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Compact quantitative optic fiber-based immunoarray biosensor for rapid detection of small analytes

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ABSTRACT

Immunoarrays have been proven to be powerful tools for high-throughput analysis of multiple analytes. In this paper, a proof-of-concept development of a novel optic fiber-based immunoarray biosensor for the detection of multiple small analytes is presented. This was developed through immobilization of two kinds of hapten conjugates, MC-LR-OVA and NB-OVA, onto the same fiber optic probe. The technique is significantly different from conventional immunoarray sensors. Microcystin-LR (MC-LR) and trinitrotoluene (TNT) could be detected simultaneously and specifically within an analysis time of about 10 min for each assay cycle. The limits of detection for MC-LR and TNT were 0.04 μg/L and 0.09 mg/L, respectively. Good regeneration performance, binding properties, and robustness of the sensor surface of the proposed immunoarray biosensor ensure the cost-effective and accurate measurement of small analytes. The change in concentration of the hapten conjugates immobilized onto the sensor surface was also proven to have no significant effection on the performance of immunoarray sensor, which is essential to the application of the immunoarray in real samples detection. This compact and portable quantitative immunoarray provides an excellent multiple assay platform for clinical and environmental samples.

1. Introduction

Analytical microarrays have emerged as powerful tools for high-throughput and rapid analysis of multiple analytes (Niessner and Seidel, 2008; Blicharz et al., 2009). Antibody and hapten arrays are specific quantitative analytical techniques using antibodies/antigens as highly specific biological recognition elements. They possess the capability to simultaneously detect numerous analytes in low sample volumes (Epstein et al., 2003; Ahn et al., 2006; Niessner and Seidel, 2008). These have been designed for a number of bioanalytical applications, such as disease diagnosis, drug discovery, proteomics assay, environmental monitoring, and detection of biological warfare agents (MacBeath, 2002; Belleville et al., 2004; Ahn et al., 2006; Kopf and Zharhary, 2007; Blicharz et al., 2009).

Despite the technological advancements made in the past decades, the successful use of microarray technology remains to be elusive for many researchers (Wingren and Borrebaeck, 2006; Kopf and Zharhary, 2007). There are many reasons for this, and a few of them are mentioned here. Conventional microarray fabrication involves spotting capture antibodies/antigens onto a heterogeneous matrix (e.g., glass slide, nylon membrane) for immobilization (Kusnezow and Hoheisel, 2002). The recognition molecules are immobilized by microprinting or microstructuring processes. Fabrication of these arrays requires expensive equipment and multiple steps, which are labor intensive and subject to some degree of variability (Wingren and Borrebaeck, 2006; Blicharz et al., 2009). Moreover, many factors influence the performance of microarray modified surfaces, including the immobilization chemistry, spotting buffer, probe concentration, and physical factors like spotter type, pins used, and environmental conditions (Ramachandran et al., 2004; Wingren and Borrebaeck, 2006). Therefore, it is essential to develop new immunoarrays which are modified by abundant bio-recognizing molecules with high activity, and have low non-specific adsorption, and good regeneration performance, and to do so without using expensive equipment and complex operation conditions.

Microarray data analysis is usually done using the complex software for image processing. It particularly presents a substantial bottleneck for many researchers (Stoeckert et al., 2002; Allison et al., 2006). Due to the inherently high variation associated with array fabrication, binding/hybridization, and image processing, the accuracy of array-based quantitative assessment is still uncertain (Clack et al., 2008; Dondrup et al., 2009). Most of microarrays cannot be regenerated, and for each multi-analyte concentration, separate microarray and positive and negative control spots are needed (Kusnezow and Hoheisel, 2002; Wingren and Borrebaeck, 2006; Allison et al., 2006).

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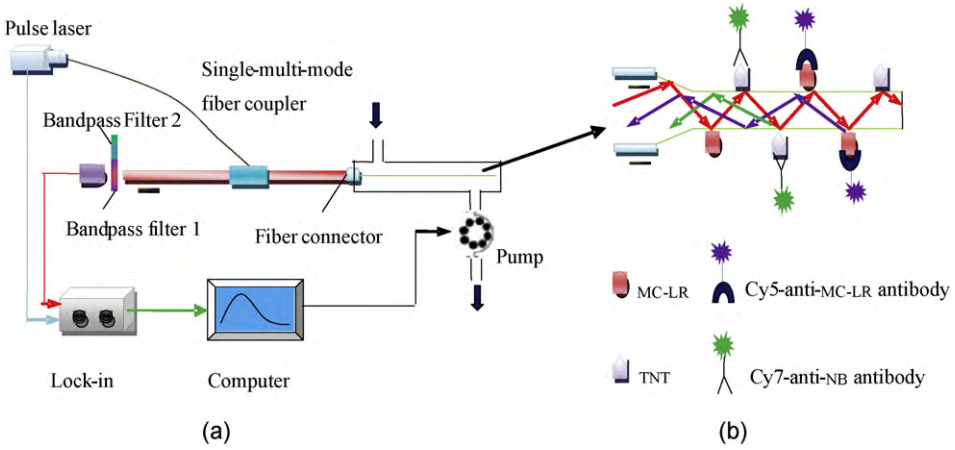

Fig. 1. (a) Schematic diagram of the structure of optic fiber-based microarray immunosensor; (b) mechanical schematic of simultaneous detection of two small analytes.

In this study, a proof-of-concept development of a compact, inexpensive, and easy-to-use optic fiber-based immunoarray biosensor is presented. This immunosensor was modified with two kinds of hapten conjugates. Its multi-analyte capabilities were demonstrated through simultaneous real-time monitoring of the binding reaction of a toxin antibody (anti-microcystin-LR or anti-MC-LR monoclonal antibody, MC8C10) and a chemistry production antibody (anti-nitrobenzene or anti-NB monoclonal antibody, NB4X10) with their counterparts (MC-LR-OVA and NB-OVA), immobilized onto the same fiber optic probe. As is already known, microcystin-LR, one of the most common microcystins, is highly toxic and contains two variable amino acids of leucin (L) and arginine (R). Many reported cases of animal poisoning and human diseases, even death, are due to MCs exposure by way of drinking and surface water (Jochimsen et al., 1998; Carmichael et al., 2001; Campàs and Marty, 2007). In this regard, the World Health Organization (WHO) has proposed an MC-LR guideline value (GV) of 1 μg/L in drinking water in order to minimize public exposure to MCs (WHO, 2004). Trinitrotoluene (TNT), another compound that is potential target for immunoassays, is a well-known explosive compound used in the preparation of landmines for military and terrorist activities (Yinon, 2002). The detection of explosives has become of tremendous importance in today's globally heightened security environment (Anderson et al., 2007). Contamination with TNT occurs through surface water runoff or ground water inflow, resulting in the accumulation of TNT in aquatic environments. Due to TNT's biological persistence, toxicity, and mutagenicity, its detection has gained considerable attention (Bromage et al., 2007). Moreover, the optic fiber-based immunoarray presented here could provide reliable quantitative results for routine application. Aside from being easy-to-use, it has faster binding kinetics, lower production cost, and regeneration and miniaturization features.

2. Experimental

2.1. Materials and reagents

Bovine serum albumin (BSA), ovalbumin (OVA), 3-mercaptopropyl-trimethoxysilane (MTS), 1-ethyl-3(-3dimethylamino-propyl) carbodiimide hydrochloride (EDC), 4-nitrohippuric acid, N-(4-maleimidobutyryloxy)succinimide, and TNT were purchased from Sigma–Aldrich (Steinheim, Germany). MC-LR was obtained from Alexis (ALX-350-012). Unless specified, all other reagents, supplied by Beijing Chemical Agents, were of analytical grade and used without further purification. Distilled deionized water was used throughout the investigation.

Monoclonal anti-MC-LR antibody (reference no. MC8C10) and anti-NB antibody (reference no. NB4X18) were produced by our research group, and labeled Cy5 and Cy7, respectively.

The hapten-carrier conjugate NB-OVA was prepared through the following processes: 101 mg 4-nitrohippuric acid was dissolved in 10 mL 0.05 M sodium phosphate-buffered saline (PBS, pH 11). Then 35 mg EDC and 20 mg OVA were added to this solution, and the reaction was incubated overnight at 4 °C under constant stirring. The NB-OVA conjugate was purified by dialysis and stored frozen at −20 °C in small aliquots until use.

MC-LR-OVA was synthesized by a modified procedure (Sheng et al., 2006). Briefly, MC-LR was treated with 2000–4000 times molar excess of 2-aminoethanethiol in a carbonate-bicarbonate buffer (pH 9.0) for 30 min at 50 °C. The product was purified from the reaction mixture on C_{18} Bond Elute cartridges with subsequent mass spectrometric identification. The resulting conjugate, H_2N-MC-LR, containing the equivalent of 1.5 mg of microcystin-LR, was then dissolved in 0.01 M PBS (pH 7.4), and mixed with 10 mg OVA. A two-step glutaraldehyde coupling procedure was carried out with 1.25% glutaraldehyde at pH 7.4. Following the coupling, the conjugate was purified by chromatography with Sephadex-G25 to ensure the absence of free toxin.

The estimated numbers of hapten molecules attached to the carrier protein (hapten-to-protein molar ratio, MR) MC-LR-OVA and NB-OVA determined by MALDI-TOF/MS were 2 and 5, respectively. 0.5 mg/mL TNT and MC-LR stock solutions were both prepared in PBS and stored at 4 °C. Standard concentrations of the analyte under analysis were prepared by serial dilutions in 0.01 M PBS from the stock solution.

2.2. Instrumentation

The schematic of the simple, compact, and portable optic fiber-based immunoarray biosensor, is shown in Fig. 1. The laser beam from a 635 nm pulse diode laser (8 mW, BWT Beijing Ltd.) with pigtail was directly launched into the single-mode fiber of the single-multi-mode fiber coupler. The laser light then entered from the single-mode fiber into the multi-mode fiber having a diameter of 600 μm and a numerical aperture of 0.22. Afterwards, the excitation light from the laser was coupled to a fiber immunosensor through the fiber connector. When the incident light propagated along the length of the immunosensor via total internal reflection, the evanescent wave was generated at the surface of the

immunosensor, and decayed exponentially with distance. It could excite the fluorophores labeled in the surface-bound analyte complex. To achieve simultaneous detection of both targets, two kinds of bandpass filters, FF01-676/29-25 (Filter 1) and FF01-775/46-25 (Filter 2) (Semrock, US), were subsequently used to filter the emission fluorescence of Cy5 and Cy7, respectively. The highest fluorescence emission for Cy5 and Cy7 is at 667 nm and 776 nm wavelength, respectively. Then the fluorescence signal was transferred into an electronic signal by a photodiode. The electronic signal of the photodiode was filtered and amplified through a digital lock-in detection system produced by our group. In this system, both the transmission of the excitation light and the collection and transmission of fluorescence were achieved by fiber optics with a single-multi-fiber optic coupler, which reduced the optical components required and rarely needed optical alignment.

In Fig. 1b, the detection mechanism of a multi-analyte using a immunoarray biosensor is shown. When two kinds of fluorescence-labeled antibodies were simultaneously delivered to the sample cell, different antibodies could bind with their respective hapten conjugates immobilized onto the surface of the immunosensor. Once the laser was switched on, both fluorescences could simultaneously be excited and emit different wavelengths of light. A small part of the excited fluorescence coupled back into the fiber optic immunosensor. Since a bandpass filter was used, only one kind of wavelength fluorescence light could pass through at a time and be detected by the photodiode. To detect the other fluorescence signal, the filter should be changed.

The immunoarray sensor was embedded in a flow glass cell with channel dimensions of 60 mm in length and 2 mm in diameter. All reagents were delivered by a flow delivery system operated with a peristaltic pump. The control of the fluid delivery system and data acquisition and processing were automatically performed by a computer.

2.3. Immunoarray biosensor fabrication

The combination tapered fiber optic probes was prepared as previously described (Long et al., 2008). To produce an immunoarray in a fiber probe for detecting multi-analytes in one sample, the hapten-carrier conjugates NB-OVA and MC-LR-OVA were used as recognition elements, which could assure the reusability of the sensor surface without depriving the binding properties of the immobilized molecule. These were simultaneously attached covalently to the sensor surface of the probes with a heterobifunctional reagent. Employing a modified procedure originally described by Bhatia et al. (1989), the hapten-carrier conjugates were immobilized onto the probe surface. Briefly, the probes were initially cleaned with Piranha reagents (concentrated H_2SO_4/H_2O_2, 2:1), rinsed with distilled deionized water, and dried in N_2. Then, the probe was placed in 2% MTS in toluene for 2 h, under an inert atmosphere, and excess MTS was eliminated with dry toluene. The thiol group of the silane was allowed to react for 1 h with a heterobifunctional crosslinker, 2 mM GMBS in ethanol. The probes were then rinsed with ethanol and PBS. To produce the immunoarray sensor, the fiber probes were then dipped in 1 mL of the mixture of 0.06 mg/mL NB-OVA and 0.06 mg/mL MC-LR-OVA for 2 h at room temperature. In this step, the succinimide group of GMBS was used to covalently bind the epsilon amino groups of the proteins. The immunoarray sensor was immersed in 2 mg/mL BSA for 20 min to block its non-specific adsorption sites.

2.4. Immunoassay

The immunoassays developed to determine TNT and MC-LR were considered binding inhibition tests, based on the conjugate coated format using NB-OVA and MC-LR-OVA as the coating conjugates. The concentrations of the antibodies used for TNT and MC-LR measurements were 0.4 μg/mL 4X18 and 0.3 μg/mL 8C10 (if not specially stated), respectively. Standard solutions were prepared in PBS (pH 7.4) from a TNT concentration of 0.5 μg/mL or an MC-LR stock solution of 0.5 μg/mL. To simultaneously detect TNT and MC-LR, two kinds of antibody solutions (anti-NB and anti-MC-LR antibody) with a volume of 200 μL each and two kinds of standard solutions with different concentrations were added and mixed thoroughly. This mixture was left for 10 min (so called pre-incubation). During this time, antibody binding sites were occupied according to the antigen. After pre-incubation, the mixture was delivered to the sample cell in 200 μL/min for 2 min. Antibodies with free binding sites left bound with their respective antigens (NB or MC-LR) that were immobilized onto the probe. When the laser was switched on, the fluorescence-labeled on the antibodies could be excited and part of them coupled back into the fiber optic immunosensor. The signal corresponding to the binding between the unbound antibody and the immobilized hapten-carrier conjugate sites was monitored. When the fluorescence signal of one target was recorded, the immunoarray sensor system automatically changed the filter and then recorded the fluorescence signal of the other one. To regenerate the obtained active probe surface, the capacity of the 0.5% SDS (pH 1.9) solution to break the antibody–analyte association was evaluated.

3. 3 Results and discussions

3.1. Fabrication of the immunoarray sensor

The optic fiber-based immunoarray sensor was fabricated for multi-analyte detection in routine analysis applications (Fig. 2). Two conjugates of small analytes (MC-LR-OVA and NB-OVA) were simultaneously and consistently immobilized onto the fiber optic probe surface. No complex modification steps and expensive equipments were required. The immunoarray was easy-to-use and versatile for laboratory or field application. This regeneration capability of the immunoarray has been proven using the results presented here.

Prior to multiple analyses, it was essential to investigate the behavior of the antibodies towards analytes, the cross-reactivity between two antibodies, and the immobilization of both hapten conjugates onto the same sensor. To validate the specificity of both antibodies, 0.3 μg/mL anti-MC-LR monoclonal antibody or 0.4 μg/mL anti-NB monoclonal antibody solutions were delivered to the microarray sensor surface or the fiber immunosensor surface modified by only one conjugate (MC-LR-OVA or NB-OVA, respectively). When taking the immunosensor modified by one conjugate, the system had only one response for its corresponding antibody and no response for the other antibody (Fig. 3a and b), proving that the cross-reactivity of both antibodies was negligible. While using the immunoarray sensor, the system had a response for either antibody (Fig. 3c), which indicated that the two conjugates were successfully immobilized onto the same fiber sensor and that the immunoarray could be used to detect two kinds of small analytes.

3.2. Immunoassay procedure

The immunoarray sensor can be operated in one of two modes. In one case, the laser is always turned on for the whole test-cycle, and fluorescence is excited continuously during binding. In another case, the excitation laser is shut off during incubation of the labeled antibodies on the immunosensor surface, so that the photobleaching of the surface-bound fluorescence molecules could be decreased. Once the fluorescence signal of one target is detected and recorded, the filter was changed to allow the signal detection of

the other target in order to achieve simultaneous detection of both objects. The typical signal trace of the immunosensor detected during a complete test-cycle using both operation methods is shown in Fig. 4.

When the laser was always turned on during the whole detection period, the typical fluorescence time trace displayed the dynamic process of the affinity interaction between the antibody and the antigen. The actual fluorescence intensity was an overlay of fluorescence excited from the labeled antibodies bound to the surface and the degradation of bound fluorophores due to photobleaching. The fluorescence signal increased as long as the binding rate to the surface was higher than the photobleaching rate of the bound fluorophores. However, upon reaching a maximum, the signal decreased, and this is when the photobleaching rate exceeded the rate of binding to the surface. When the incubation reaction was over, the immunoarray sensor immediately recorded the fluorescence signal, and changed the filter to detect the fluorescence signal another target.

When the laser was switched off during incubation, the influence of photobleaching on the surface-bound dye molecules could be reduced, and a higher fluorescence signal might be obtained. However, the tradeoff was loss of information on the binding kinetics of the antibodies to the immunosensor surface. From Fig. 4, when the laser was turned on, the fluorescence signal did not obviously decrease and the immunoarray sensor system immediately recorded the fluorescence signal of the anti-MC-LR antibody. The filter was then changed to detect the fluorescence signal of the anti-NB antibody. The difference between the signal value of switching on the laser after the binding process and of the background measurement was taken as the effective result of detection for the calibration of the sensor. As seen in Fig. 4, the signal of the second operation mode was about 30% higher than that of the first operation modes after the same incubation time. It was beneficial for improving the performance of the immunoarray sensor, particularly for sensitivity in analyte detection. Therefore, the second method was used in the following experiments.

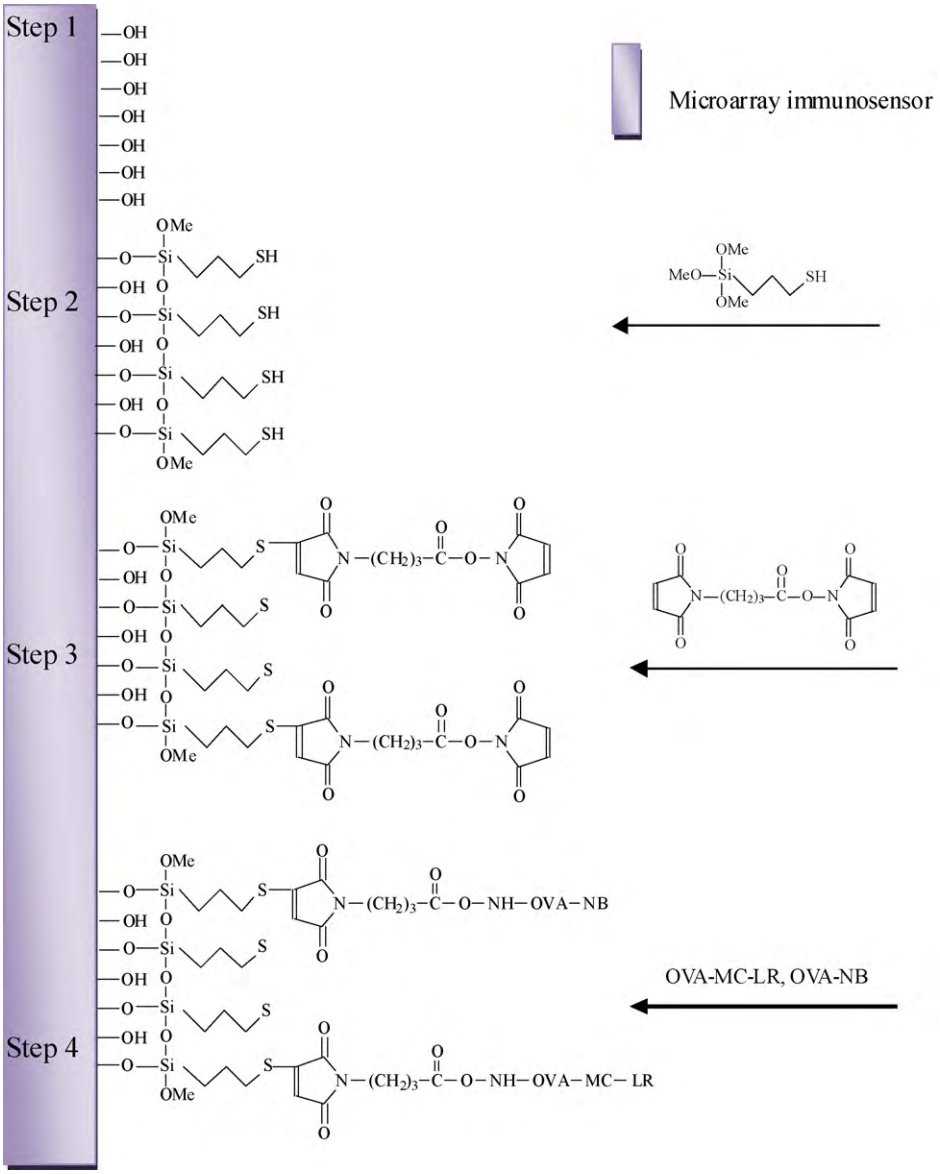

Fig. 2. Modification of the microarray immunosensor: the hydroxyl groups exposed on the probe by sulfuric acid (step 1) covalently attached with MTS molecules (step 2). The thiol group of silane was then allowed to react with the maleimide function of the bifunctional crosslinker GMBS (step 3). Then, the ester moiety of the GMBS allowed the covalent linking of the OVA protein (MC-LR-OVA, NB-OVA) through an amino group (step 4). Finally, the non-specific binding sites of the sensor surface were blocked by adding the 2 mg/mL BSA solution.

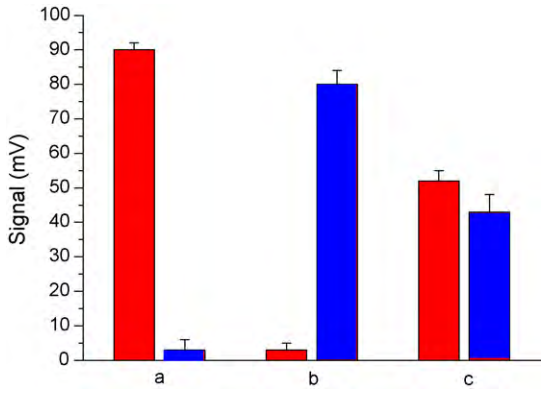

Fig. 3. Response of the immunosensor for both antibodies: Responses of the immunosensor modified by only MC-LR-OVA (a) or NB-OVA (b) for the anti-MC-LR antibody and anti-NB antibody, respectively; response of the microarray immunosensor modified by MC-LR-OVA and NB-OVA for both fluorescence-labeled antibodies (c); the red bar and the blue bar represent the response of the immunosensor for the anti-MC-LR antibody and anti-NB antibody, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.3. Analytical performance of the immunoarray sensor

The analytical performance of the immunoarray sensor was demonstrated by obtaining the binding inhibition standard curves for TNT and MC-LR. To perform binding inhibition assays, the analyte TNT solutions of various concentrations in the range of 0–100 mg/L and MC-LR solution of various concentrations in the range of 0–1000 μg/L, mixed with a fixed concentration of anti-NB antibody 4X18 (0.4 μg/mL) and anti-MC-LR antibody 8C10 (0.3 μg/mL), were incubated for 10 min. During this time, the binding sites of the anti-NB antibody and anti-MC-LR antibody were occupied according to the concentration of TNT or MC-LR, respectively. The mixture was then delivered to the sample cell at a flow-rate of 200 μL/min for 2 min. Antibodies with free binding sites left bound their corresponding antigens (NB or MC-LR) that were immobilized onto the sensor surface. The incubation reaction time was 4 min. Then, the laser was turned on and surface-bound fluorescence was excited by the evanescent wave. Once the fluorescence signal of one target was recorded, the filter was immediately changed into the other filter to detect the other analyte. After this, regeneration of the sensor surface was carried out by using 0.5% SDS (pH 1.9) for 2 min and then rinsing with PBS. A complete test-cycle took about 10 min for completion (Fig. 4).

The inhibition standard curves for TNT and MC-LR, which were normalized by expressing the signal of each standard point as the ratio of the maximum response [fluorescence signal/fluorescence signal max], are shown in Fig. 5. The error bars in the figure corresponded to the standard deviation of the data points in three repeat experiments ($n = 3$). The inhibition standard curves for TNT and MC-LR were fitted with a four-parameter logistic model (Long et al., 2009). The limit of detection (LODs) determined for TNT and MC-LR using three times standard deviation of the mean blank values was 0.09 mg/L and 0.04 μg/L, respectively. The IC_{50} of test was 1.06 ± 0.05 mg/L for TNT and 0.96 ± 0.02 μg/L for MC-LR. The standard deviations of all data points were within 4.0% ($n = 3$). On the other hand, when the fiber sensor was modified by OVA, no significant fluorescent signal was observed no matter which antibodies were used.

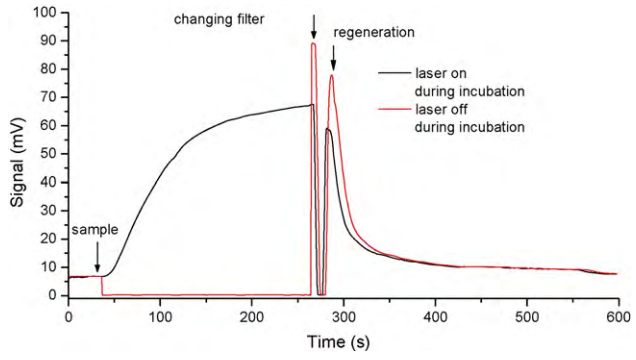

Fig. 4. Typical signal traces for measurements taken with the excitation laser turned on and off during the binding reaction (the concentration of the anti-NB antibody and anti-MC-LR antibody used was 0.6 μg/mL and 0.5 μg/mL, respectively).

Sensitivity is a critical parameter that impacts the effectiveness of an immunoarray-based approach for detecting small analytes (Zhou and Thompson, 2002). Although the same immunoarray sensor was used for determining the TNT and MC-LR, their LODs were very different. The LOD for MC-LR was less than the guideline value of 1 μg/L in drinking water proposed by the World Health Organization (WHO, 2004), and was highly comparable to most of the other immunoassays demonstrated for MC-LR (Lindner et al., 2004; Sheng et al., 2006; Campàs and Marty, 2007). However, the LOD for TNT was a little high. These indicated that the sensitivity for detecting small analytes was not only related to the performance of instrument, but also greatly depended on the nature of the antibodies used.

From Fig. 5, an interesting appearance pattern was found: when the immunosensor was modified with one conjugate (MC-LR-OVA or NB-OVA) or with the mixture of two conjugates, the standard curves for TNT or MC-LR were basically consistent. Specifically, the concentration of the conjugate immobilized onto the fiber optic immunosensor did not apparently affect the detection performance of the system. The binding reaction between the conjugate immobilized onto the probe surface and the fluorescence-labeled antibody in solution can be given by (Squires et al., 2008):

$$C + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C_s \tag{1}$$

where C is the concentration of the free binding sites at the surface, B is the concentration of fluorescence-labeled antibody in the bulk solution, C_s is the concentration of the antigen–antibody complexes at the surface, and k_1 and k_2 are association rate constant and dissociation rate constant, respectively.

Assuming first-order Langmuir kinetics for simplicity, the surface concentration of the complexes C_s obeys,

$$\frac{\partial C_s}{\partial t} = k_1(C_m - C_s)B - k_2 C_s \tag{2}$$

where C_m is the initial concentration of the free binding sites at the probe surface. If both convective and diffusive transport supply fluorescence much more quickly than the reactions can bind them, the detection kinetic is reaction-rate-limited and the antibody concentration is uniform everywhere above the sensor surface, $B = B_0$. Based on this, Eq. (2) can readily be solved:

$$C_s(t) = \frac{C_m B_0/K_d}{1 + B_0/K_d}(1 - e^{-(k_1 B_0 + k_2)t}) \tag{3}$$

where $K_d = k_1/k_2$. Then, the rate equation of the fluorescence signal (S) becomes:

$$S = \frac{S_m B_0/K_d}{1 + B_0/K_d}(1 - e^{-(k_1 B_0 + k_2)t}) \tag{4}$$

where S_m is the maximum fluorescence signal when the free binding sites at the probe surface are saturated, which is obviously closely related to the concentration of the receptor immobilized onto the probe surface. When detecting the small analytes, indirect competitive flow immunoassays are usually employed, in which

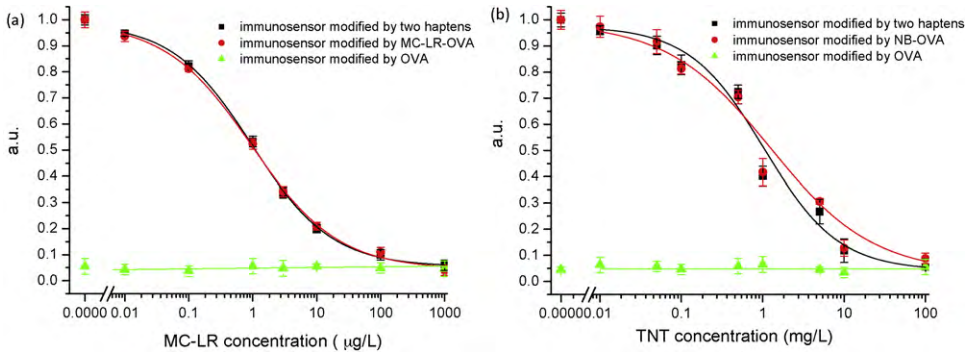

Fig. 5. Inhibition standard curves for the detection of MC-LR (a) and TNT (b), where the immunosensors were modified by two, one hapten or OVA.

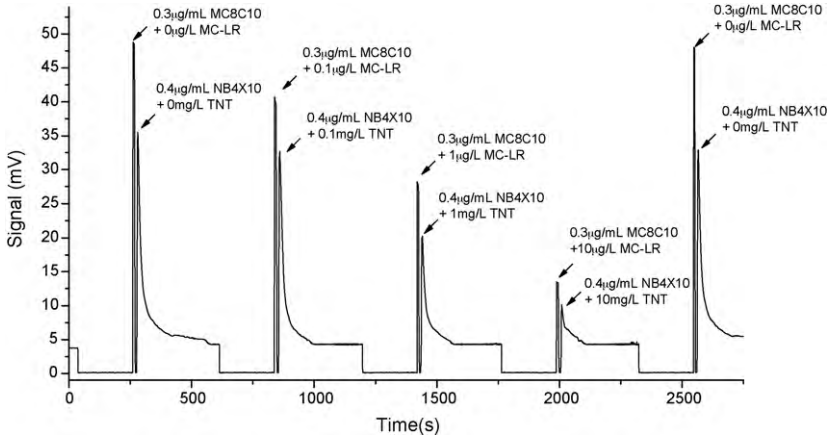

Fig. 6. Response curves of the microarray immunosensor to the sequential flow of different concentration of MC-LR and TNT over the sensor surface; all the analyte solutions were incubated with anti-MC-LR and anti-NB antibody solutions. After each run, the sensor surface was regenerated with the injection of 0.5% SDS (pH 1.9) buffer for 2 min.

the antigen in the unknown sample mixes with the labeled antibody of a fixed concentration (pre-incubation period), and the mixture is delivered to the receptor surface. The antibodies left with free binding sites bind to the antigen immobilized onto the solid phase. In this method, the detection signal will be inversely proportional to the concentration of antigen in the unknown. To produce the standard curves, all measurements are normalized with respect to the blank signal (that is, the antigen concentration is zero, and the maximum fluorescence signal can be obtained). The normalized ratio (R) can be expressed as:

$$R = \frac{S_x}{S_0} \tag{5}$$

where S_x is the fluorescence signal when the antigen concentration is given, and S_0 is the fluorescence signal when the antigen concentration is zero. From Eq. (5), R is found related only to the concentration of the antibody in the bulk solution, the association rate constant, and the dissociation rate, but not to the concentration of the conjugate immobilized onto the sensor surface. Therefore, it may be concluded that the concentration of the hapten conjugates immobilized onto the sensor surface does not affect the performance of the sensor, such as the LOD, IC_{50}, and quantitative detection range. This provided the possibility for proposed immunoarray biosensor to be expanded to detect more small analytes simply by adding more haptens to the existing array.

3.4. Regeneration and reusability

Regeneration and reusability are undoubtedly important aspects of the immunoarray. However, to date, most arrays, such as DNA chips and antibody/protein arrays, cannot be reused. This difficulty not only increases the detection price but also decreases the accuracy of array-based quantitative detection. In the current work, the hapten conjugates, MC-LR-OVA and NB-OVA, used as recognition elements were simultaneously attached covalently to the sensor surface, which could prevent the compromise of immobilized molecules' binding properties (Shriver-lake et al., 1995). The stability and activity of the immobilized immunosurface were evaluated through a large number of immunoassays (Fig. 6). In this paper, the immobilized recognition element could retain at least 150 successive assays without compromising sensor performance. After each MC-LR or TNT determination cycle, the complete removal of the non-covalently bound antibody was achieved and the active sensor surface was regenerated using 0.5% SDS solution. Good regeneration performance, excellent binding properties, and robustness of the sensor surface of the proposed immunoarray sensor are all critical for the cost-effective and accurate measurement of small analytes.

4. Conclusion and outlook

In this paper, a proof-of-concept hapten array is developed to detect multiple small analytes. For the current version, two highly regulated small pollutants (MC-LR and TNT) can simultaneously be detected. Compared with optic fiber array sensors that usually detect different targets using separate probes (Golden et al., 1997), the fiber-based immunoarray biosensor developed is obviously advantageous. Firstly, this immunoarray has simple and compact structure, and the variation of different probes can be overcome. Secondly, multi-analyte detection can easily be achieved by a fiber immunosensor with simple immobilization of more haptens on the same immunosensor. More importantly, compared with conven-

tional immunoarray biosensors, the proposed immunoarray sensor has a simple array format and gives quantitative results. Finally, the fiber-based immunoarray biosensor is inexpensive to fabricate, easy-to-use, and has regeneration and miniaturization features. The compact optic fiber-based immunoarray shows great potential for the rapid detection of small analytes in disease diagnosis, drug discovery, proteomics, and environmental monitoring, especially for individual laboratories and clinics in developing countries.

The proposed immunoarray biosensor can be expanded to detect more small analytes simply by adding more haptens to the existing array. However, organic dyes are not good for the multicolor detection due to their intrinsic photophysical properties such as susceptibility to photobleaching, broad emission spectra and small Stokes shifts (Resch-Genger et al., 2008). Fortunately, these disadvantages can be avoided due to the emerging development of quantum dots (QDs), which are highly fluorescent semiconductor nanocrystals. They exhibit remarkable brightness and photostability, narrow emission spectrum, and excellent biological compatibility (Cui et al., 2007). Most importantly, QDs are the ideal candidates for a multiplexed fluoroimmunoassay at a single excitation wavelength because of their unique flexibility in excitation and their narrow emission bands (Chan and Nie, 1998). Therefore, the application of QDs to high-throughput or parallel multiple assay formats can be anticipated. Currently, various efforts are ongoing on the development of QDs-based immunoarray biosensors for quantitative multiple assays.

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