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Acousto-optic tunable filter—based surface plasmon resonance biosensor for determination of human factor B

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Abstract

A surface plasmon resonance (SPR) biosensor based on wavelength modulation with a fixed angle of incidence incorporating an acousto-optic tunable filter (AOTF) is described. The AOTF is used as the wavelength selector of the AOTF–SPR biosensor system. The wavelength range of the AOTF is 440–790 nm. The wavelength is modulated by inputting AOTF 0–5 V voltage and one voltage corresponds to one wavelength. The SPR spectrum is shown in terms of reflected light intensity versus voltage supplied to AOTF. The intensity of reflected light is the minimum at the resonant voltage. Molecular self-assembling on the surface of the sensor is applied to form the sensing membrane on the gold substrate. The staphylococcal protein A (SPA) is modified on the gold substrate. The temperature effect of human factor B (Bf) antibody and antigen reaction was studied. The human factor B was determined in the concentration range of 2–80 μg/ml.

Keywords: AOTF; SPR; Biosensor; Bf

1. Introduction

An acousto-optic tunable filter (AOTF), which is all-solid-state, compact, electronically tunable with no moving components, narrow band light filter, is a new wavelength selector. The main component of the AOTF is acousto-optic crystal, where the interaction between the acoustic wave and optical radiation occurs, and on the anisotropic acousto-optic crystal a piezoelectric transducer is bonded. The piezoelectric transducer generates an acoustic wave when a radiofrequency (RF) is applied to it. When a light wave travels in the anisotropic crystal, it is diffracted by the acoustic wave through the acousto-optic effect [1–5]. The wavelength of the diffracted light is selected by tuning of the RF applied to the crystal and wavelength can be changed by changing the frequency. The electronic tunability of the AOTF provides it with the most compelling advantages over the more conventional spectroscopic devices that are mechanically tuned to change wavelength. The scanning speed of the AOTF is determined by the speed of the acoustic wave in the crystal and optical aperture which is on the order of microseconds (μs). In practice, the access time will be longer if limited by electronic switching speed and the settling times of RF generator. Not only monochromatic but also polychromatic light can be diffracted from the AOTF when more than one RF signals are simultaneously applied to the AOTF. So, the AOTF can be used as a polychromator, for example, multidimensional fluorimeter [6,7]. Because of so many advantages, the AOTF has been applied in near-infrared (NIR) spectromerty [8], fluorescene spectromerty [9], Raman spectromerty [10], UV-Vis spectromerty [11], spectral imaging [12], thermal lens spectrophotometry [13] and atomic spectromerty [14].

In recent years, surface plasmon resonance (SPR) biosensors have been extensively applied to the analysis of biomolecular interactions in real time without labeling [15–17]. With these sensors, biomolecular interactions are detected via a change in the refractive index or layer thickness at the sensor surface. SPR biosensors have also been applied to determine reaction kinetic and affinity constants for molecular interactions and the active concentration of biomolecules in solution. Most commercial SPR biosensing devices are based on angular modulation, which includes two modes. One is angular scan, which uses a machinery rotating table. But angular scans usually take several

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minutes and thus even slow changes cannot be monitored in real time [18]. The other method is using a convergent light beam covering a suitable ranges of incidence angles reflected at the prism and exciting the surface plasmons and the resonant angle is measured using a photodetector array [19,20]. In contrast, the wavelength modulation SPR biosensor is based on the measurement of changes in the resonant wavelength induced by SPR. SPR spectrum is shown in terms of reflected light intensity versus wavelength of the incident light. The intensity of the reflected light is the minimum at the resonant wavelength. The change of resonant wavelength depends on the change of refractive index at the sensor surface. So, in SPR biosensor, anyone change in concentration or kind of analyte, which will lead to the change of the refractive index at the sensor surface, will make resonant wavelength change. The SPR sensors based on angle and wavelength modulation have the approximately the same resolution [21]. The best angular accuracy of the goniometer is about 0.001°, which corresponds to a shift in optical wavelength of 0.6 nm [22,23] if a fixed angle of incidence system is used. Several couplers including fiber optic SPR sensor [24,25], grating-based SPR sensor [26] and prism-based SPR sensor [27–29] have been employed in the SPR sensor based on the wavelength modulation. A theoretical analysis and comparison of the sensitivity of both grating and prism-based SPR sensors indicated that grating-based SPR sensors using wavelength modulation are much less sensitive than their prism-based counterparts [30]. Compared to the angle modulation, the wavelength modulation method has more potential for sensor miniaturization and analysis to remote location [27].

A surface plasmon-based optical sensor using an AOTF was developed by Jory et al. [26] in 1995. A gold-coated diffraction grating was used as optical coupler. Compared with other traditional SPR biosensor, it has two obvious characteristics: firstly, there is no moving part in the biosensor. Secondly, the biosensor is measuring reflected light intensities at all wavelengths almost simultaneously. That is to say, it can monitor reaction in real time. By adding a chemically active overlayer to the system NO_2 in N_2 was detected. In 1998, Carusoa et al. [31] studied measurements of thin film on gold by acousto-optic surface plasmon resonance. An equilateral sapphire prism was used as optical coupler. Adsorption of a poly(ethylene glycol) monododecyl ether surfactant (C12E8) and a 30-mer DNA oligonucleotide with a mercaptohexyl group at the 5′-phosphate end (DNA-SH) onto gold from water have been examined. Angle-dependent reflectivity measurements taken on the same system provide complementary SPR data, allowing the sensitivity of the two techniques to be compared. The AOTF SPR system was found to be more than two orders of magnitude more sensitive than conventional SPR measurements.

In this paper, AOTF–SPR biosensor was applied in immunosensing tasks. The sensor is designed on the basis of fixing angle of incidence and measuring the change of resonant wavelength. A halogen tungsten lamp is used as the light source and photomultiplier tube (PMT) is used as detector. The most obvious characteristics is to use AOTF as wavelength selector. The AOTF offer such advantages as being all-solid state (contains no moving parts), having rapid scanning ability (μs), so the AOTF–SPR setup is no moving parts, compact (the AOTF used is only 60 mm × 40 mm × 30 mm), and can monitor reaction in real time. AOTF used as the selector of wavelength is a new method in wavelength modulation SPR biosensor.

2. Experimental

2.1. Reagents

Staphylococcal protein A (SPA), bovine serum albumin (BSA), rabbit anti-human factor B antiserum (rabbit IgG fraction to human Bf, titer 1:40) and human factor B (Bf) were purchased from Shanghai Biology Product Research Institute. All other chemicals were of analytical reagent grade. All solutions were prepared with ultrapure water (resistivity >18.3 MΩ cm) supplied by an EASY-pure RF compact ultrapure water system (Barnstead Thermdyme, USA).

A 0.01 mol/l 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 ultrapure water. A 0.3 mol/l 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 ultrapure water.

2.2. Equipment

The halogen tungsten lamp and adjustable optical device were purchased from Changchun Fifth Optics Precision Instrument.

Generally, the AOTF is composed of an anisotropic crystal and an array of piezoelectric transducer which is bonded onto the anisotropic crystal. The piezoelectric transducer is in conjunction with a RF generator, from which a RF signal is generated and applied to the transducer which, in turn, generates an acoustic wave propagating through the anisotropic crystal. In this paper, the AOTF used consists of an anisotropic TeO_2 crystal and the piezoelectric transducer is $LiNbO_3$. In different, the piezoelectric transducer is in conjunction with the voltage controlled oscillator (VCO). A direct voltage signal from 0 to 5 V is supplied to the VCO of AOTF by digital to analog converter controlled by computer. Then, the signal corresponding to RF is produced and transmitted to the $LiNbO_3$ piezoelectric transducer which generates an acoustic wave propagating through the TeO_2 crystal. The propagating acoustic wave produces a periodic modulation of the index of refraction. When a light beam travels through the TeO_2 crystal, only a very narrow band of optical frequencies can satisfy the phase matching condition and be diffracted. When the RF signal, namely, voltage signal, is changed, the center of optical passband is

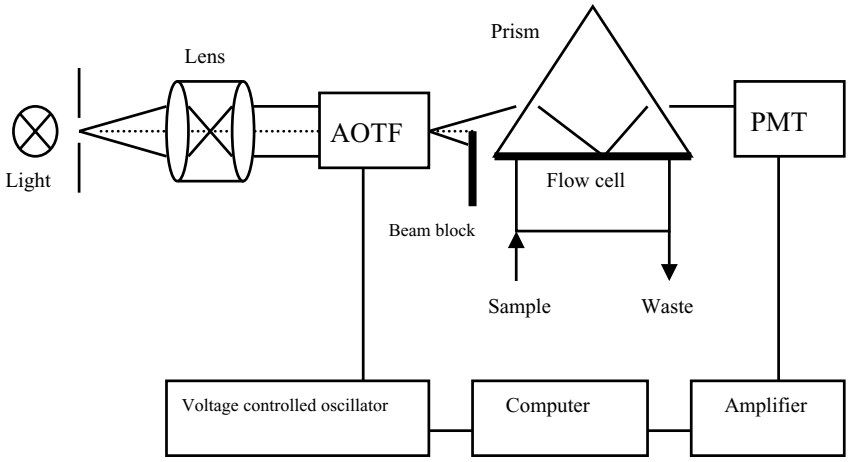

Fig. 1. The experimental set-up for AOTF–SPR biosensor.

changed accordingly so that the phase-matching condition is maintained. That is, when the RF signal is changed, the wavelength of diffracted light is changed. That is to say, one voltage corresponds to one wavelength. The wavelength range of AOTF is 440–790 nm. The AOTF is obtained from Shanghai Silicate Research Institute.

The schematic diagram of the SPR biosensor based on wavelength modulation using AOTF as the wavelength selector is shown in Fig. 1. The light source is a halogen tungsten lamp powered with a constant voltage source. The light emitted from the lamp passes through a aperture and a parallel optical tube that consists of two lenses is employed to make the light parallel. The parallel polychromatic light travels through AOTF whose wavelength range is 440–790 nm, then is diffracted into TM and TE polarized light beam and a undiffracted zero-order light beam. TM polarized light is applied to produce SPR, therefore the dark beam block is used to shut out TE polarized light beam and zero-order light beam. The parallel TM polarized monochromatic light beam passes through an optical prism with a thin gold film and excites surface plasmon at the interface between the gold film and the analytes. The output light from the prism enters photomultiplier tube and is detected. The prism was vacuum-deposited with a gold film of 50 nm. A 180 μl flow cell was used for the reaction. A water bath connected with a thermostatic circular pump is used to keep the temperature constant.

The incident angle is fixed at a suitable value to ensure that the surface plasmon resonance phenomenon occurs. SPR spectrum is shown in terms of reflected light intensity versus applied voltage of AOTF. When the plasmon resonance phenomenon occurs, The voltage at which reflected light intensity is minimum is the resonant voltage. The resonant voltage is corresponding to the resonant wavelength of conventional wavelength modulation SPR biosensor. A smaller change in refractive index or layer thickness at the sensor surface will cause a shift of the resonant voltage in SPR reflected spectra.

2.3. Assay procedure

Molecular self-assembling was applied to form sensing membrane on the gold substrate. Before immobilization of SPA, the biosensor surface was washed with PBS to keep the resonant voltage constant, and then the SPA was injected into the flow cell. The process of the monolayer formation was monitored by taking the SPR spectra at different time. After the SPA monolayer formation, PBS was injected into the flow cell to wash off the unbound SPA. Then, 5% (w/v) of BSA PBS solution was applied to block the unoccupied sites on the gold film.

The diluted rabbit anti-human factor B antiserum was injected into flow cell. After immobilization of Bf antibody on the biosensor surface prepared with the SPA, the BSA solution was injected into the flow cell to block the non-specific binding sites on the sensor surface. Then, the different concentrations of human factor B were injected into the flow cell and the shifts of the resonant voltage were measured. To test the effects of temperature on Bf antibody and antigen reaction, additional experiments were performed at 10, 14, 16 and 20 °C. The temperature is controlled by a thermostatic circular pump.

3. Results and discussion

3.1. Relationship between voltage and wavelength of the AOTF

In this paper, the output wavelength of AOTF is corresponding to the voltage inputting to AOTF VCO. If the input voltage is changed, the output wavelength will changed. Fig. 2 shows the relationship between supplied voltage of AOTF and transmitted wavelength. It can be seen from Fig. 2 that inputting AOTF VCO 0–5 V voltage, the wavelength range of diffracted light is 440–790 nm. The low voltage corresponds to long wavelength, while the high voltage

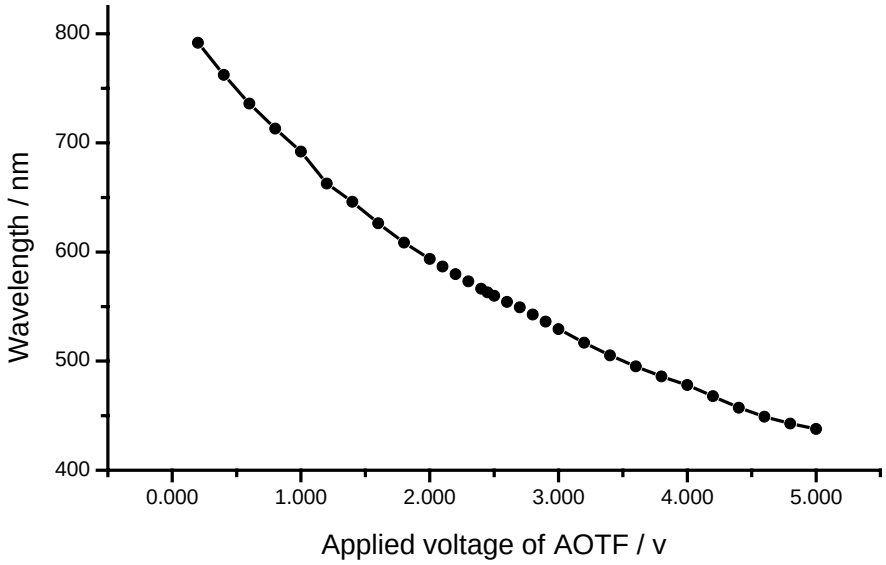

Fig. 2. The relationship between applied voltage of AOTF and transmitted wavelength.

corresponds to short wavelength. In conventional wavelength modulation SPR biosensor, a less increase of refractive index of analyte in the proximity of the gold surface will cause shift of the resonant wavelength in SPR reflected spectra towards longer wavelength. However, in this AOTF–SPR biosensor, the less increase of refractive index will cause the shift of resonant voltage towards lower voltage.

3.2. Assembling of SPA on the gold surface

SPA whose molecular weight is about 40 kDa is a polypeptide isolated from staphylococcus cell wall. It was found that SPA can bind specifically protein A antibody, as well as antibody else not protein A. SPA which has four binding sites with antibodies binds antibody by two sites can form polycomplex with antibody. The binding process of SPA and antibody has four significant characteristics: (1) binding sites of SPA and antibody locate on the Fc fragment without interacting at antigen binding sites. So, the association capability of antibody and antigen cannot be changed. (2) SPA will resume its character readily. (3) The affinity of SPA and antibody is very high, however, the bond antibody can be eluted from SPA at acidic solution [32]. (4) SPA is also easily attached to gold and gold–SPA complexes are highly stable, as make SPA adaptation to becoming a membrane used to connect antibody and Au in SPR biosensor. In this paper, the biosensor surface was modified with SPA.

To observe the SPA assembling on gold substrate, a 0.1 g/l SPA solution was injected into the flow cell at different temperature. It is shown that the lower the temperature is, the higher the rate of SPA attached to gold is, the shorter the time of getting to association equilibrium is. In this paper, the assembling temperature was chosen at 13 °C. SPR spectra of SPA assembling on the surface of gold film at different time is show in Fig. 3. When the flow cell was filled with PBS, the resonant voltage is 2.235 V. Injecting 0.1 g/l SPA solution, after 1 min, the resonant voltage is 2.185 V, that is, the shift of the resonant voltage is −0.050 V; after 30 min, the resonant voltage is 2.177 V, the shift of the resonant voltage is −0.058 V. It can be seen from Fig. 3 that the increase of the assembling time of SPA causes a shift of the resonant voltage towards lower voltage, that is, the resonant wavelength moves towards longer wavelength. The adsorption curve of SPA at surface of gold is shown in Fig. 4. The shift of the resonant voltage reaches about 95% of its total shift within 10 min. After 10 min, the resonant voltage keep almost constant. This means the self-assembling is complete and the monolayer formed. The maximum shift of the resonant voltage is −0.058 V, corresponding to 4.00 nm. The 5% (w/v) of BSA solution was applied to block the unoccupied sites on the gold film.

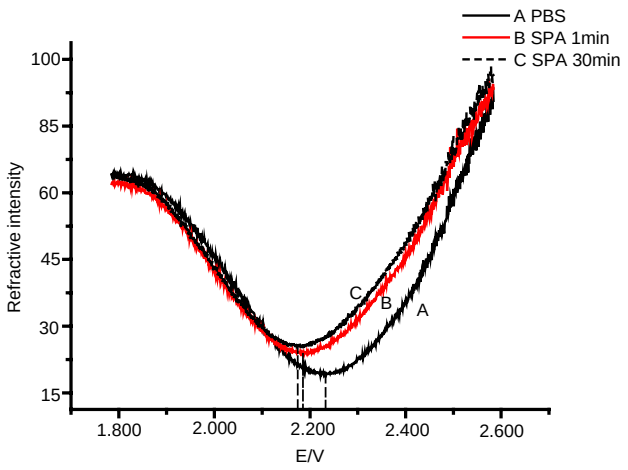

Fig. 3. SPR spectra of 0.1 g/l SPA at different time.

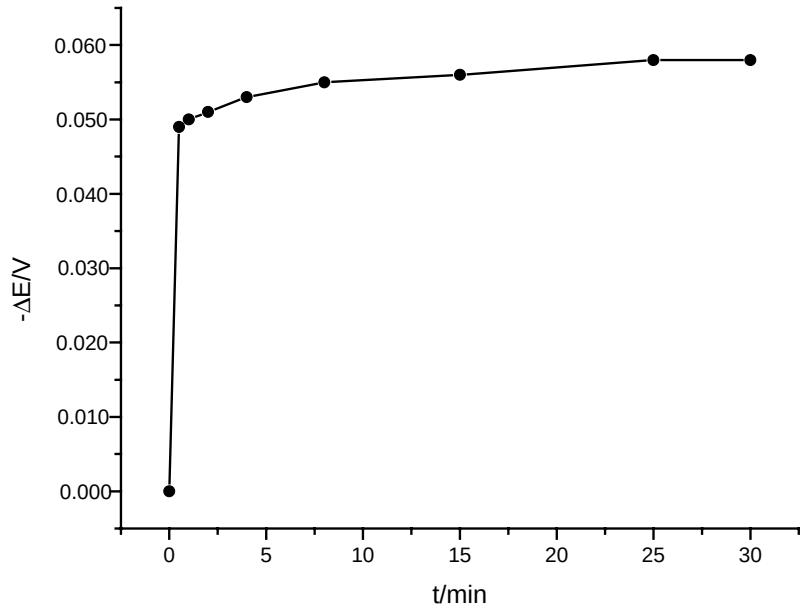

Fig. 4. The kinetic adsorption curve of protein A on the gold film at 13 °C.

3.3. Immobilization of Bf antibody

The diluted rabbit anti-human Bf antiserum was injected into flow cell. By fast voltage scanning of AOTF, the antibody assembling was monitored. To test influence of temperature on the association between SPA and Bf antibody, the diluted rabbit anti-human Bf antiserum was injected into flow cell after the mobilization of SPA on gold at different temperature. It is shown that the lower the temperature is, the more amount of association is, that is, the greater the shift of resonant voltage is. In the experiment, the temperature of association between SPA and Bf antibody is chosen at 13 °C. The best dilution titer of Bf antiserum was 1:160. At this dilution titer, the maximum shift of the resonant voltage is −0.217 V, namely 15.77 nm. If the antibody titer is too low, the shift of the resonant voltage will be too small. If the antibody titer is too high, the shift of the resonant voltage will not change, keeping −0.217 V. Fig. 5 shows the time dependence of SPA monolayer assembled with rabbit anti-human factor B antibody. The assembling of the antibody (1:160) was carried out for 20 h to organize the processing antibody molecular on

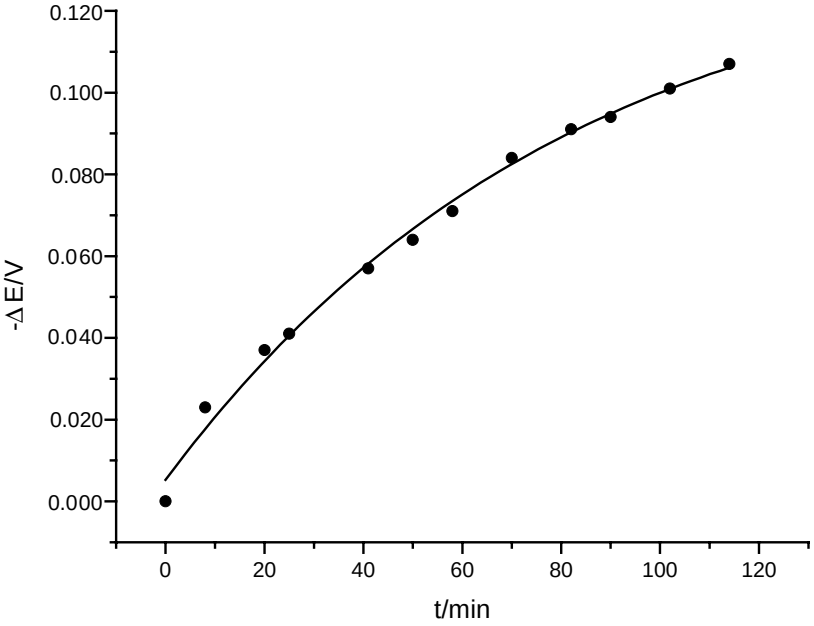

Fig. 5. The kinetic adsorption curve of Bf antibody on the SPA monolayer at 13 °C, Bf antiserum titer (1:160).

the biosensor surface and thus the sensor membrane was stable.

3.4. Temperature effect on Bf antibody and antigen reaction

To detect the effect of the temperature on Bf antibody and antigen interaction, 20 μg/ml human factor B was injected into the flow cell after immobilization of Bf antibody on the biosensor surface prepared with the SPA at 10, 14, 16 and 20 °C, respectively. Fig. 6 shows the sensorgram of binding between same concentration Bf antigen and antibody at different temperature. As shown in Fig. 6, when the temperature is lower, the shift of resonant voltage is greater, the time of getting to association equilibrium is longer, while the binding between Bf antibody and antigen is stable and compact. When the temperature is higher, the time of getting to association equilibrium is shorter, the shift of resonant voltage is less.

3.5. Determination of human factor B

Human factor B, which is also named pre-complement activator of complement 3 (C3), plays a crucial role in the activation of the alternative pathway of C3 on the surface of biomaterials during extra-corporeal procedures. Bf is a monochain carbohydrate protein, which is composed of 733 amino acids. The molecular weight of Bf is 93,000. These amino acids pucker and then form three volume proximate global regions. One is Ba, the other two are Bb of dumbbell shape. In recently years, it has been found that the two fragments have immuno-measurement function. That is, Bb can promote the proliferation of B cell which is golden staphylococcal crowan I, while Ba can restrain the proliferation of B cell which is induced by B cell growth factor (BCGF) and turning into activation state. The content of Bf is related with diabetes, brain apoplexy, hypertension, and so on [33–35]. Some traditional assay methods such as enzyme-linked immunosorbent assay (ELISA) [36], Western blot [37] have been applied to determine factor B. However, these traditional assay methods have either low sensitivity or long analysis time and complicated operation.

In this paper, the AOTF–SPR biosensor has been applied to determine the human factor B. Because the time of getting to association equilibrium is long at low temperature, while the shift of resonant voltage is too little at high temperature, the reaction temperature of Bf antibody and antigen is chosen at 16 °C. Bf was diluted with PBS buffer to obtain different concentrations. Fig. 7 shows association and dissociation curve of Bf antigen and antibody reaction at 16 °C. It can be seen from Fig. 7 that the binding of Bf antigen and antibody has been close to equilibrium at 70 min. Therefore, the determination condition for Bf was chosen at 16 °C for 90 min. Fig. 8 shows the relationship between Bf concentration and the shift of resonant voltage. The Bf was determined in the concentration range from 2 to 80 μg/ml. Repeating the determination of 20 μg/ml Bf solution for 11 times, a relative standard deviation of 1.8% is obtained. Mu et al. [38] used a wavelength modulation SPR sensor in which a grating was used as wavelength selector to determine the Bf. By using avidin–biotin system, the Bf was determined in the concentration range of 0.5–100 μg/ml. In our design, if using a high resolution AOTF, the sensitivity of AOTF–SPR instrument can been improved.

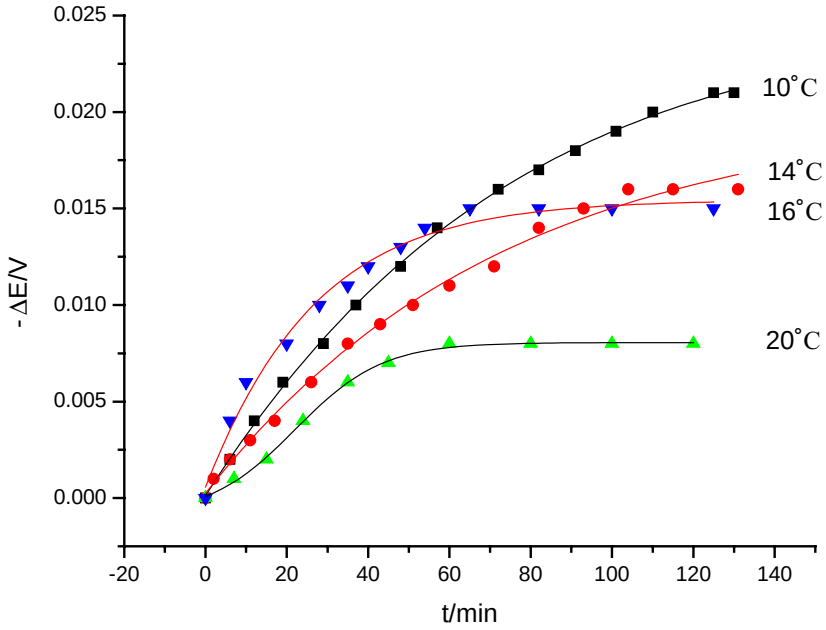

Fig. 6. Kinetic curve of the interaction between Bf antigen and antibody at different temperature.

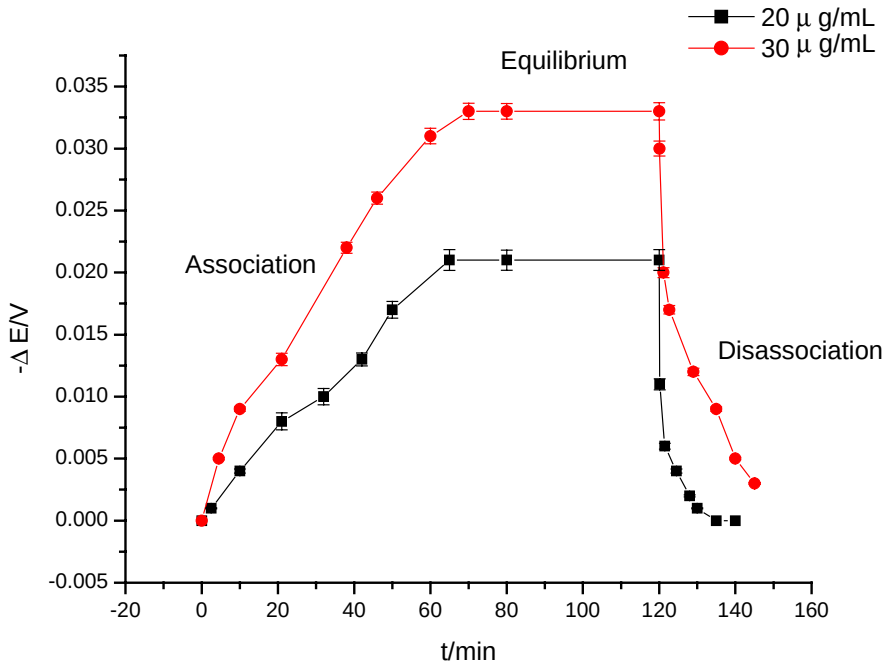

Fig. 7. Kinetic curve of the interaction between Bf antigen and antibody at 16 °C, Bf concentration.

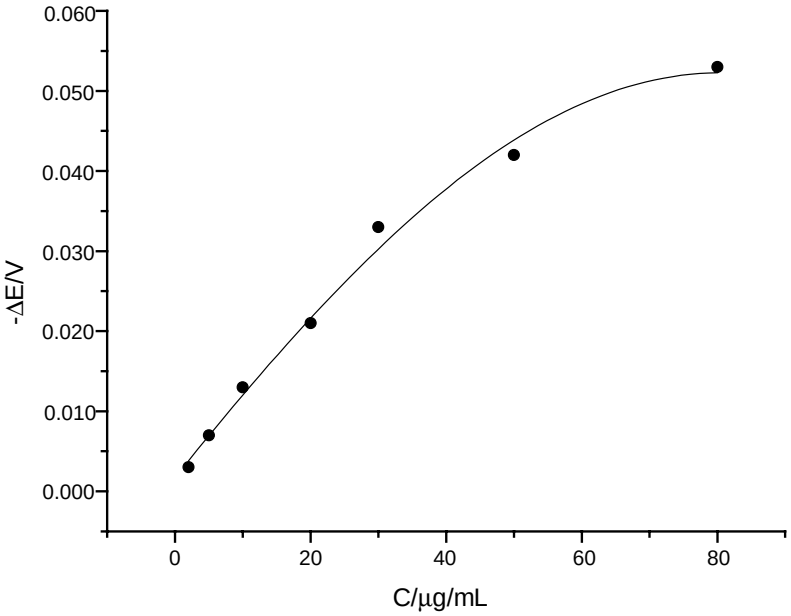

Fig. 8. The relationship between concentration of Bf and resonant voltage shift at 16 °C.

3.6. Regeneration

The bound Bf antibody can be desorbed from SPA surface with acidic solution. After being rinsed for 10 min with 0.3 mol/l citrate buffer (pH 2.7), the Bf antibody–antigen complex is desorbed from SPA monolayer and the biosensor can be applied repeatedly.

4. Conclusion

As a new wavelength selector, AOTF has been used to all kinds of spectroscopy. However, the reports for AOTF-based SPR biosensor is little. In this paper, an AOTF is used to control the wavelength of TM-polarized light beam made incident on a gold-coated prism. By choosing an appropriate

angle of incidence and monitoring the reflectivity of the light, a surface plasmon resonance was observed. The AOTF–SPR biosensor was used to determining Human factor B and obtain a good result. Because of the characteristic of rapid scanning speed of AOTF, the AOTF–SPR system can realize determining the biomolecular interactions in real time.

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