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Correspondence and requests for materials should be addressed to Z.X.-H. (xhzhou@mail.tsinghua.edu.cn) or S.H.-C. (hanchang@mail.tsinghua.edu.cn)

A reusable evanescent wave immunosensor for highly sensitive detection of bisphenol A in water samples

Zhou Xiao-hong^{1,2}, Liu Lan-hua¹, Xu Wei-qi¹, Song Bao-dong¹, Sheng Jian-wu¹, He Miao^{1,2} & Shi Han-chang^{1,2}

¹State Key Joint Laboratory of ESPC, School of Environment, Tsinghua University, Beijing 10084, China, ²Collaborative Innovation Center for Advanced Water Pollution Control Technology and Equipment, Nanjing, China.

This paper proposed a compact and portable planar waveguide evanescent wave immunosensor (EWI) for highly sensitive detection of BPA. The incident light is coupled into the planar waveguide chip via a beveled angle through undergoing total internal reflection, where the evanescent wave field forms and excites the binding fluorophore-tagged antibodies on the chip surface. Typical calibration curves obtained for BPA has detection limits of 0.03 µg/L. Linear response for BPA ranged from 0.124 µg/L–9.60 µg/L with 50% inhibition concentration for BPA of 1.09 ± 0.25 µg/L. The regeneration of the planar optical waveguide chip allows the performance of more than 300 assay cycles within an analysis time of about 20 min for each assay cycle. By application of effective pretreatment procedure, the recoveries of BPA in real water samples gave values from $88.3\% \pm 8.5\%$ to $103.7\% \pm 3.5\%$, confirming its application potential in the measurement of BPA in reality.

Bisphenol A (BPA), also known as 2,2-bis-(4-hydroxyphenyl) propane or 4,4'-isopropylidenediphenol, has been widely used in the production of epoxy resins and polycarbonate plastic used in food packaging although it has been proven that BPA could mimic the effect of endogenous hormones, estrogens and androgens by binding to the estrogen receptor and proliferation^{1,2}. Due to its harmfulness, BPA has been identified as endocrine disrupting compounds by the U.S Environmental Protection Agency (EPA), World Wide Fund for Nature (WWF) and is declared as a social, environmental and global issue³. It is discharged into the aquatic environment (both freshwater and marine waters), not only from the migration from BPA-based products, but also through effluent from wastewater treatment plants and landfill sites^{2,4}. Majority of studies have revealed that levels of BPA detected in the aquatic environment were lower than 1 µg/L⁴; therefore, developing an easy and sensitive method for on-site detection of BPA is of critical significance for food safety and human public health.

Traditional analytical methods for BPA monitoring in water include gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS)^{5–7}. These methods, although accurate and reliable, are labor-intensive and require expensive and sophisticated instrumentation, as well as complicated and multistep sample pretreatment, which prohibit on-line and real-time monitoring. Besides these classical methods, the application of various enzyme linked immunosorbent assays (ELISA), which is followed by the recently developed fluorescence-linked immunoassay (FLISA), for the determination of bisphenol A in aqueous samples has also been reported^{8–10}. However, the methods depend on heavy manual labor and need to consume relatively large amounts of reagents. Therefore, much effort has been devoted to cost-effective, rapid, and on-site detection of BPA, including colorimetric¹, electrochemical^{11–13}, and optical sensors employing the native fluorescence of BPA¹⁴, functional polymers¹⁵, antibodies^{16–20}, oligonucleotides²¹, and enzyme²² as sensing elements.

Among the optical sensors, evanescent wave sensing systems have long been favored because they confine the interactions between light input/output and fluidics inflow/outflow to a single interface, allowing greater flexibility in the overall design^{23–26}. Evanescent wave biosensors using planar waveguides as the transducer have evolved into two major groups. One is based on the refractive index changes caused by mass adsorption within the evanescent field, such as the outstanding configuration of traditional surface plasmon resonance (SPR)^{17–20}, which are associated with the attractive features of in-situ and label free; however, the sensitivities of these methods are inferior²⁷. The other is using evanescent fields to probe specifically sensitized films on the waveguide surface,

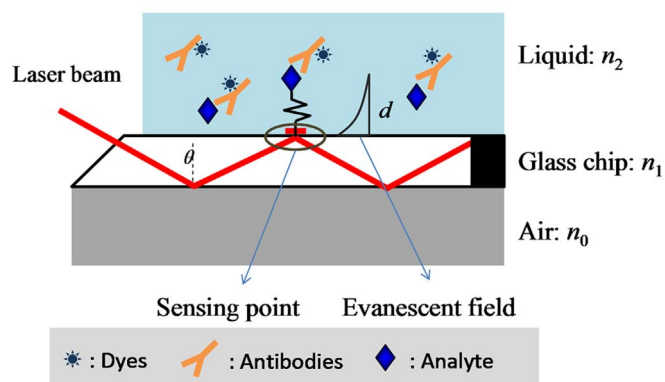


Figure 1 | Principle schematic of evanescent wave planar optical waveguide chip.

named as evanescent wave excitation, such as the RIANA and AWACSS devices^{26,28,29} achieved in the EU projects. Compared to the label-free methods, the luminescence-based sensors offer the advantage of improved sensitivity, optimized detection limit and independent of the molecular size^{27,30}. In terms of BPA detection, the detection limit of the evanescent wave excitation system was reported to be 0.014 $\mu\text{g/L}$ ¹⁶, which was 1–2 orders of magnitude lower than that obtained by the label-free SPR system^{18,20}.

Therefore, we propose a compact and portable planar waveguide evanescent wave biosensor, which employs fluorescence-based detection of the binding of fluorophore-tagged antibodies to the surface of a planar optical waveguide chip. The incident light is coupled into the planar optical waveguide chip via a beveled angle to form reflection spot for biosensing. In combination with bio-affinity assays between antibodies and antigens, the biosensor with this transducer geometry offers a broad number of advantages, such as reusable and cheap chip, enhanced sensitivity, ease of sensor handling and preparation, sample volume reduction, versatility with the potential prospects of developing multi-channel detection for online, laboratory and portable applications and low cost of no more than 20 RMB per test.

Results

Theory and sensor configuration. When a light beam propagating through a transparent medium of high index of refraction (e.g. a solid

glass prism) encounters an interface with a medium of a lower index of refraction (e.g. an aqueous solution), it undergoes total internal reflection (TIR) for incidence angles greater than the “critical angle” of θ_c , which is given by³¹

$$\theta_c = \sin^{-1}(n_2/n_1) \quad (1)$$

Where n_2 and n_1 are the refractive indices of the liquid and the solid, respectively. Although the incident light beam totally internally reflects at the interface, the electromagnetic field called the “evanescent wave” still penetrates a small distance into the liquid medium and propagates parallel to the surface in the plane of incidence. The evanescent wave is capable of exciting fluorescent molecules that might be present near the interface.

In the proposed evanescent wave biosensor, we adopt a simple transducer geometry (Fig. 1) using a rectangular K9 glass (Jinji optical glass processing center, Beijing) with a high refractive index of 1.5163 as the waveguide core; however, only air and liquid bulk phase surrounding the chip with low refractive index of 1.33 function as the upper and lower cladding. Chip sizes are 60 mm \times 15 mm with depth of 2 mm. The incident light is coupled into the waveguide chip via a beveled edge with angle of 45°. The present transducer geometry resulted in a θ_c of 61.3°.

The evanescent electric field intensity decays exponentially with perpendicular distance z from the interface. This penetration depth (d) can be calculated from the incident wavelength (λ) and the incidence angle (θ) using

$$d = \frac{\lambda}{4\pi} (n_1^2 \sin^2 \theta - n_2^2)^{-1/2} \quad (2)$$

In the proposed system as shown in Fig. 2, laser diode (Huanyuan-Star Laser Ltd., Beijing) light with 635.0 nm wavelength and 10 mW power is coupled into the beveled edge of the biochip and propagates along the sensitive area of the biochip by the TIR. The penetration depth of evanescent wave is nearly 100 nm, which could allow studies of turbid or highly adsorbing solutions^{25,31}.

In the TIR point, the evanescent wave field interacts with the surface-bound fluorescence-labeled biomolecules, causing the excitation of fluorophores. The emitted fluorescence light is collected by high-numerical-aperture polymer fibers (NA = 0.46) located beneath the biochip opposite the biosensing layer. The collected light is filtered by a highpass filter (HF01-700, CDHC-Optics, China) to reject any lost and scattered laser light, and then the light signal is detected by the photodiodes through a lock-in amplifier.

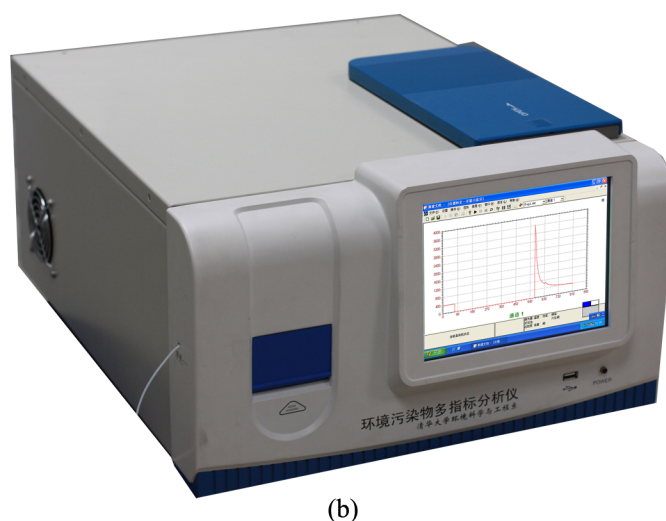
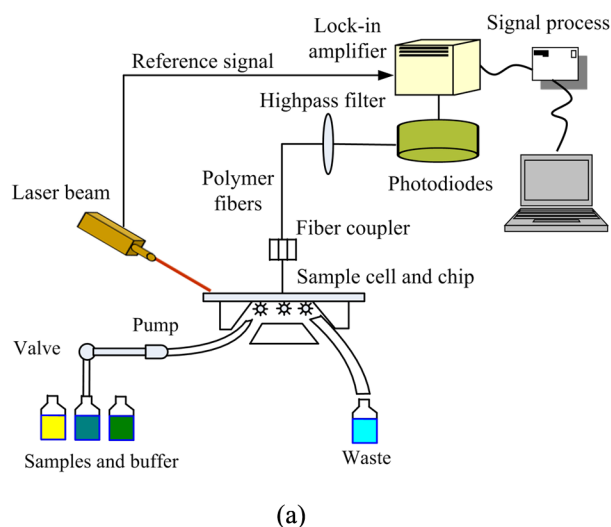


Figure 2 | (a) Schematic of planar waveguide evanescent wave immunosensing platform. (b) Photograph of the planar waveguide evanescent wave biosensing platform for the reusable detection of Bisphenol A (Photo by Bao-dong Song).

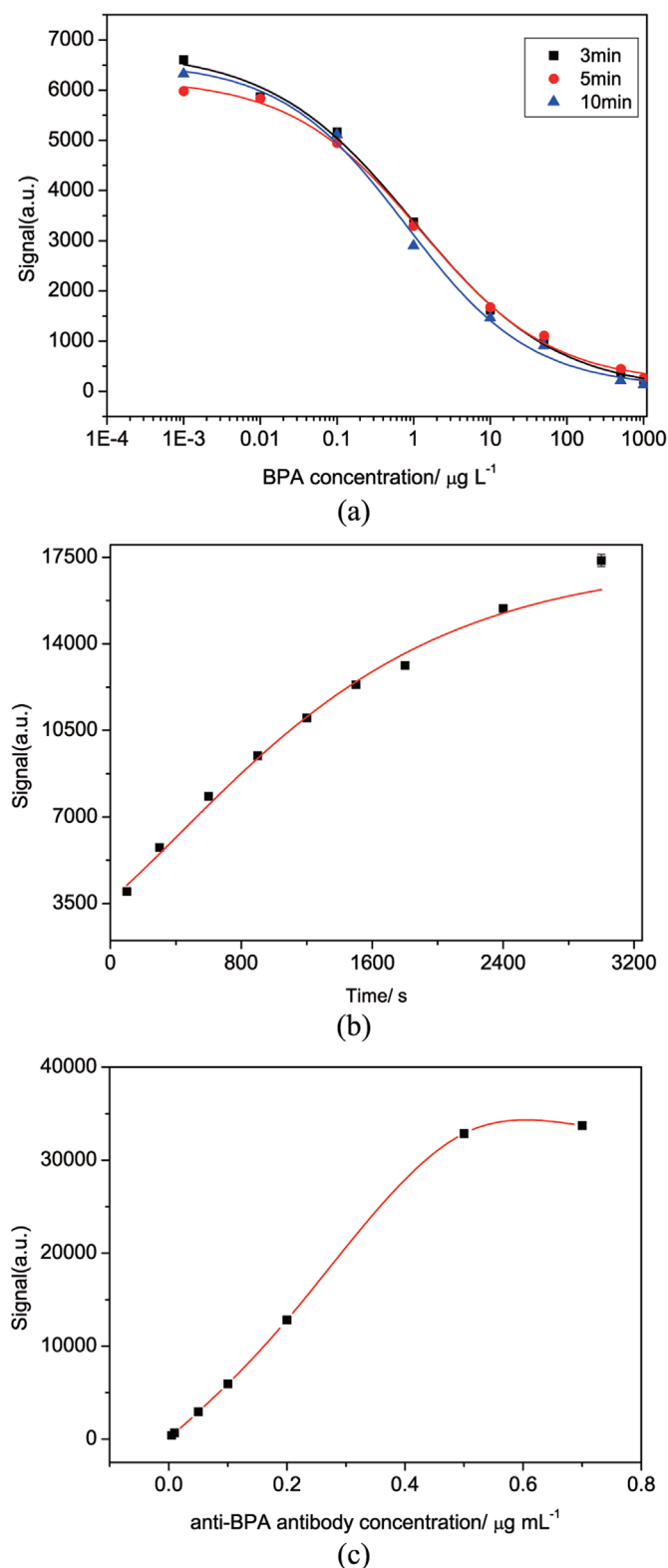


Figure 3 | Effect of pre-incubation time (a), incubation time (b) and Cy5.5-labelled antibody concentration (c) on the calibration curve or signals of EWIS system for the BPA detection.

The flow injection system consists of a syringe pump, a six-way injection valve, a prereaction loop (1 mL), a solenoid valve, and a sample cell. To ensure the activity and stability of bioreagents, the antibody storage and prereaction loop are kept in two individual thermostats, where the temperatures are adjusted to be 4°C and

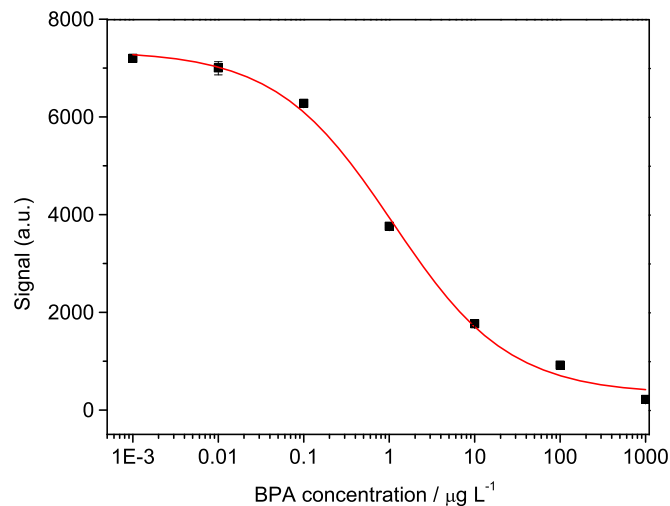


Figure 4 | Typical calibration curve of EWIS system towards BPA detection ($n = 3$).

37°C , respectively. All assay-related parameters are controlled by a special parser embedded within the user interface of the measurement software. Fluid handling and data acquisition are fully automated and computer controlled.

Optimized detection conditions. Fig. 3a shows the effect of pre-incubation time on the calibration curve of EWIS for the detection of BPA. All calibration curves accorded well with the logistical model (Eq. 3). The quantitative detection range and sensitivity of EWIS were slightly improved when the pre-incubation time was over 5 min. Therefore, 5 min was adopted as the appropriate pre-incubation time through considering both the improved accuracy and the reduced test time.

The effect of incubation time on the signals of EWIS for the detection of BPA was also investigated (Fig. 3b). Obviously, the longer incubation time resulted in the higher output signal, which could improve the sensitivity of EWIS system. However, also considering reducing the whole test time, an incubation time of 300 s was adopted in our experiments, where the signal reached over 50% of the relatively stable value.

The antibody concentration has a strong effect on the performance of competition immunoassays^{26,35} as shown in Fig. 3c. Our previous studies revealed that the EWIS system could reach relatively high sensitivity when the maximum signal was in the range of 6000 a.u. to 7000 a.u. Therefore, in order to save the reagent cost and simultaneously achieve high detection sensitivity, 0.05 $\mu\text{g/mL}$ of antibody was adopted in the following experiments.

Immunoassay. The performance of the biosensor towards the detection of BPA was demonstrated by measuring the response to seven known concentrations of BPA in PBS solution, ranging from 0.001 $\mu\text{g/L}$ to 1000 $\mu\text{g/L}$. Fig. 4 shows the average value and standard derivation (S.D.) of three individual calibration curves for BPA detection under the optimum testing conditions. The IC_{50} of immunoassay is $1.09 \pm 0.25 \mu\text{g/L}$. The quantitative detection response for BPA ranged from 0.124 $\mu\text{g/L}$ to 9.60 $\mu\text{g/L}$ with the limit of detection (LOD), using the 90% of the signal difference region (A_1 - A_2), of 0.03 $\mu\text{g/L}$. The biosensor performance meets the requirement for the determination of BPA in the drinking water set by the Chinese government. During a complete test cycle, the entire test time is no more than 20 min.

Cross-reactivity. In order to validate the reliability of proposed EWIS system to specifically detect BPA in real aqueous samples, cross-reactivity signals of the system towards atrazine, MC-LR and 2,4-D

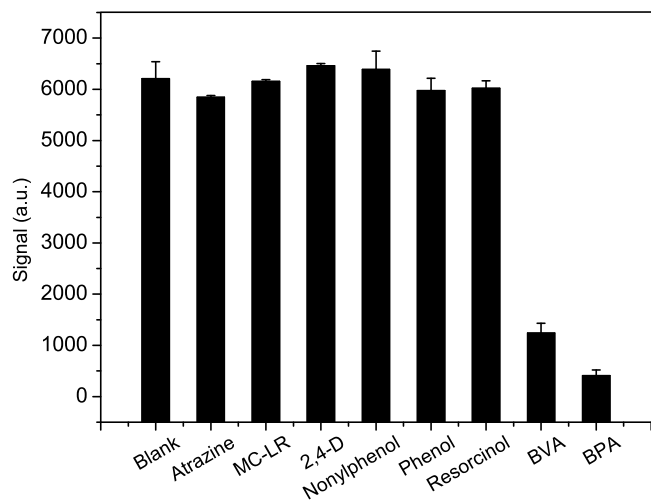


Figure 5 | Signal responses towards 500 $\mu\text{g/L}$ BPA and interferences in water samples.

commonly existed in the pollutant water were investigated. The results demonstrate that the cross-reactivity responses (CR) decrease by less than 6% compared with the blank value when the added concentration of interferences is up to 500 $\mu\text{g/L}$ (Fig. 5). The adverse effects of investigated organic matters on the EWI system response are negligible, which confirms that the EWI system appears little susceptible to interferences in the water samples during BPA determination.

We also evaluated the cross-reactivities of one phenolic compound (BVA) and three simple phenolic/phenolic xenoestrogens compounds (phenol, nonylphenol, resorcinol) as shown in Fig. 5. From the results, it could be clearly seen that the CR responses decrease by 79% and 93% for BVA and BPA, respectively, compared with the blank value, while the cross reactivities for the other three compounds were almost negligible. The results are comparable with the study previously reported by Feng *et al.*⁹. The possible reason is the produced antibody specifically recognized the common part of the structure of BPA and BVA, i.e. two phenolic rings bridged through a carbon atom as stated by Feng *et al.*⁹. However, more detailed investigations towards the cross-reactivity of the monoclonal antibody (BPA-MAb, 4D11) are needed to be undertaken and described in future.

Regeneration and sensor reusability. In the immunoassay herein described, the hapten-carrier conjugate is covalently coated on the

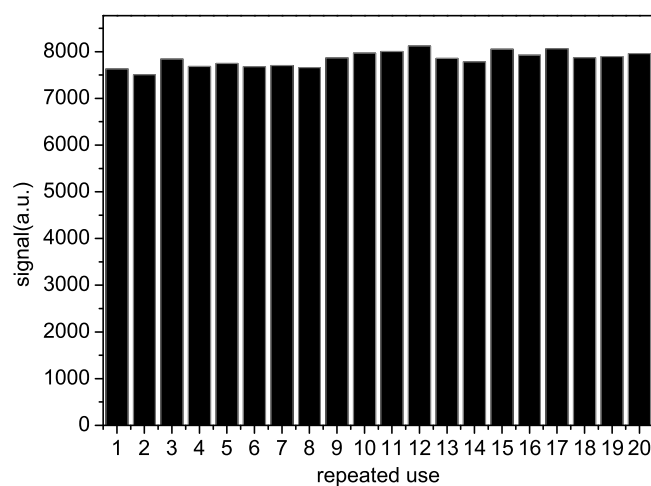


Figure 6 | Signal recovery after twenty times of measurements regenerated by using 0.5% SDS solution at pH = 1.9.

chip surface, which binds to the dye-labelled antibody. After one test cycle, 0.5% SDS solution (pH 1.9) was used to remove the bound antibody. The signal recovery after twenty consecutive determinations when using regeneration solution of SDS at a constant flow rate of 1 mL/min gives a relative standard deviation of 2.1% (Fig. 6).

Based on our long experiences, the surface regeneration was carried out up to 300 times with a less than 10% decrease of the registered signal observed, indicating no significant degradation of the surface chemistry during the regeneration process.

Interference, recovery study and analytical application. Interferences caused by the ionic strength, pH value and humic acid were investigated on the sensitivity and stability of the EWI system as shown in Fig. 7. In order to make the values more comparable, relative signal value, i.e. the ratio of signal with the maximum value (A_1), was used in the following figures.

In an immunoassay, the pH of solutions obviously do not only affect the stability and biological activity of antibodies, but also the binding efficiency between antibody and antigen^{26,35,37–39}. From Fig. 7a, we can see that acid solution result in higher IC_{50} value compared with the control pH value of 7.4. At lower pH values (<5) or higher pH values (>8), a decrease of the signal was observed in the EWI determination of BPA, indicating that the pH values of solution strongly affected the stability and sensitive of the proposed immunoassay system. Moreover, the immunoassay was the most sensitive between pH 5 and pH 8 and almost no changes in the LOD and IC_{50} were observed in the BPA determination. As a result, when the pH of the real water samples tested is too high or too low, pH value adjustment should be considered to prepare samples and antibody solution to eliminate its impact on immunoassay. Ionic strength is also one of the important factors for the performance of immunoassay although the reported experimental results were contradictory^{37–39}. In the proposed EWI system as shown in Fig. 7b, using a higher ionic strength PBS buffer (20 mM or 50 mM) would result in a higher IC_{50} value, indicating the system was sensitive to the ionic strength.

It is well-known that the organic matter (dissolved and suspended) content in surface waters may have a negative effect on the immune determination^{37,40}. To evaluate this effect, the calibration curves of BPA were prepared in 10 mM PBS containing 30 mg/L humic acid, which represented organic matters in natural waters at high level⁴⁰. As shown in Fig. 7c, the consistency of calibration curves with and without humic acid is satisfactory, and the maximum signal value and IC_{50} did not change significantly. Previous study confirmed that the porous structure of humic acid polymer can absorb or chemically react with organic pollutants, therefore resulting into the impact of immunoassay⁴⁰. However, the detection performance was not affected under the investigated conditions of adding BSA into the antibody solution. The BSA played a role in shielding the non-specific adsorption of the antibody³⁷.

Moreover, another interference study was performed, in triplicate, by comparing the calibration curves in real water matrix. The system was sensitive to the ionic strength (Fig. 7b), which was mainly affected by the hardness (Ca^{2+} or Mg^{2+} concentrations) in natural waters. As a result, the chelating reagent should be considered in the assay of real water samples with high hardness. We used the tap water matrix to prepare the calibration curves (Fig. 7d) with and without adding 0.5% (w/w) EDTA in the samples. The tap water comes from deep ground water sources and the hardness of tap water is in the range of 290–350 mg/L in calcium carbonate. In china, the allowed harness in drinking water is below <450 mg/L in calcium carbonate (GB5749–85). Only the hardness of 13% surface water in China is higher than 450 mg/L⁴¹. Therefore, under the obviously adverse condition, a significant matrix effect was observed by comparing the two calibration curves. The IC_{50} values were 4.6 $\mu\text{g/L}$ for tap water matrix with

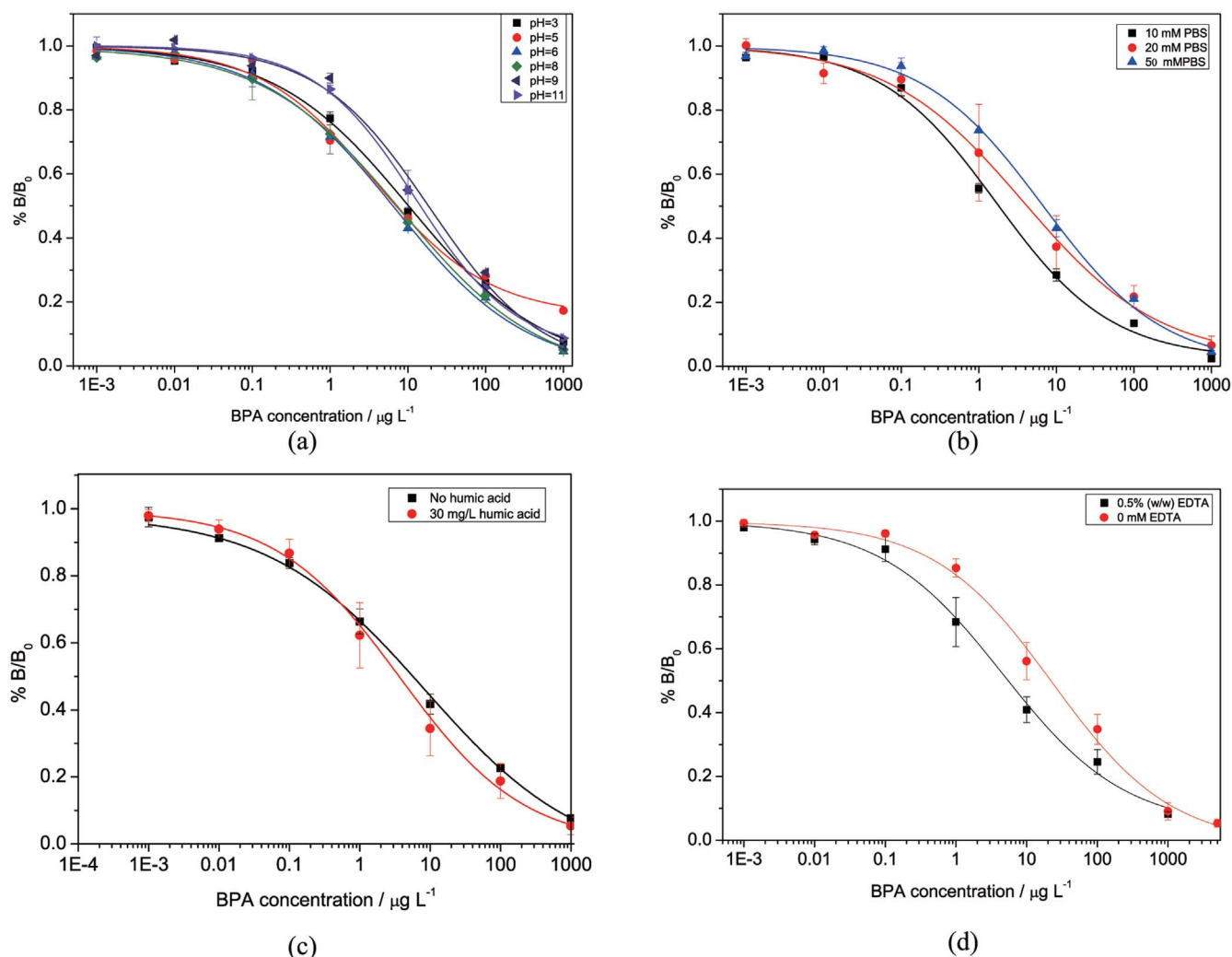


Figure 7 | Effects of pH value (a), ionic strength (b), humic acid (c) and addition of EDTA (d) on the BPA immunoassay based on EWI system.

EDTA addition and 23.3 $\mu\text{g/L}$ for matrix without EDTA addition, respectively. The calibration curve in the tap water was influenced by the new environment. However, the impact can be eliminated by adding 0.5% (w/w) EDTA. Furthermore, according to the chelating ratio of approximate 1:1 between the EDTA and Ca^{2+} or Mg^{2+} , the used EDTA 0.5% (w/w) can eliminate the impact of hardness up to 1700 mg/L as calcium carbonate, which could cover most water matrixes, including the surface and ground water in China.

Under the conditions of adding the chelating reagent EDTA of 0.5% (w/w) into the real water samples and preparing the antibody solution in 10 mM PBS containing 5.0 mg/mL BSA and 0.1 mg/mL thiomersal, a recovery study was performed, in triplicate, using three real water samples including bottled pure water, tap water and lake water taken from the campus of Tsinghua University, respectively, spiked with three different standard concentrations (0, 1 and 2 $\mu\text{g/L}$) of BPA. The concentrations measured were compared with the concentrations added and results are summarized in Table 1. It shows that the average recoveries vary from $88.3\% \pm 8.5\%$ to $103.7\% \pm 3.5\%$, demonstrating the satisfactory accuracy of the developed biosensor and confirming the application potential of our method to measure BPA in real samples.

Discussion

This study proposed an evanescent wave immunosensor system which realized the quick, sensitive and selective detection of BPA in water samples. It is no doubt that the immunoassay would have a

high level of sensitivity if extremely high-affinity antibodies were used³⁶. Therefore, one of the critical success factor is attributed to the high quality anti-BPA antibody. Our group has accumulated abundant research experiences in the production of monoclonal and polyclonal antibodies for microcystin, 2,4-D, atrazine and BPA *et al.*, which ensures the antibody supply in the construction of biosensors.

Table 1 | Recovery of BPA in different real water samples using the proposed EWI system

Sample	BPA ($\mu\text{g/L}$)		Recovery \pm RSD(%)
	Added	Found ^a	
Bottled pure water	0.00	0.37 \pm 0.007	
	1.00	1.41 \pm 0.04	103.7 \pm 3.5
	2.00	2.35 \pm 0.06	99.1 \pm 3.0
Tap water	0.00	0.09 \pm 0.007	
	1.00	1.07 \pm 0.10	98.2 \pm 10.0
	2.00	2.09 \pm 0.14	100.3 \pm 7.0
Lake water	0.00	0.04 \pm 0.006	
	1.00	0.93 \pm 0.08	88.3 \pm 8.5
	2.00	2.05 \pm 0.10	102.3 \pm 5.2

^an = 3.



Compared with the common analytical methods, such as GC-MS or HPLC-MS, the proposed EWI system requires no sample pre-concentration. By employing of the evanescent field, fluorescent dyes near the chip surface are excited and the generated fluorescence signals quantitatively relate with the concentrations of analyte. In the EWI system, a binding-inhibition assay with an immobilized BPA derivative, and a monoclonal antibody to BPA as biological recognition element are adopted. The indirect competitive immunoassay is proven to be highly sensitive with a low detection limit, because the decrease in analyte sample concentration increases the binding site of hapten conjugate captured with the antibody^{23,27,30}, which results in a high sensitive detection of BPA with IC_{50} of $1.09 \pm 0.25 \mu\text{g/L}$.

Through proposing an effective pretreatment procedure by adding the chelating reagent EDTA of 0.5% (w/w) into the real water samples and preparing the antibody solution in 10 mM PBS containing 5.0 mg/mL BSA and 0.1 mg/mL thiomersal, the recovery study confirmed its application potential in the measurement of BPA in real water samples. It also paves the way for the biosensing of chemical and biological parameters in water quality monitoring, food safety detection, and biochemistry analysis.

Methods

Materials and reagents. N-(4-Maleimidobutryloxy) succinimide (GMBS), bovine serum albumin (BSA), 3-mercaptopropyl-trimethoxysilane (MTS), 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC), BPA and humic acid were purchased from Sigma–Aldrich (Germany). The atrazine, microcystin-LR (MC-LR) and 2,4-dichlorophenoxyacetic acid (2,4-D), which are the commonly existed organic pollutants in the water bodies, and four phenolic compounds of 4,4-bis (4-hydroxyphenyl) valeric acid (BVA), nonylphenol, phenol and resorcinol, used for cross-reactivity tests were also purchased from Sigma–Aldrich. All other reagents, if not specified, were supplied by the Beijing Chemical Agents; they were all at analytical grade and used without further purification. Distilled deionized water was used throughout the experiments.

1 mg/mL BPA stock solution was prepared in mixture of 4 : 6 (v/v) methanol and deionized water and stored at 4°C. Series of BPA standard solutions at different concentrations were freshly prepared by serial dilutions of stock solution using 0.01 M PBS buffer (pH = 7.4). Monoclonal anti-BPA antibody (BPA-MAb, 4D11) was produced by our research group and labeled by Cy5.5 (GE Healthcare Life Sciences) as previously described by Mujumdar *et al.*³². The hapten conjugate of BPA and carrier protein was synthesized according to the procedure proposed by Moorhead *et al.*³³.

Planar optical waveguide chip preparation. Sizes of the K9 glass chip are 60 mm × 15 mm and 2 mm in depth (Jinji optical glass processing center, Beijing) with a polished 45° bevel on one endface used for incident light coupling. The other endface is coated with black paste to absorb the reflection of light. In order to facilitate the following bimolecular modification, a thin layer of SiO₂ film (35 nm) was deposited on the K9 glass chip by the magnetron sputtering (PVD coating technology).

Immunoassay. An indirect competitive immunoassay for the trace concentration of BPA detection is developed and stepped as follows. The coating antigen BPA-BSA is covalently immobilized on the chip surface by a similar procedure described by Long *et al.*³⁴. When performing the test cycle, 0.8 mL of sample solution and 0.2 mL of Cy5.5-labelled antibody solution (in 10 mM PBS containing 5.0 mg/mL BSA and 0.1 mg/mL thiomersal) is firstly transferred to the pre-incubation loop for a certain time to make the antibody-binding sites occupied with the analyte, which is called “pre-incubation”. Subsequently, the mixture is delivered into the sample cell. Antibodies still left with free binding sites would bind the coated antigen immobilized on the biochip. The process is called “incubation”. To reduce the effect of free antibody in solution and its non-specific adsorption on the detection result, the fluorescence signal is detected after the mixture is washed with PBS solution. The amount of antibody immobilized on the chip is proportional to the concentrations of analytes and in turn is proportional to the fluorescence intensities excited by the evanescent wave field.

In order to achieve optimum detection performances for the antigen-coated indirect immunoassay, comparative experiments were validated under various operation conditions, such as the antibody concentration, pre-incubation time and incubation time. The main criterion used to evaluate immunoassay performance was IC_{50} defined as follows.

Evaluation of calibration curves and cross-reactivity. In order to obtain a comprehensive reversed S-shape curve, the concentration of standard BPA ranged from 0.001 $\mu\text{g/L}$ to 1000 $\mu\text{g/L}$, containing seven calibration points. Standard curves of EWI system towards BPA detection were obtained by fitting calculated means ($n = 3$) for logistic model, a five-parameter function³⁵.

$$SI = \frac{A_1 - A_2}{1 + (x/x_0)^p} + A_2 \quad (3)$$

Where x is the BPA concentration; SI is the signal intensity of EWI system; A_1 is the maximum signal (upper asymptote, $x \rightarrow 0$) and A_2 is the minimum absorbance (lower asymptote, $x \rightarrow \infty$). x_0 is the midpoint or inflection point (IC_{50}), p is the slope of the tangent at this point.

The quantitative detection range is defined as the signals from 20% to 80% of the signal difference region ($A_1 - A_2$), which should be a linear range. The limit of detection (LOD) is determined using the 90% of the signal difference region ($A_1 - A_2$).

Cross-reactivity was used to assess the specificity of the EWI system against several commonly existed organic pollutants to BPA and stepped as follows. 500 $\mu\text{g/L}$ BPA and interferences were detected using the EWI system in succession to obtain the respective signals (SI), which were compared with the blank signal (SI_0) using PBS buffer. The cross-reactivity (CR) was calculated using the drop of signal compared with the blank one according to function (4):

$$CR(\%) = [(SI_0 - SI)/SI_0] \times 100 \quad (4)$$

Interference, recovery study and analytical application. In terms of a practical and quantitative immunoassay technology, evaluation of matrix effects is of great importance because antigen and antibody binding depends mainly on van der Waals forces and hydrophobic interactions, which are greatly affected by effects existing in real water samples, such as pH, ionic strength, organic contents and so on^{37–40}. Interferences were performed under a set of sequential experimental parameters, such as ionic strength, pH and real water matrix, to assess their impacts on the sensitivity and stability of the BPA fluorescence immunosensor, and demonstrate that with the choice of a proper elimination method, the influence of interfering substances can be eliminated to the acceptable extent, which resulted in a satisfactory recovery performance.

To determine the effect of salt concentration on the assay performance, PBS at 10, 20, 50 mM with constant pH of 7.4 was tested. The effect of pH was evaluated using 10 mM PBS solutions with different pH values, ranging from pH 3.0 to 11.0. 30 mg/L humic acid was selected as the organic interference substances in water samples and its impact on the immunoassay was investigated. We also compared the influences of real water matrix on the calibration curves of BPA determination, as well as the EDTA addition in water samples on the optimization of immunoassay. The main criterion used to evaluate immunoassay performance was IC_{50} . In the last stage, a serial concentrations of BPA were spiked in real water samples (bottled water, tap water and 0.22- μm filtered-lake water), and the recoveries were analyzed.

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Author contributions

X.-H.Z., L.-H.L. and W.-Q.X. designed and performed all the experiments, and wrote the manuscript. B.-D.S. drew and summarized Figure 1 and Figure 2. J.-W.S. prepared the antibody used in the experiment. M.H. discussed the results and commented on the manuscript. X.-H.Z. and H.-C.S. designed and managed the project. All the authors reviewed the manuscript.

Additional information

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