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Highly sensitive and selective optofluidics-based immunosensor for rapid assessment of Bisphenol A leaching risk

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ABSTRACT

Bisphenol A (BPA), a xenoestrogenic endocrine-disrupting chemical, is used in many consumer products worldwide and is widely detected in the environment and in food. Combining the advantages of evanescent wave fiber optic sensor and microfluidic technology, an all-fiber optofluidics-based bioassay platform (AFOB) was developed for the rapid immunoassay and assessment of BPA. The captured molecular BPA–bovine serum albumin was covalently immobilized on the surface of the fiber optic sensor. A mixture of different concentrations of BPA and a certain concentration of fluorescence-labeled anti-BPA monoclonal antibodies after pre-reaction was introduced to the optofluidic cell. A higher concentration of BPA reduced the fluorescence-labeled antibodies bound to the sensor surface and thus reduced fluorescence signals. Under optimal conditions, the BPA quantified as 0.5–100 μg/L, with a detection limit of 0.06 μg/L. The high selectivity of the sensor was evaluated in terms of its response to several potentially interfering chemicals. The potential interference of an environmental sample matrix was assessed by spiked samples, and the recovery of BPA ranged from 90% to 120% with relative standard deviation values of $<9.1\%$. The AFOB and high-performance liquid chromatography had a desired correlation ($R^2=0.9958$). The sensing platform was successfully used to assess BPA leaching from polycarbonate bottles at 45 °C and 80 °C, indicating that more BPA was substantially leached at elevated temperature and extend time. Thus, the developed sensing strategy can be an alternative method to rapidly analyze and assess the migration mechanism and fate of BPA or other pollutants.

1. Introduction

Bisphenol A (BPA), a xenoestrogenic endocrine-disrupting chemical, is principally used as a monomer in polycarbonate (PC) plastics and as a constituent of epoxy resins (European Food Safety Authority (EFSA), 2006). BPA is widely detected in the environment and in food because of manufacturing processes and leaching from consumer products (Rudel et al., 2011; Vom Saal and Myers, 2008; Yang et al., 2011). Humans are exposed to BPA by many different mechanisms, predominantly through the consumption of contaminated food and beverages that has had contact with PC plastics or epoxy resins (Van Winkle et al., 2013). Thousands of consumer products, including food, drink containers, baby bottles, and water supply pipes, have been found to have residual BPA of <1 ppm to $>$ 600 ppm (Kruger et al., 2008). The adverse effects of BPA leaching from PC baby bottles on infant health have attracted considerable public attention (Jo et al., 2011). Consistent with its widespread presence, urinary BPA was detected in more than 90% of people in population representative samples (Calafat et al., 2008). The potential risks of BPA to human health are cardiovascular disease, diabetes, neurobehavioral disorders, carcinogenic hypersensitivity, and reproductive impairment (Rogers et al., 2013; Frye et al., 2012). Recent studies have demonstrated that BPA poses a potential health risk to humans and wildlife from exposure even at very low levels (Soto and Sonnenschein, 2010). Thus, the ultrasensitive and rapid detection of BPA is important for ensuring food safety and human health.

The primary analytical techniques used to determine BPA levels are high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS), and HPLC–MS (Watabe et al., 2004; Altamirano et al., 2011). Solid-phase extraction coupled with isotope dilution-HPLC is used as the 'gold standard' for BPA detection by the Centers for Disease Control and Prevention in the United States (Jo et al., 2011; Bucher, 2009). Although these analytical techniques are accurate with low detection limits, they require sophisticated and expensive instrumentation,

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multistep and complicated sample preparation, and expert personnel for operations. These requirements limit the practical applications of these methods to rapidly assess BPA on site. Biosensors are ideal alternative analytical tools for the rapidly monitoring environmental pollutants on site (Song et al., 2011). Various colorimetric, electrochemical, and optical biosensors that employ antibodies (Hegnerová et al., 2010; Lu et al., 2012), enzyme (Han et al., 2013), oligonucleotides (Jo et al., 2011), and/or metal nanoparticles (Song et al., 2011) have been used to detect BPA. However, these biosensors are associated with several problems, including low sensitivity and selectivity, complicated design and operation, and inconvenience. Therefore, challenges still remain in developing BPA biosensors that are highly sensitive and selective, cheap, reusable, and suitable for the rapid detection and risk assessment of BPA at low levels.

This study combined the advantages of evanescent wave fiber optic biosensor and microfluidic technology to develop an optofluidics-based biosensor for rapidly detecting and assessing BPA. Microfluidic technology precisely and rapidly detects environmental pollutants with small sample, a small amount of reagent, low unit cost, short reaction time, and a possibility of parallel operation (Thorsen et al., 2002). The evanescent wave optical sensor is one of the most important optofluidic technologies (Fan and White, 2011). When light transmits in the optical waveguide at the total internal reflection (TIR), the evanescent wave generated at optical waveguide surface penetrates essentially into the surrounding solution of low refractive index and exponentially decays with distance (Psaltis et al., 2006). Because the effective depth of the evanescent wave is about 100–200 nm, the free fluorescence-labeled biomolecules in the bulk solution do not contribute to the fluorescent signals and thus enable the real-time measurement of the binding reaction on the sensor surface and eliminate the need for washing. When the captured molecules (e.g., BPA–BSA) are immobilized on the sensor surface, the BPA in samples can be quantitatively detected in real time and at a rapid rate on the basis of the mechanism of the binding inhibition reaction. Experimental conditions, such as pre-reaction time, incubation time, and antibody concentration, were optimized. The optofluidics-based immunosensor showed potential for detecting BPA in real samples and for assessing the risk of BPA leaching from plastic bottles.

2. Experiments

2.1. Materials and chemicals

Bovine serum albumin (BSA), 3-aminopropyl triethoxysilane (APTES), glutaraldehyde, N,N′-Dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), Atrazine (ATZ), N,N-Dimethylformamide (DMF), 4,4′-(1-Phenylethylidene)bisphenol (AP), Nonylphenol (NP), Triclosan, 4,4 bis-(4-hidroxyphenyl) valeric acid (BVA), sodium dodecyl sulfate (SDS), 2,4-Dichlorophenoxyacetic acid (2,4-D), Bisphenol B (BPB), Bisphenol F (BPF), Biophenal S (BPS), and Bisphenol A (BPA) were purchased from Sigma-Aldrich (Steinheim, Germany). All other reagents, unless otherwise specified, were supplied by the Beijing Chemical Agents; they were also analytical grade and used without further purification. Distilled deionized water was used throughout the investigation. BPA was dissolved with methanol as the stock solution and stored at 4 °C. Standard concentrations of the analyte were prepared from the stock solution by serial dilutions in 0.01 mol/L PBS.

Anti-BPA monoclonal antibody (anti-BPA-MAb) was produced by our research group and was labeled with Cy5.5 as in previous research (Mujumdar et al., 1996). Because BPA has no functional groups that can be coupled to carrier proteins, we used BVA, a structural analog of BPA with a carboxyl group. The hapten–carrier conjugate BPA–BSA was synthesized as follows: BVA (22 mg) was dissolved in 1 mL DMF, and 13.3 mg of NHS and 17.4 mg of EDC was then added to this solution. The reaction mixture was incubated overnight at 4 °C while constantly stirred. Afterward, 2.5 mL of this mixture was added dropwise to 5 mL of BSA solution (10 mg/mL in 0.01 M PBS buffer at pH 7.4). The BPA–BSA conjugate was purified by dialysis and was frozen at −20 °C in small aliquots until use. MALDI–TOF MS revealed that the estimated number of hapten molecules attached to the carrier protein (i.e., the hapten-to-protein molar ratio) BSA was 24.

2.2. Preparation of fiber optic sensor

The preparation of a combination tapered fiber optic sensor has been described in previous research (Long et al., 2009). The hapten–carrier conjugate BPA–BSA used as a recognition element was covalently attached to the sensing surface of the fiber optic sensor by covalent coupling via glutaraldehyde, which ensures the reusability of the sensor surface without affecting the binding properties of the immobilized molecules. First, the sensor surface was cleaned with a piranha solution [H_2SO_4/H_2O_2, 3:1 (v/v)] for 30 min. The sensor was rinsed with ultrapure water and then dried in an oven at 105 °C. Second, the fiber optic sensor was aminated by immersion in a 2% (v/v) APTES acetone solution for 1 h to coat a reactive silane layer with an aminated-terminal silane. Excess APTES was removed with acetone to ensure the order and uniformity of the self-assembled monolayer. Third, the sensor was immersed in a 5% (v/v) glutaraldehyde solution at 37 °C for 1 h and was then washed with water. Fourth, the captured BPA–BSA molecules were covalently coupled to the sensor surface by immersing the fiber sensor overnight in 1 mL of a solution of 0.5 μM BPA–BSA in PBS (pH 7.4) at 4 °C. Finally, the sensor was immersed in 2 mg/mL BSA for 20 min to block non-specific binding sites on the sensor.

2.3. Instrument: portable all-fiber optofluidics-based biosensing platform

A portable all-fiber evanescent wave optofluidics-based biosensing platform (AFOB) was used to rapidly detect BPA (Fig. 1). This system comprised a microfluidic system, an all-fiber optical system, and a signal processing and control system. The microfluidic system consisted of a poly (methyl methacrylate) microfluidic chip and a peristaltic pump. The combination tapered fiber optic sensor (effective part: 15 mm length × Φ 225 μm) was embedded into a microfluidic channel 15 mm long, 600 μm wide, and 600 μm high. An integrated single-multi-mode silica fiber optic coupler was used to transmit excitation and to collect fluorescent light with high efficiency. A pulse laser light (635 nm, 5 mW) was coupled to the tapered fiber optic sensor through a single multi-mode fiber optic coupler. Incident light was propagated along the entire length of the fiber optic sensor via TIR. The evanescent wave was generated at the surface of the fiber optic sensor and excited the surface-bound fluorescence-labeled analyte complexes. The collected fluorescence signal was filtered by a bandpass filter (FF01-692/40, Semrock, USA) to eliminate any lost or scattered laser light and was subsequently monitored by photodiodes through digital lock-in detection. This optofluidic platform can rapidly detect pollutants at field sites because it has few optical components and reduce unnecessary optical alignment. Furthermore, this platform increases the efficiency of light transmission, reduces light loss, and improves signal-to-noise (S/N) ratio (Long et al., 2009). All reagents were supplied by a flow delivery system operated by a peristaltic pump. This fluid delivery system was automatically controlled by an embedded computer, which also acquired and processed the data. All relevant assay parameters were controlled by a special parser embedded within the user interface of the measurement software.

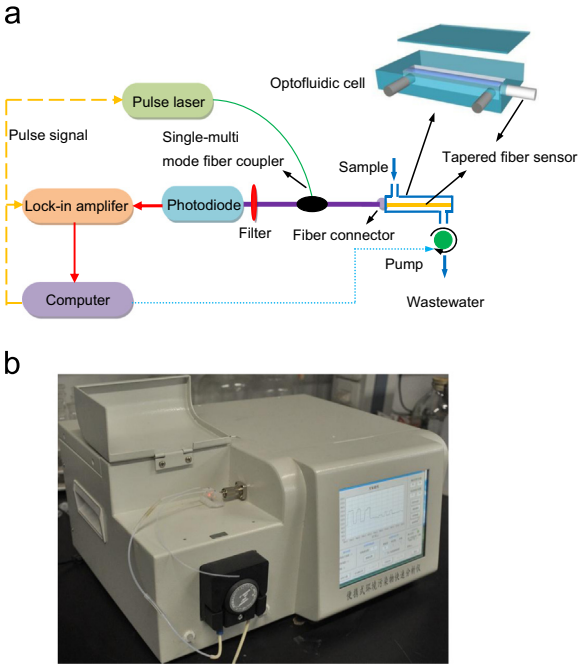

Fig. 1. (a) Schematic of AFOB system; (b) photo of AFOB system.

2.4. Assessment of BPA leaching

Four PC bottles of different brands were purchased from a local store in Beijing. Two of the brands were baby bottles (B1 and B2), and the other two brands were reusable water bottles (W1 and W2). All bottles were rinsed with boiling water. For the BPA leaching experiment, the bottles were completely filled with tap water from Tsinghua University, and were placed in an oven at 45 °C and 80 °C to simulate the temperatures used to reconstitute baby milk powder and to brew Chinese tea, respectively. Drinking tea is a daily habit of many Chinese, and the temperature for brewing tea water is often higher than 80 °C. Water samples of 100 μL were taken from each bottle after the bottles were incubated for 0.5, 1, 2, and 6 h.

3. Results and discussions

3.1. Sensing mechanism and characterization of BPA biosensor

The indirect competitive immunoassay mechanism of BPA is shown in Fig. 2. First, 50 μL of samples containing various concentrations of BPA were pre-reacted with 50 μL of fluorescence-labeled anti-BPA-MAb in PBS at a fixed concentration for a certain time. During that period, some antibodies specifically bound to BPA and the occupied antibody binding sites were proportionate to the BPA in the samples. Second, the mixture was delivered to the optofluidic cell at 2.5 μL/s in 20 s, and the antibodies with the remaining binding sites were bound to the BPA–BSA immobilized on the sensor surface (Phase II in Fig. 2) and thus increased the fluorescence signals detected. Finally, the sensing surface was regenerated with a 0.5% SDS solution (pH 1.9) for 120 s and was then washed with a PBS solution (Phase III in Fig. 2) to prepare the sensor for reuse.

Fig. 2 shows a typical signal profile of one cycle of BPA analysis. To determine subsequent dose–response relationships, the net fluorescence intensity I of each assay was calculated by subtracting the baseline value according to the following equation:

$$I = \text{peak fluorescent intensity} - \text{baseline fluorescent intensity} \quad (1)$$

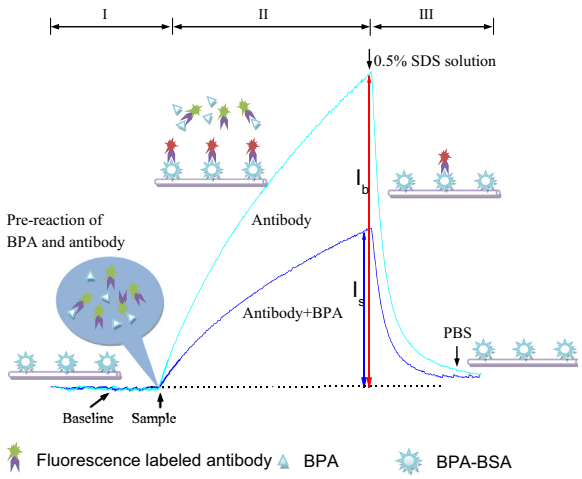

Fig. 2. Schematic diagram of sensing mechanism of BPA detection and representative signal profile of BPA detection with AFOB.

The normalized response was calculated as

$$I' = \Delta I/I_b = (I_b - I_s)/I_b \text{(a.u. fluorescence intensity)} \quad (2)$$

where I_b is the net fluorescence intensity of the blank without BPA and I_s is the net fluorescence intensity of the samples.

To confirm the observed fluorescence signal that originated from the binding between BPA on the sensor surface and fluorescence-labeled anti-BPA antibodies, fluorescence labeled anti-BPA antibodies and non-specific antibodies (e.g., anti-MC–LR antibodies) were introduced to the sensor surface (Fig. S1). When 10 ng/mL of Cy5.5 dye-labeled anti-BPA antibody was introduced, a strong fluorescence signal was detected, indicating the binding of the antibody to the sensor surface. When 60 ng/mL of Cy5.5 dye-labeled anti-MC–LR antibody was introduced, few fluorescence signals were observed. These results indicate that the contribution of fluorescence signals from non-special adsorption was negligible and that minimal free fluorescence in the solution was excited.

The selectivity of the sensor was also assessed by evaluating its responses to interferences from other endocrine disrupting chemicals (EDCs), such as BPB, BPS, BPF, estriol, 4-nonylphenol, and triclosan, and from other chemicals, such as simazine, diuron, and 2,4-D, all at 200 μg/L (Fig. S2). Except for BPB, the developed biosensor system had low signal intensity for all other EDCs and chemicals tested. This selectivity must contribute to the specificity of the antibody, an important factor that affects the rapid assessment of environmental pollutants without separation.

To evaluate the stability and reusability of the sensor, a series of immunoassays was performed (Fig. S3). After each cycle of BPA determination, the surface of the active sensor was regenerated with a 0.5% SDS solution (pH 1.9) to completely remove non-covalently bound antibodies. This regeneration method enabled the sensor surface to be reused for more than 100 measurements without damaging its physical and chemical properties. When the sensor was stored at room temperature and measured every day, the sensor retained approximately 80% of its original response after 30 days of storage (data not shown). These results show that the sensor presented had the desired reusability and stability, which were critical for ensuring the accuracy of detection results.

3.2. Optimization of BPA detection conditions

To examine the effect of pre-reaction time on the BPA immunoassay, experiments were conducted at different pre-reaction times of 5, 10, and 20 min. After 10 μg/L of BPA and 6 ng/mL of anti-BPA antibodies were pre-mixed at 5, 10, and 20 min and the mixture was

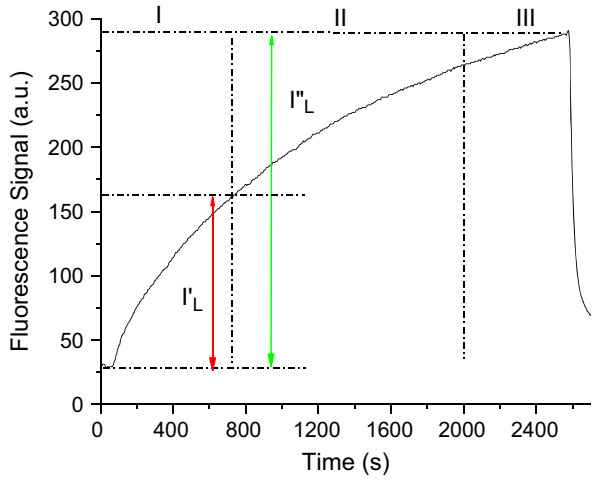

Fig. 3. Typical sensorgram of anti-BPA antibody (6 ng/mL) bound with antigen immobilized on sensor surface over incubation time.

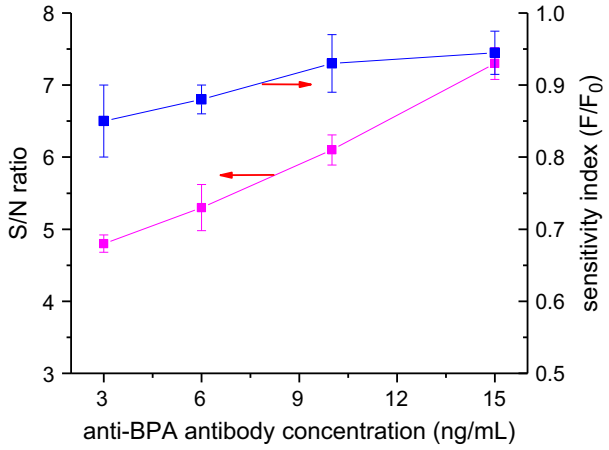

Fig. 4. Changes in *S*/*N* ratio (magenta) and sensitivity index (blue) in relation to concentration of fluorescence-labeled anti-BPA antibodies. (For interpretation of references to color in this figure legend, the reader is referred to the web version of this article.)

pumped into the cell. The fluorescence signal detected barely varied with variation in pre-reaction time (Fig. S4), indicating that the antibody and the sample were well mixed and that the binding balance between antigen and antibody was rapidly obtained. Therefore, a pre-reaction time of 5 min was selected to shorten the detection period.

To investigate the effect of incubation time on the detection signal, 6 ng/mL of Cy5.5-BPA-MAb was tested and the fluorescence signal was recorded (Fig. 3). When incubation time was shorter than 700 s (Phase I, Fig. 3), the fluorescence signal detected by AFOB linearly increased with increasing incubation time, and then slowly increased until the incubation time was longer than 2000 s (Phase II, Fig. 3). Over this time, the fluorescence signal only slightly increased and a platform was reached (Phase III, Fig. 3). For heterogeneous immunoassay, the antigen–antibody reaction rate was mass transport-limited, and the detection of low concentration samples (nM or lower) required a long time (Hegnerová et al., 2010; Lu et al., 2012). Fig. 3 shows that the binding balance between the antibody and antigen was obtained after more than 40 min. Considering the goal of reducing the assay duration and the signal intensity required, a signal value of 650 s, about 50% of the maximum (I_L''), was the desired result.

A sensitivity index (F/F_0), the ratio between the net fluorescence intensity obtained in the presence of an analyte ($F=I_b-I_s$) and that obtained in the absence of an analyte ($F_0=I_b$), was introduced to determine the optimum antibody concentration. Fluorescence-labeled antibodies at 3 ng/mL to 15 ng/mL were optimized for competitive immunological recognition and further generation of the fluorescence signal (Fig. 4). The sensitivity index was calculated from the fluorescence intensity at 1 μg/L BPA (F) and in the absence of BPA (F_0). Several experiments were performed, and the optimum concentration of the fluorescence-labeled antibody was determined according to the following criteria: (1) the *S*/*N* ratio was as high as possible, and preferably higher than 5.0 to generate reasonable fluorescence intensity, and (2) the sensitivity index was as low as possible, and preferably below 0.90. Therefore, the most acceptable antibody concentration was 6 ng/mL. For competitive immunoassay, low concentrations of fluorescence-labeled antibodies resulted in effective analyte competition, and enhanced sensitivity (Long et al., 2012).

3.3. Dose–response curves

Once the general assay parameters had been optimized, a quantification assay of the analyte BPA was performed on the basis of the mechanism of the binding inhibition immunoreaction. BPA standard solutions at 0–500 μg/L were explored to obtain BPA dose–response curves. The temporal fluorescence signal during a typical test cycle for BPA detection using the AFOB system is shown in Fig. 5A. The mixture containing BPA at different concentrations and 6 ng/mL of fluorescence labeled antibodies after pre-reaction was introduced to the optofluidic cell, and a high concentration of BPA reduced the fluorescence-labeled antibodies bound onto the sensor surface and thus to the reduced fluorescence signal. No fluorescence signal was observed when BPA flow was greater than 500 μg/L. This observation confirms that fluorescence signals were obtained neither by the non-specific adsorption of Cy5.5-labeled anti-BPA-MAb on the sensor surface nor by the excitation of free Cy5.5-labeled anti-BPA-MAb in the solution.

Fig. 5B shows that the calibration curve (the average of three independent curves) for BPA was normalized by expressing the signal of each standard point as a ratio to the signal of the blank sample without BPA. The signal intensities were fitted to a four-parameter logistic equation,

$$y=\frac{A_1-A_2}{1+([Ag]/[Ag_0])^p}+A_2 \quad (3)$$

where y is fluorescence intensity; $[Ag]$ is the BPA concentration; A_1 and A_2 are the upper and lower asymptotes (background signal) to the titration curve; $[Ag_0]$ is the analyte concentration at inflection; and p is the slope at the inflection point. The error bars in Fig. 5B correspond to the standard deviations of the data points in triplicate experiments; the standard deviations of all these data points were within 7.5%. The limit of detection (LOD) for BPA was 0.06 μg/L according to the calibration curve based on an *S*/*N* ratio of 3. The quantification value of BPA ranged from 0.5 μg/L to 100.0 μg/L, with a percentage relative standard deviation of 3.57–6.12%. The typical LODs for SPR biosensors for BPA are around 1.0 μg/L (Soh et al., 2003), whereas a compact SPR biosensor based on binding inhibition detection method has an LOD of 0.08 and 0.14 μg/L for PBS and wastewater, respectively (Hegnerová et al., 2010). The LODs for competitive ELISA were estimated to be 10.0 μg/L (Mujumdar et al., 1996). An amperometric nano-biosensor for BPA demonstrated a sensitivity of 0.2 μg/L under optimized experimental conditions (Han et al., 2013). The proposed biosensor had high sensitivity because of the following. First, the surface chemistry of the BPA–BSA conjugates on the sensor surface maintained the activity of the immobilized BPA toward anti-BPA-MAb and diminished non-specific adsorption. Second, the high number of hapten molecules attached to each

carrier protein allowed many fluorescence labeled antibodies to bind to the sensor surface and low antibody concentration to be used, and thus benefited the effective competition of analyte in competitive immunoassay and increased sensitivity. Meanwhile, the present AFOB system also manifested its other advantages, such as the regeneration of the sensor without compromising immunoreactivity for multiple analyses (more than 100 times). This regeneration is better than that of the reported SPR immunosensor (only fewer than 10 times) (Hegnerová et al., 2010) and that of the nano-biosensor (reused only about 5 times) (Long et al., 2009). Another important feature of this optofluidic biosensing system is that only a small amount of sample solution is required for analyses. Without any loss in sensitivity, the dimensions of the optofluidic device ensure a total volume ($<10\,\mu L$) less than that of conventional ELISA, which requires at least 100 μL to be filled in the microwell (Mujumdar et al., 1996). These dimensions significantly reduce the assay cost because several reagents (e.g., antibodies) are very expensive. Compared with the traditional immunosensors, the method proposed in the present study for BPA detection is simple and time-saving (only 15 min of detection, including measurement and regeneration).

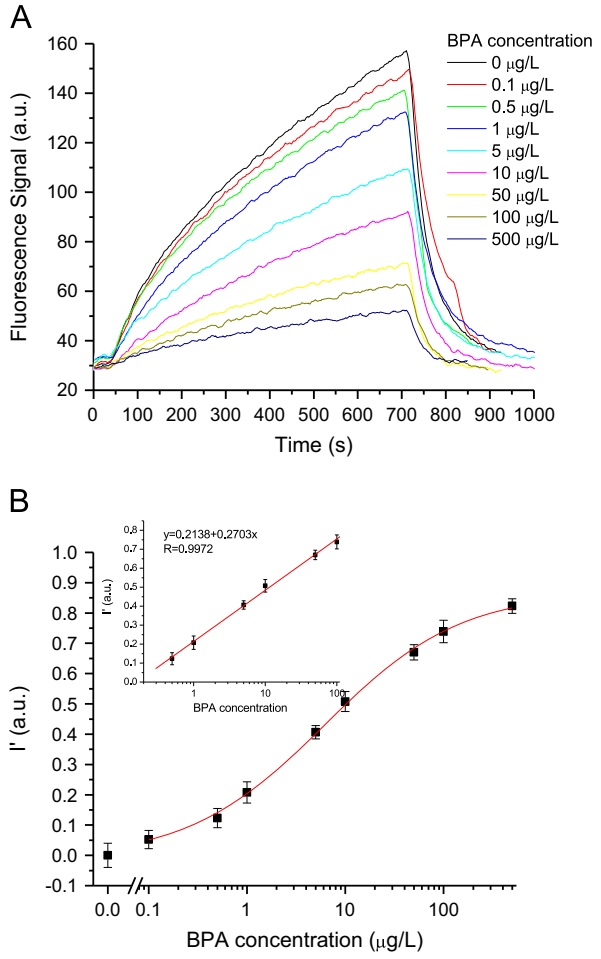

Fig. 5. (A) Typical sensor response curves and signals obtained at various concentrations of BPA. (B) Logarithmic calibration plot for determination of BPA based on optofluidics-based immunosensor. Inset: Linear relationship between BPA concentration and fluorescence intensity.

3.4. Assay of spiked water samples and calibration of AFOB

Because the binding between the antibody and antigen was affected by the pH, ionic strength and organic matter of natural water, the evaluation of the matrix effect is very important for the assessment of newly developed methods. In this study, spiked tap water (Tsinghua University) and Chinese tea were tested to evaluate the potential effect of real environmental water. Unspiked samples were measured to ensure that they were uncontaminated ($<0.06\,\mu g/L$). These environmental samples were then spiked with BPA solution at concentrations of 0.5, 2.0, and 10.0 μg/L. BPA content was measured by AFOB (in triplicate) and simultaneously evaluated by HPLC as previously described (Watabe et al., 2004; Altamirano et al., 2011).

The results are shown in Table 1. The recoveries of the spiked samples and the relative standard derivations were in the range of 90–120% and 3.8–9.1%, respectively. A comparison between the biosensor and HPLC yielded desired correlation results ($y=1.2726x-0.1064$). The slope (1.2726) of this linear regression equation indicates that, with respect to the measured HPLC values, the AFOB results were overestimated. The reason for the underestimated HPLC results may be the unavoidable loss of BPA when the water samples were pre-concentrated. Nevertheless, the correlation coefficient ($R^2=0.9958$) demonstrated satisfactory agreement between methods. All these data confirm that the proposed optofluidics-based immunosensor is applicable to BPA detection with acceptable precision and accuracy, even in a real environmental sample matrix.

3.5. BPA leaching of PC bottle

Residual BPA and degradation BPA by hydrolysis of carbonate linkages in PC products may migrate into food, especially at elevated temperature for extended periods (Li et al., 2010). Herein, tap water (pH 7.1, from Tsinghua University) was used as a food simulant to mimic aqueous foodstuffs. Fig. 6 shows the concentrations of BPA in water in the four PC bottles after these bottles were heated at 45 °C or 80 °C for different durations. Because only a 100 μL water sample was taken from each bottle for analysis each time, thus it should not affect the concentrations of BPA in water samples during subsequent migration periods.

Table 1
Water sample analysis by AFOB and HPLC.

No.	Spiked concentration (μg/L)	Spiked water tested by HPLC	Spiked water tested by AFOB	Recovery of AFOB (%)	CV of AFOB (%)
Tap water	0.50	0.46	0.56	112	5.2
	2.00	1.71	2.16	108	7.2
	10.0	8.57	9.62	96.2	5.1
Chinese tea	0.50	0.45	0.58	116	3.8
	2.00	1.63	1.85	92.5	9.1
	10.0	8.20	11.3	113	8.7

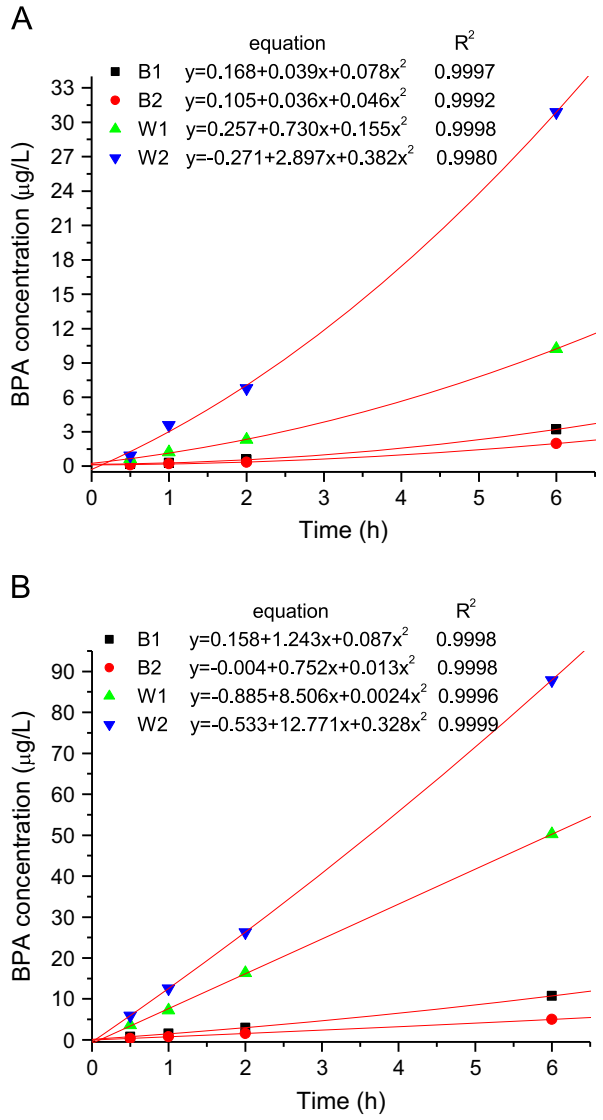

Fig. 6. Effect of temperature on BPA leaching from PC bottles. (A) The water temperature is 45 °C. (B) The water temperature is 80 °C.

Table 2
Leaching rate of BPA from PC bottles heated at 80 °C.

Bottle brand	Surface area (cm^2)	Volume (mL)	Leaching rate (ng/cm^2 h)					
			0.5 h	1 h	2 h	6 h	Average	RSD, (%)
B1	130	150	1.78	1.78	1.71	2.06	1.83	7.38
B2	139	150	0.86	0.89	0.89	0.96	0.90	4.02
W1	335	550	8.35	8.32	9.41	9.66	8.94	14.60
W2	429	600	13.66	14.53	15.18	16.90	15.07	16.99

Fig. 6 shows that BPA leaching increased with time for all PC bottles in a similar trend when the water temperature was 45 °C or 80 °C. BPA leaching kinetics can be fitted well to the quadratic equations (second-degree polynomials) with R^2 values greater than 0.9980. The leaching amount of reusable water bottles was higher than that of the baby bottles. The leaching level of BPA rapidly increased when the water temperature increased from 45 °C to 80 °C. At the water temperature of 80 °C, the leaching levels of BPA at 6 h was W2 (87.9 μg/L) > W1 (50.2 μg/L) > B1 (19.2 μg/L) > B2 (13.0 μg/L). To minimize exposure to BPA, the contact time of infant formula with the PC bottles should be a minimum as well as the water temperature for reconstituting baby milk powder should be as low as possible. Furthermore, the leftovers should be discarded to prevent accumulation of BPA in the formula.

If the leaching rate was defined as the amount of BPA leaching from the unit surface area of the bottle per unit of time, the leaching rate of BPA at 80 °C can be calculated as shown in Table 2. The BPA leaching rates of all bottles almost increased over time, which is consistent with the results reported in literature (Li et al., 2010; Cao and Corriveau, 2008). The leaching rates of all bottles ranged from 0.96 $ng/cm^2 \cdot h$ for brand B2 bottles to 16.90 ng/cm^2 h for brand W2 bottles. Because the relationships between the residual levels of BPA in the products and the migration levels were well-established (Kawamura et al., 1998), we assumed that the differences between the BPA leaching levels were likely due to the different levels of residual BPA in the products and that the high residual levels of BPA in the products increased the leaching levels of BPA under the same conditions. The degradation of PC at elevated temperatures may be another factor. Such degradation may vary from one product to another (Li et al., 2010). Therefore, for PC bottles, filling with hot water or heating to high temperatures increases the level of BPA leaching and is thus discouraged.

On the basis of the average value of the W2 bottle in Table 2, an adult ingests 129 μg/d of BPA through drinking water only if an adult (60 kg body weight) consumes 2 L of water per day (European Food Safety Authority (EFSA), 2006). Although this daily intake of BPA is lower than the tolerable daily intake, 50 μg/kg body weight for BPA (European Food Safety Authority (EFSA), 2006), exposure from drinking water is merely one of the many sources of EDC contamination. Other sources, such as food and beverages, should be included to properly assess risk for BPA. Health risk assessment with a single compound may also only partially reflect the effects of exposure to EDC mixtures in the real environment (Li et al., 2010).

4. Conclusions

In summary, a portable AFOB system was built to enable the rapid detection and risk assessment of BPA with high sensitivity and selectivity. This biosensing system has a number of distinct advantages over traditional detection technologies. First, the AFOB provides rapid and high-frequency measurements of BPA because one whole testing period (including regeneration) is shorter than 15 min, which provide new insight for analyzing and assessing the migration mechanism and fate of BPA in combination with physicochemical parameters (e.g., temperature and pH). Second, this system demonstrates sufficient sensitivity at an LOD of 0.06 μg/L, and few matrix effects were noted to occur during the BPA determination process even in real environmental samples. Third, the AFOB exhibits robustness and ruggedness with long-term use, and the fiber optic sensor modified by BPA–BSA conjugate is reproducible and verifiable. More importantly, to the best of our knowledge, this report is the first on a sensitive, rapid, on-site, real-time detection biosensor for the assessment of BPA leaching risk. Thus, the AFOB is a vital routine analysis that satisfies the high demand for ensuring the safety of drinking water and food.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.11.069.

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