cleaning Instruments Co. Halogen tungsten lamp and collimating device were purchased from Changchun Fifth Optics Precision Instrument. The lenses, prisms and various optical adjusting frames were purchased from the Precision Optical Instrument Factory of Changchun (PR China).

A 0.01 mol l^{-1} phosphate-buffered saline solution (PBS, pH 7.4) was prepared by dissolving 0.2 g KCl, 8.0 g NaCl, 0.24 g KH_2PO_4 and 1.44 g Na_2HPO_4, in 1000 ml water.

A 0.3 mol l^{-1} citrate buffer (pH 2.7) was prepared by dissolving 21 g $C_6H_8O_7{\cdot}H_2O$ and 11 g Na_2HPO_4 in 350 ml water.

2.2. Apparatus

Traditional SPR biosensing devices are based on fixing a discrete excitation wavelength and measuring the angle of incident light (Fig. 1A), therefore, the SPR reflected spectra are shown in terms of reflected light intensity versus angle of the incident light (Fig. 1B). In practice, the surface plasmons are excited by a convergent beam of monochromatic light and the resonance angle is measured using a photodetector array. The best angular accuracy of this SPR biosensor is about 0.001° which corresponds to a shift in optical wavelength of 0.6 nm [25], which makes that its sensitivity is limited. Such an instrument is available from BIAcore, which is much more expensive than the one described here.

In this study, the SPR biosensor developed is based on fixed incident angle and measures resonance wavelength

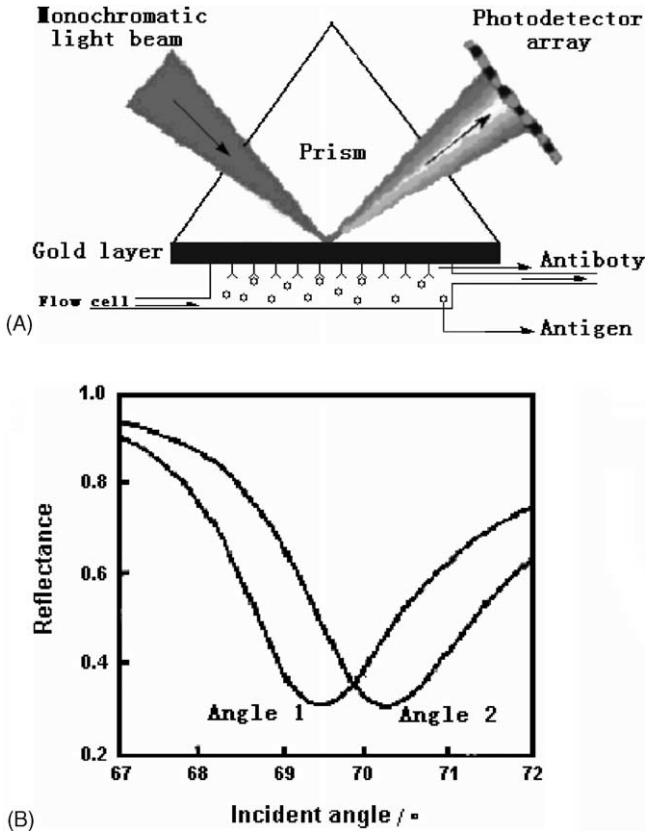

Fig. 1. (A) SPR optical biosensor based on incident angle modulation. (B) Spectral reflectivity for incident angle modulation SPR biosensor.

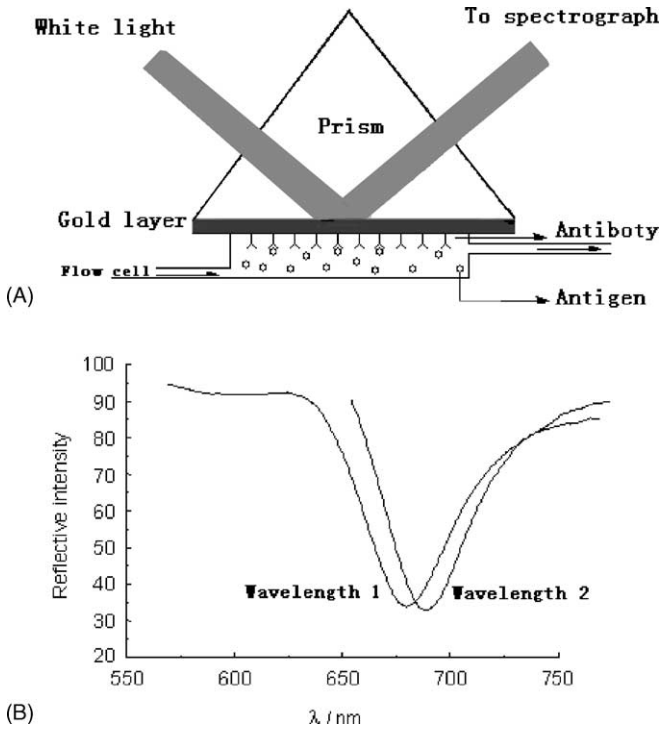

Fig. 2. (A) SPR optical biosensor based on wavelength modulation. (B) Spectral reflectivity for wavelength modulation SPR biosensor.

instead of employing a fixed wavelength and modulating the incident angle of light (Fig. 2A). The SPR spectra are shown in terms of reflected light intensity versus wavelength of the incident light (Fig. 2B). The intensity of the reflected light is the minimum at the resonant wavelength. In this SPR biosensor configuration, a halogen tungsten lamp is used as the light source in conjunction with a constant voltage transformer and it is much cheaper than the laser light source used in the traditional SPR biosensor. The light from this source passes through a polarizer and becomes TM polarized light. In order to make the light be parallel one, two lenses are employed. The parallel polychromatic light beam passes through an optical prism with a thin gold film and excites surface plasmon at the interface between the gold film and analytes. The excitation of surface plasmons is accompanied by the transfer of optical energy into surface plasmons and its dissipation in the metal layer, which results in a narrow dip in the spectrum of reflected light. The output light from the prism is guided into the optical fiber and then into the Fullwave Spectrophotometer (Ocean Optics Inc., USA). The spectrophotometer was inserted into the computer. Therefore, the apparatus is compact and small. The detector is a 1024 element charge coupled device (CCD) linear-array detector and the wavelength range to be measured simultaneously is 400–800 nm. The prism was vacuum-deposited with approximately 50 nm gold film. The gold surface of the biosensor was cleaned by exposure to ethanol for 5 min, and dried under nitrogen.

The incident angle is fixed at a suitable value to ensure the surface plasmon resonance phenomenon to occur. A smaller

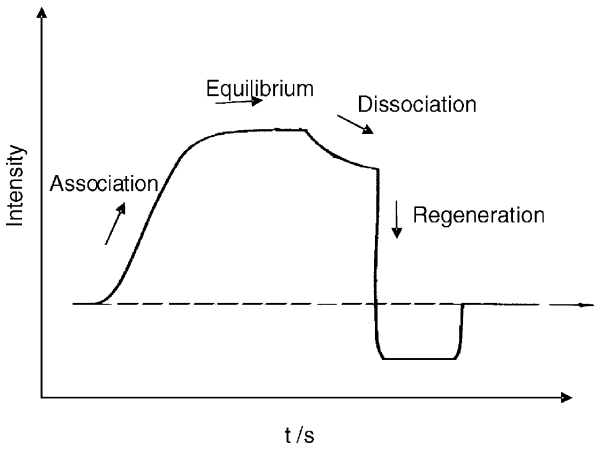

Fig. 3. A typical binding cycle obtained from an of SPR biosensor.

change in refractive index or layer thickness at the sensor surface would cause a clear wavelength shift of the resonant wavelength in SPR reflected spectra. A 90 μl volume flow cell (18 mm × 5 mm × 1 mm) was used for reaction.

2.3. Assay design

SPR biosensor analysis involves immobilizing one of the molecules of interest on the biosensor surface and injecting the second molecule across the biosensor surface. In the current experiment, the reaction biosensor surface was prepared by a standard amine coupling method (see Section 3).Tetanus antitoxin was diluted 1:10 (v/v) with PBS buffer at pH 7.4 and was injected into the flow cell. Then the tetanus toxin was flowed across the tetanus antitoxin surface. SPR detectors monitored the change in refractive index of the solvent layer near the surface induced by association and dissociation of the tetanus toxin. Tetanus toxin binding to the immobilized tetanus antitoxin generates an increase in response. The response intensity levels off over time as equilibrium between tetanus antitoxin-bound and free tetanus toxin in solution is achieved. To monitor dissociation of the tetanus antitoxin and tetanus toxin complex, PBS buffer alone is injected over the surface. As tetanus toxin dissociated from the tetanus antitoxin surface, the response intensity returns to baseline. Fig. 3 is schematic representation of a typical SPR response obtained during a binding interaction cycle. Fig. 4 shows the design of the assay. All processes were performed at room temperature (25 °C).

3. Results and discussion

3.1. Assembling of the MPA on the gold sufrace

The SPR biosensor based on the 3-mercaptopropionic acid membrane with active terminal carboxyl is studied in

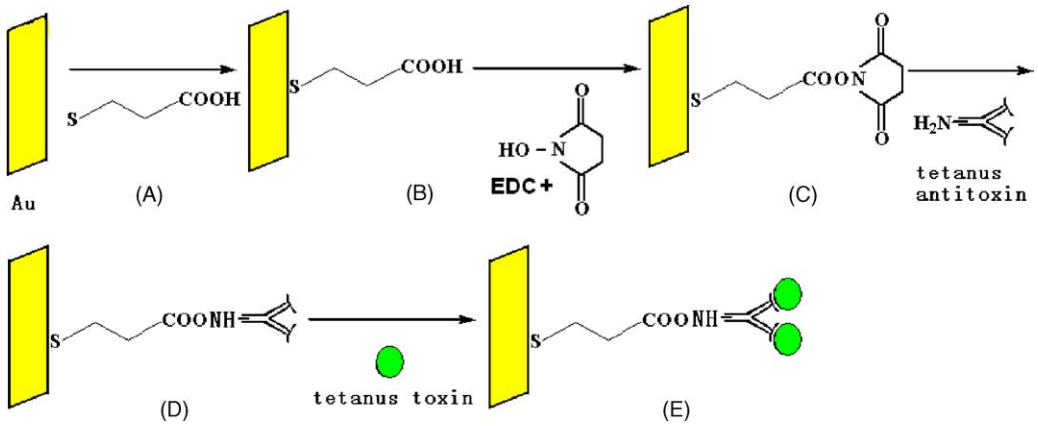

Fig. 4. The design of the assay: (A) assembling of the MPA on the gold surface; (B) acylation of the MPA; (C) assembling of the tetanus antitoxin on the surface of MPA acylated; (D) tetanus toxin binding to the immobilized tetanus antitoxin.

this paper because the MPA monolayer can stick firmly to the gold surface via its sulfide bonds. To study the MPA self-assembly on the gold substrate, a solution containing MPA was injected into the flow cell and the changes of resonant wavelength were measured in real time. The shift of the resonant wavelength reached about 99% of its total shift within 5 min in the 10 mmol l^{-1} MPA solution. Further increasing the self-assembly time resulted in a virtually constant resonant wavelength, which suggested that a complete monolayer had self-assembled. The adsorption curve of MPA at the surface of gold film is shown in Fig. 5. This experimental result implied the MPA forms a good monolayer. The resonant wavelength maximum shift for the formation of this monolayer is 11.56 nm. It showed that the sulfide bond of MPA is easy to form S-Au binding.

3.2. Acylation of the MPA

The biosensor surface was rinsed with PBS buffer until the resonance wavelength kept constant after the MPA assembling on the gold surface. This showed that non-special adsorption was wiped off on the gold surface. Then solutions containing NHS (40 mg ml^{-1}) and EDC (40 mg ml^{-1}) were mixed and injected immediately into flow cell. Meanwhile –COOH group reacted with NHS. NHS reacted with MPA under the catalysis of EDC. Observing SPR spectra's shift as a function of time, the reaction between MPA and NHS is very rapid in the initial 5 min. After 15 min, the resonance wavelength remains almost constant. The maximum shift of resonant wavelength is 39.33 nm. The kinetic curve of the reaction of NHS and MPA is shown in Fig. 6. In fact,

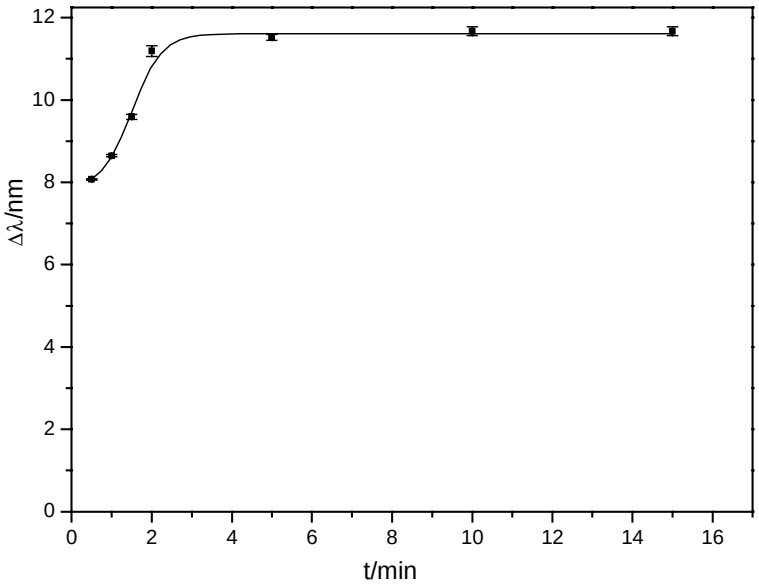

Fig. 5. The kinetic adsorption curve of MPA on the gold surface. The error bars represent the standard deviation of the values determined from three the same assays.

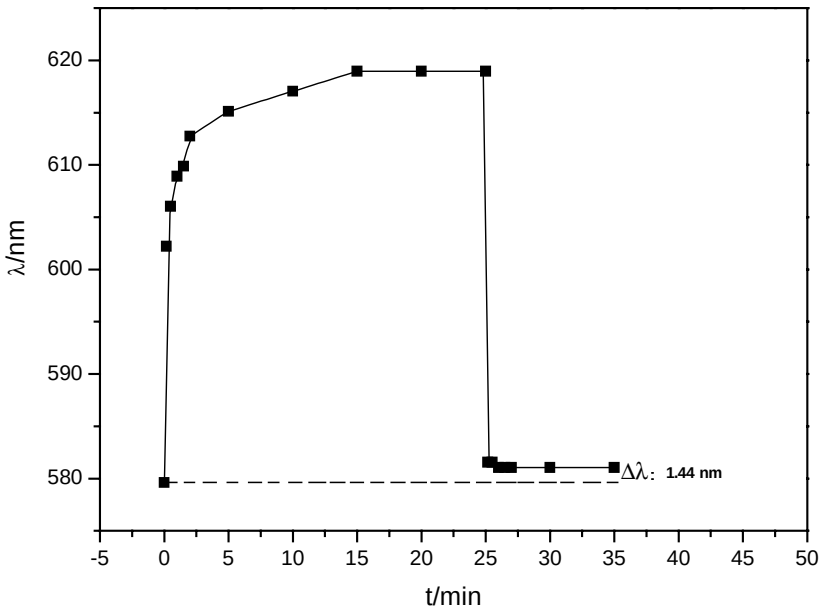

Fig. 6. Dependence of resonance wavelength on the time for reaction of EDC and MPA on the surface of the biosensor.

the shift of the resonance wavelength is 1.44 nm after the biosensor surface was rinsed with PBS buffer in order to clean out non-specific adsorption.

3.3. Immobilization of tetanus antitoxin

The solution containing tetanus antitoxin was injected into flow cell to monitor its assembling on the surface of MPA acylated. Tetanus antitoxin concentration is very important for the tetanus antitoxin assembling. If the tetanus antitoxin concentration is too low, the shift of resonance wavelength will be too small. In this assay, tetanus antitoxin was diluted 1:10 (v/v) with the PBS buffer at pH 7.4. The tetanus antitoxin well assembled on the biosensor surface under the concentration. The assembling of the tetanus antitoxin was carried out for 12 h to organize the processing tetanus antitoxin molecular on the solid surface and thus the biosensor membrane was stable. Fig. 7 shows the adsorption curve of tetanus antitoxin on the MPA monolayer. As expected, a biosensor surface prepared with MPA is exposed to tetanus antitoxin and shows a strong response. The shift of the resonant wavelength is 8.18 nm

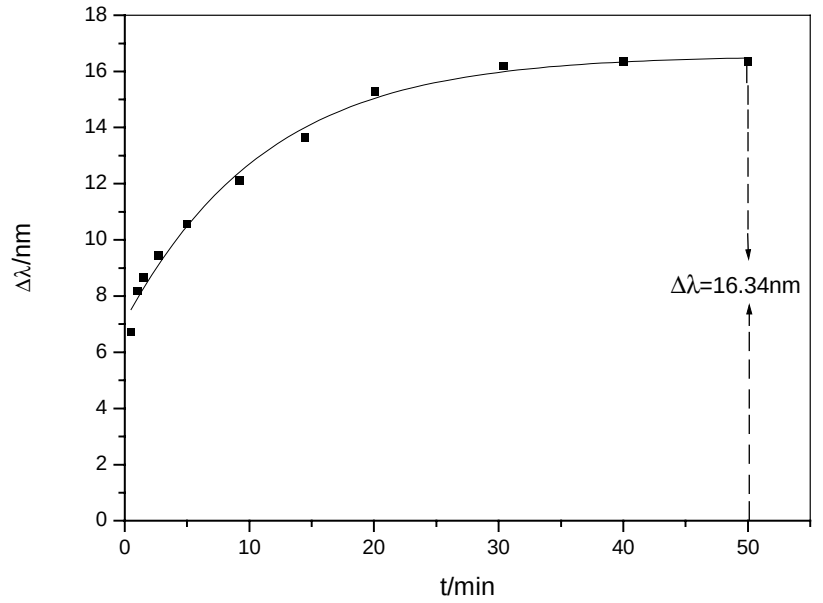

Fig. 7. The kinetic adsorption curve of tetanus antitoxin assembling on the MPA monolayer.

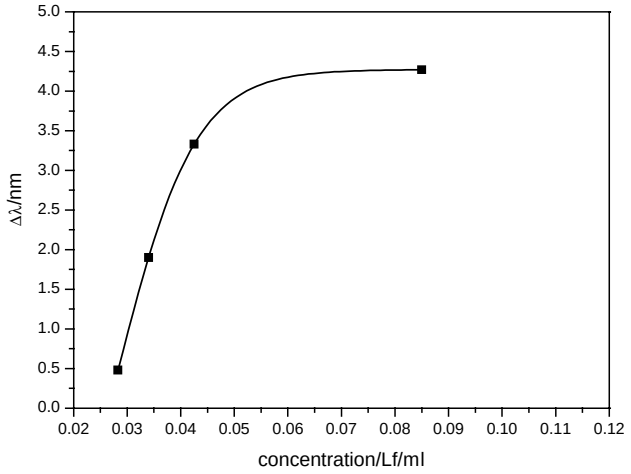

Fig. 8. The relationship between C (concentration) of tetanus toxin and $\Delta\lambda$ (resonance wavelength shift).

within 1 min. After 30 min, the resonant wavelength almost keeps constant. The maximum shift of resonance wavelength is 16.34 nm. Almost no change in the resonant wavelength was observed when washing the biosensor surface with PBS buffer. This suggested that the tetanus antitoxin has bound tightly to the MPA on the biosensor surface.

3.4. Determination of tetanus toxin

Based on the assay design, tetanus toxin was diluted with PBS buffer to obtain different concentrations. The optimum determination conditions for this biosensor are in PBS buffer (pH 7.4) at room temperature for 40 min. Fig. 8 shows the relationship between C (concentration) of tetanus toxin and $\Delta\lambda$ (resonance wavelength shift). The tetanus toxin is determined in the concentration range from 0.028 to 0.085 Lf ml^{-1}.

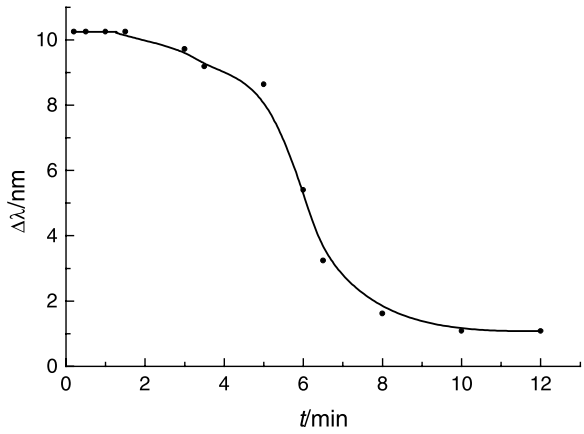

Fig. 9. The elution curve of tetanus antitoxin–tetanus toxin complex from MPA.

3.5. Regeneration

The bound tetanus antitoxin can be desorbed from MPA surface with an acidic solution [26]. After being rinsed for 10 min with 0.3 mol l^{-1} citrate buffer (pH 2.7), the tetanus antitoxin–tetanus toxin complex is desorbed from MPA monolayer. At the same time, the surface of MPA may stably exist; because the acid rinsing can make the resonance wavelength value recovery to that of MPA (see Fig. 9). Therefore, the biosensor can be used repeatedly.

4. Conclusions

Using the wavelength modulation SPR optical biosensor, tetanus toxin was detected. Experimental results indicate that the SPR biosensor can detect tetanus antigen at low concentration 0.028 Lf ml^{-1}.

In summary, some features of SPR biosensor analysis, such as the lack of labeling requirements, low sample consumption and high sensitivity, along with the ability for high-throughput screening, make this technology particularly suitable for these biomolecular studies [27].

Acknowledgements

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