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A Portable Optic Fiber Aptasensor for Sensitive, Specific and Rapid Detection of Bisphenol-A in Water Samples

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A portable optical fiber aptamer-based biosensor for fast, cost-effective, sensitive, and selective for BPA detection in water samples is described. The detection limit of BPA is better or comparable to current analytical methods and has the potential for direct and on-site analysis without any pre-concentration and treatment steps.

A Portable Optic Fiber Aptasensor for Sensitive, Specific and Rapid Detection of Bisphenol-A in Water Samples

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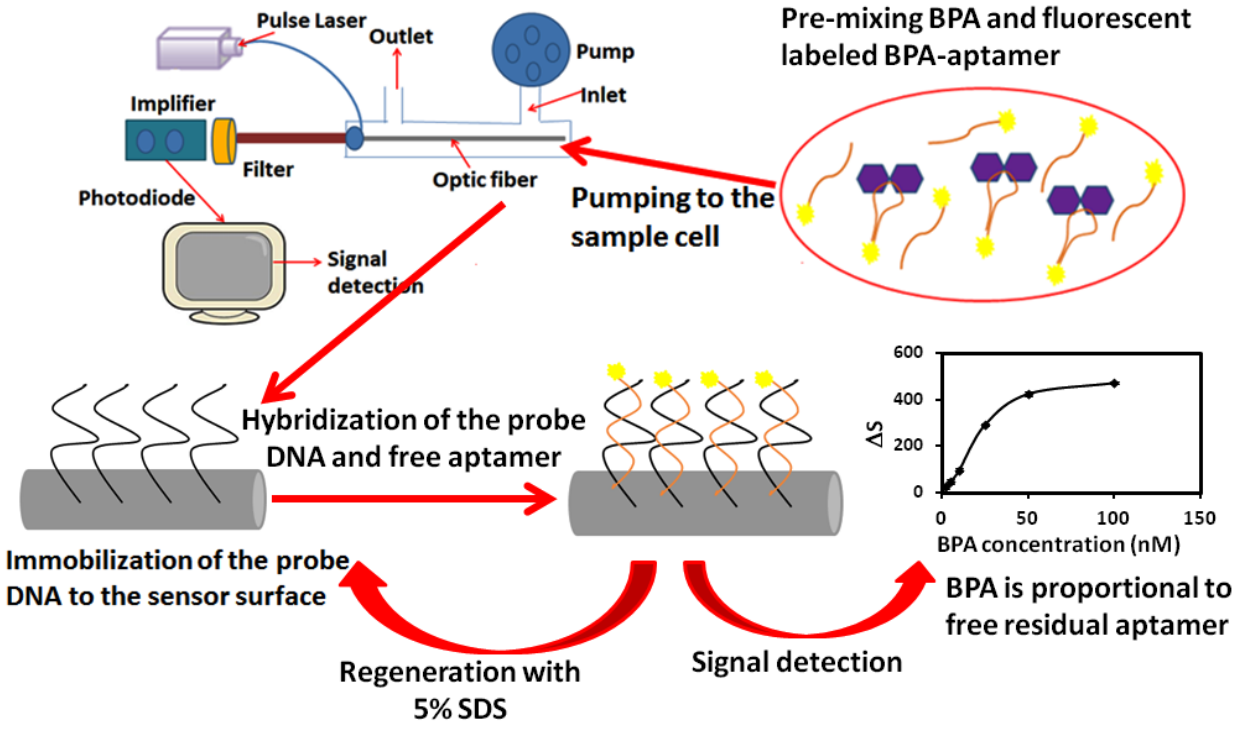
Abstract

Bisphenol A (BPA) is a known endocrine disruptor and one of the most serious environmental contaminant, often presents at low levels in various water sources. Therefore, it is very important and necessary to develop a fast, cost-effective, sensitive, and selective method for on-site detection of BPA. Herein, we developed a portable evanescent wave fiber-optic aptasensor for rapid, on-site detection of BPA with high sensitivity and selectivity. In this system, the probe DNA molecule, which is the complementary sequence of a small part of the BPA-aptamer, was covalently immobilized onto the optical fiber sensor surface. With an indirect competitive detection mode, samples containing different concentrations of Bisphenol A were premixed with a given concentration of fluorescence-labeled BPA-aptamer, which highly specifically binds to Bisphenol A. Then, the sample mixture is pumped to the sensor surface, and a higher concentration of BPA leads to less fluorescence-labeled BPA-aptamer hybridized with surface immobilized probe-DNA and thus to lower fluorescence signal. The developed sensing system exhibits a sensitive response to BPA in the range of 2 nM to 100 nM with a low detection

limit of 1.86 nM (0.45 ng/ml) under the optimal conditions. The biosensors were characterized to show good reproducibility, stability, and good selectivity for BPA detection. Finally, this proposed sensor was successfully employed to determine BPA in waste water samples.

Keywords; Bisphenol A, aptamer, biosensor, optical sensor, environmental analysis.



1. Introduction

Bisphenol A (BPA) has been used in chemical industry for production of polycarbonate, epoxy resin, polysulfone resin, polyphenylene oxide resin, and unsaturated polyester resin. These are extensively employed for nursing bottle, food can linings, beverage container, from which BPA can lead to human exposure[1]. BPA itself has been produced in the amount of 6.4 billion pounds per year[2] and its levels in the low µg/L range were detected in clinical, food and water samples [3, 4]. BPA is one of the known endocrine disrupting compounds (EDCs) that binds to the estrogen receptors and induces activation of the estrogen receptor [5, 6, 7] and its effects on human

and other organisms have become of growing concerns. Exposure of BPA to human fetuses has been reported [8]. In addition, BPA is postulated to cause reproductive disorders including decline in sperm counts, birth defects due to fetal exposure, various kinds of cancers, such as prostate, testicular, and breast cancer, and has diverse pleiotropic actions in the brain and cardiovascular system [9]. Thus, simple, selective, and sensitive analytical methods for the detection of a trace amount of BPA in the environment are in urgent need.

Until now, the most widely used methods for the detection of BPA include high performance liquid chromatography (HPLC)[10], liquid chromatography coupled with electrochemical detection (LC-ED)[11], liquid chromatography coupled with mass spectrometry (LC-MS)[12], gas chromatography (GC)[13], and gas chromatography coupled with mass spectrometry (GC–MS)[14]. Although these methods can offer good selectivity and detection limit, they often require advanced and expensive instrumentations, complex pre-treatment steps and skilled personnel, which prohibit their application for real-time or on-site analysis of large number of environmental samples. In recent years, various enzyme linked immunosorbent assays (ELISA) for the determination of BPA has been reported[15, 16]. However, the use of immunosensors has certain limitations as well, related to the low stability of the biological material, complicated multistage step, and large and expensive equipment needed. Particularly, the specific antibodies and proteins required are obtained from killing animals or by recombinant techniques [17]. The complexity of matrix encountered in environmental samples renders detection of trace BPA and it analogues a formidable challenge. Except for ELISA-based methods, molecular imprinting based detection approaches also developed in recent years. For example, Yin et al reported the selective screening of trace bisphenols in river water by using molecularly imprinted polymer[18].

Another type recognition agent, nucleic acid based aptamer, has been shown to provide excellent alternatives to antibodies as immune specific agents[19]. Aptamers are single-stranded (ss) oligonucleotides that can bind to their target molecules with high affinity and selectivity by folding into distinct secondary and tertiary structures. They are identified from an initial library containing 10^{13}–10^{16} random ssDNA or ssRNA sequences through an *in vitro* selection process termed SELEX (systematic evolution of ligands by exponential enrichment). Aptamers can be isolated against most targets (referred to as 'aptamerogenic' targets) without involvement of animals, even those that are toxic or have a low immunogenicity[20, 21]. A specific ssDNA aptamer that binds specifically to Bisphenol A (BPA), but not to Bisphenol B (BPB) or other structurally similar molecules, was reported by Jo et al. recently [22].

In the present study, we developed an optical fiber platform-based portable biosensor for the detection of BPA via using a fluorescence-labeled aptamer specifically binds BPA. Easy-to-use evanescent wave fiber-optic biosensor platform was used for this rapid, highly specific and sensitive detection of BPA. The biosensor's sensing time, sensitivity, specificity, resistance to background interference and reusability were evaluated. The developed portable BPA sensing system exhibits a sensitive response concentration range and detection limit comparable to BPA levels in environmental water samples and therefore potentially applicable for direct and on-site analysis without any pre-concentration and treatment steps.

2. Materials and Methods

2.1. Reagents

Bisphenol A (BPA), 3-aminopropyl triethoxysilane (APTS), and glutaraldehyde (GA) were purchased from Sigma-Aldrich (St. Louis, MO). Fluorescent dye labeled single-stranded DNA aptamer against BPA, which was isolated by SELEX process from a random ssDNA

library[21] were purchased from Eurofins MWG Operon (AL, USA). The sequences for the aptamer, probe-DNA and control-DNA are: 5'-Cy5.5-CCGGTGGGTGGTCAGGTGGGATAGCGTTCCGCGTATGGCCCAGCGCATCACGGGTTCGCACCA-3' (aptamer), 5'- NH_2-$(CH2)_6$-TGGTGCGAACCCGTGATGCGCT-3' (Probe-DNA), and 5'-Cy5.5-TCCCGAGA-3' (non-specific DNA sequence used for control).

Both aptamer and non-specific DNA oligonucleotides were dissolved in 100 mM PBS and kept frozen at -20ºC for storage. Buffer solution of 100 mM PBS was used for dissolving all DNA sequences, BPA (stock in methanol) and water sample effluents, which contained 200 mM NaCl, 25 mM KCl, 10 mM $MgCl_2$ and 5% ethanol and had a pH of 8.0. For sensor specificity tests a number of chemicals containing phenolic groups 2, 4-dichlorophenol, bromophenol blue, phenol and phenol red (Thermo Fisher Scientific Inc. PA, USA), and other environmental pollutants such as estriol and 17β-esradiol (Sigma-Aldrich, St. Louis, MO, USA) were tested.

2.2. Instrumentation: evanescent wave all-fiber biosensing platform

The portable evanescent wave all-fiber biosensing platform was as previously described[23, 24]. Briefly, the laser beam from a 635-nm pulse diode laser with pigtail was directly launched into a single-mode fiber of a single multi-mode fiber coupler. The laser light then entered the multi-mode fiber with a diameter of 600 μm and numerical aperture of 0.22 from the single-mode fiber. The excitation light from the laser, through the fiber connector, was coupled to a fiber probe. The incident light propagated along the length of the probe via total internal reflection. The evanescent wave generated at the surface of the probe then interacted with the surface-bound fluorescently labeled analyte complexes and caused excitation of the fluorophores. The collected fluorescence was filtered by means of a bandpass filter and detected by photodiodes through a lock-in detection. The probe was embedded in a glass flow cell with a

flow channel having a nominal dimension 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 controls of fluid delivery system, data acquisition and processing were automatically performed by the built-in computer.

2.3. Immobilization of probe-DNA (complementary to BPA aptamer sequence) onto fiber optic sensor surface

Details of the fabrication and preparation of the combination tapered fiber optical sensor were described previously[24]. Figure 1 depicts the steps for immobilizing a probe-DNA that complement to a partial sequence of the BPA-recognizing aptamer, onto the optical sensor fiber surface. The sensor fiber was pre-cleaned with a piranha solution (H_2SO_4/H_2O_2, 3:1 (v/v)), then aminated by immersion in a 2% (v/v) APTS acetone solution for 60 min, followed by an acetone wash (three times), ultrapure water wash, and drying in an oven for 30 min at 110 ◦C. For immobilization of the probe-DNA, the aminated sensor was first immersed in a 5.0% (v/v) GA solution for 1 h at 37 ◦C for adding aldehyde functional group, washed with water, and then immersed in 0.5 µg/ml aminated probe-DNA sequence (5'- NH_2-$(CH2)_6$-TGGTGCGAACCCGTGATGCGCT-3') in PBS (pH 7.4) solution overnight at 4 ˚C. The sensor surface was then dipped in a 2mg/mL BSA solution for 1 h to block the remaining aldehyde sites.

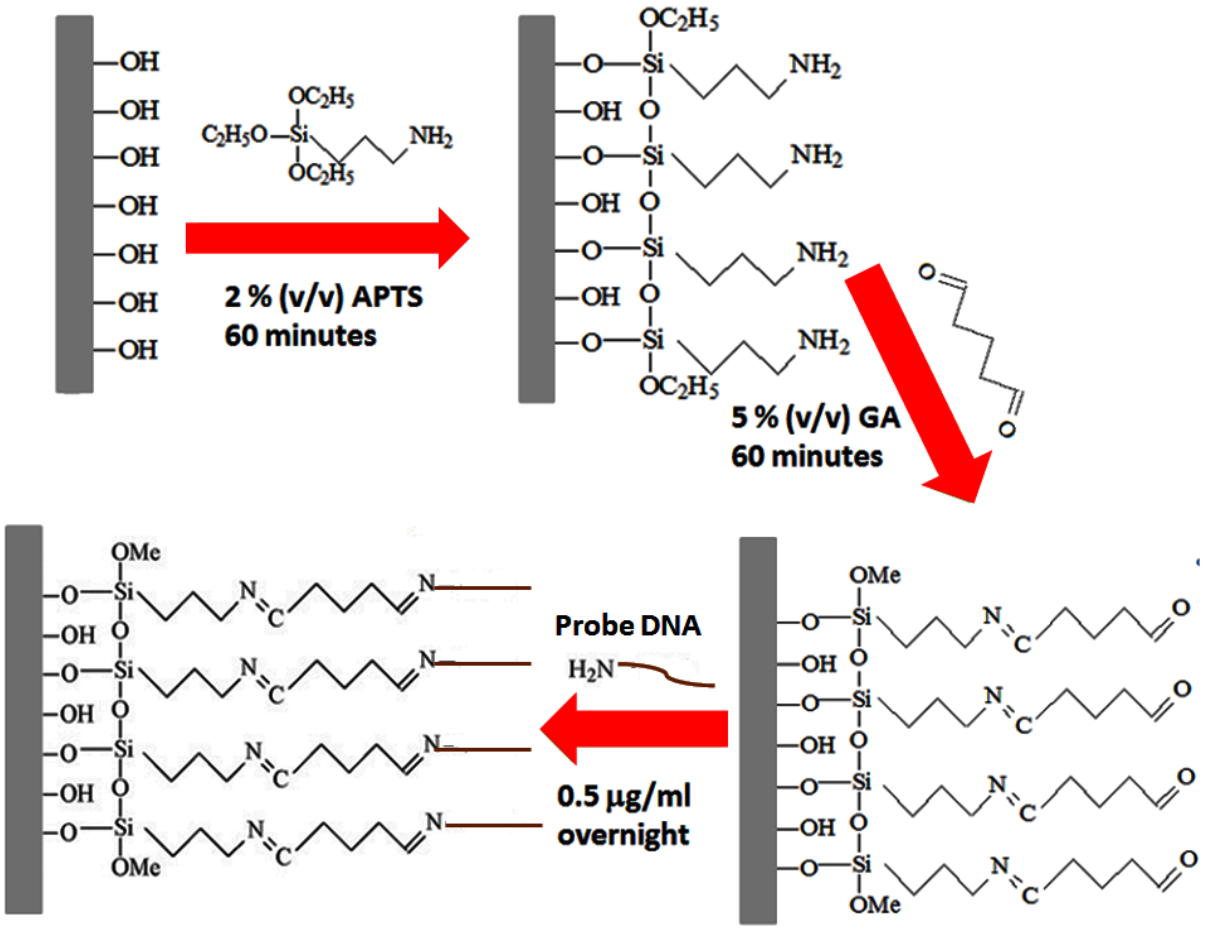


Fig. 1- Schematics of the process employed for immobilizing the probe-DNA that can complementarily bind to a partial sequence of BPA- aptamer, onto the sensor fiber surface, using GA covalent coupling approach.

2.4. Sensing mechanism

The proposed sensing mechanism of the evanescent wave aptamer-based biosensor for detection of BPA (Bisphenol A) and the exemplary signal profile for BPA detection is represented in Figure 2 and Figure 3, respectively. The sensing procedure started with pre-injection of BSA for blocking the rest of the open places from first BSA blocking step in order to avoid the non-specific binding of fluorescent labeled BPA-aptamer to the sensor surface. We

employed an indirect detection mode that includes a pre-mixing step to incubate samples containing various concentrations of BPA with a fixed amount of fluorescence-labeled BPA-aptamer (see details in sensor optimization section). Upon the completion of binding between BPA and its specific aptamer, the remaining free aptamers concentration is inversely proportional to that of BPA in the water sample. The sample mixture is then pumped through the optical fiber sensor surface for 30 seconds at a rate of 300 μL/min, and the remaining free aptamers are allowed to bind to the immobilized probe-DNA that is complementary to a certain section of the BPA-aptamer (reaction time of 6 minutes, see details in sensor optimization). The fluorescence signal was recorded real-time during the sensing process. To reuse the sensor, the sensing surface was regenerated with a 0.5% SDS solution for 90 seconds and washed with a PBS solution (pH=7.2).

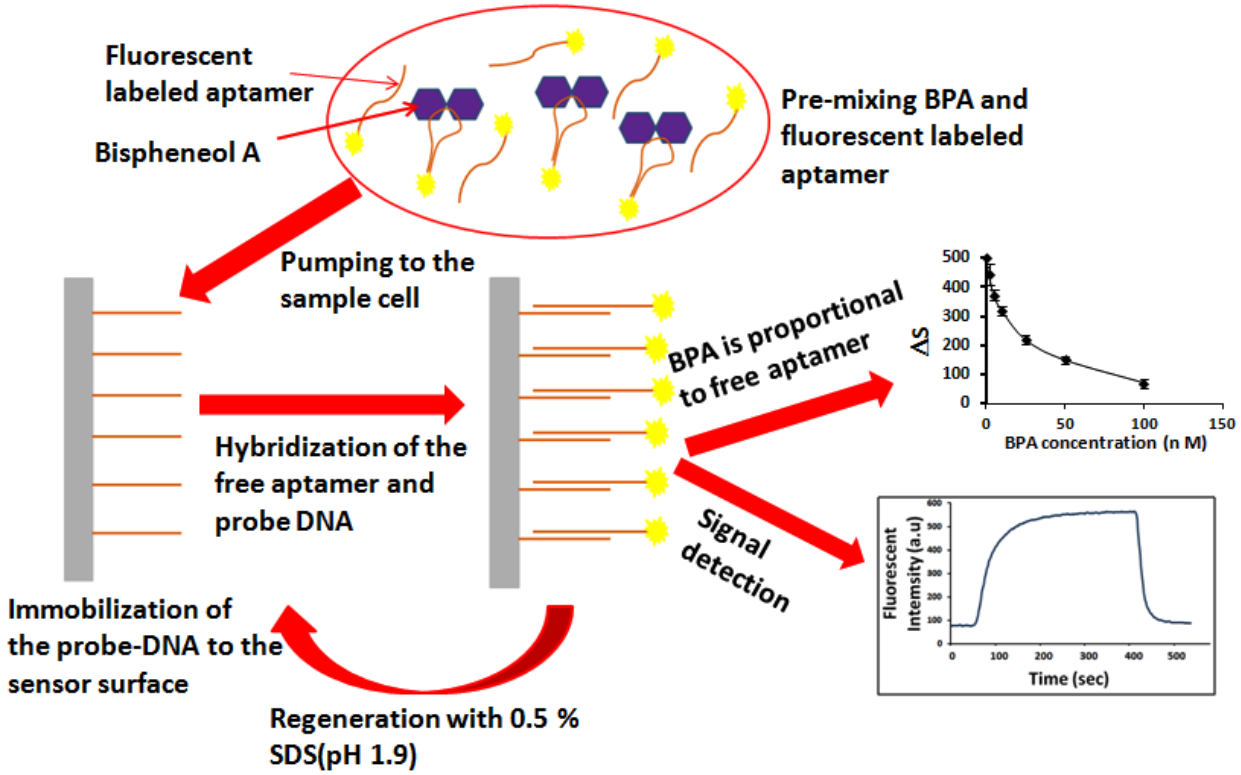


Fig. 2 Schematic representation of sensing mechanism for BPA (Bisphenol A) detection using a optical fiber sensing platform and employing DNA aptamer as the BPA recognition agent. The sensing mechanism and procedure involves pre-mixing and incubation of water sample with known concentration of aptamer, hybridization of residual free BPA-aptamers with probe-DNA immobilized on optical fiber sensing surface, real-time signal detection and regeneration steps.

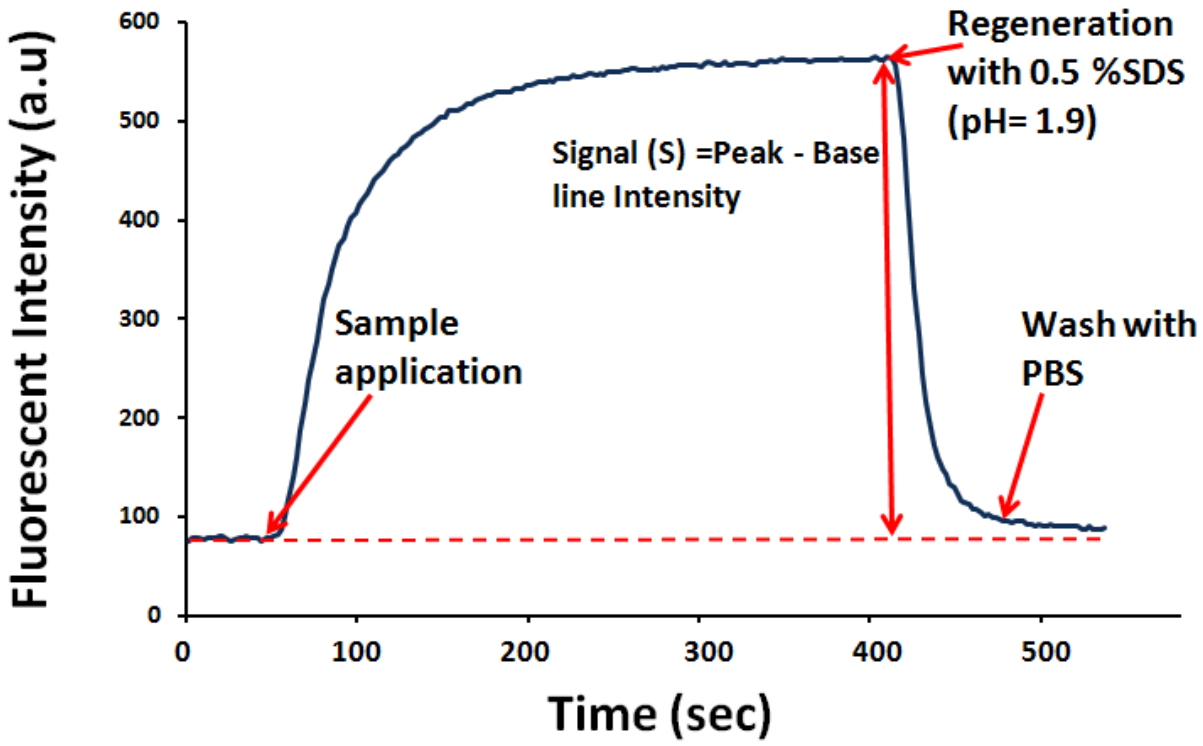


Fig. 3 Exemplary fluorescence intensity profile for one complete Bisphenol A detection cycle with the aptamer-based optical biosensor including baseline checking, initiation of sample application, recording fluorescence signal reflecting the hybridization of residual fluorescence-labeled aptamer with probe-DNA immobilized on sensor surface, and sensor regeneration using SDS followed by PBS. Signal (S) is the difference between fluorescent intensity of signal peak and signal base-line.

2.5. Optimization of the sensing conditions

Sensing condition optimization studies were performed for various sensing steps. First, the incubation time length and optimal aptamer concentration for the pre-mixing step with the BPA-containing sample was optimized. A varying incubation time of 1, 3, 6 and 10 minutes was conducted and compared. Tests with a series of different aptamer concentrations (10 nM to 200 nM) in the pre-mixing step were performed to determine the optimal aptamer concentration.

Second, for evaluating the effectiveness of non-specific binding sites blocking with BSA, sensing with or without BSA blocking step were performed and compared. Before detecting samples, BSA (1mg/ml) was pumped through the sensor cell for 30 seconds and the sensor surface was washed via PBS buffer solution (30 seconds) to remove the residual of BSA. Fluorescently labeled non-specific DNA (represents other DNA rather than the specific aptamer) was also tested in comparison with BPA-aptamer to confirm the specific binding of BPA-aptamer with probe-DNA on sensor.

2.6. Assessment of sensor specificity

To determine the specificity of the aptamer biosensor for detecting Bisphenol A, a number of chemicals containing phenolic group as well as several other environmental pollutants are evaluated and they include 2, 4-dichlorophenol, Bromophenol blue, Phenol, Phenol red, Estriol and 17β-Estradiol.

2.7. Analysis of spiked wastewater treatment effluent samples

To evaluate the potential matrix effect of real environmental water sample on the sensor performance, we analyzed spiked samples that contained different concentrations of Bisphenol A (10 nM, 25 nM and 100 nM) in tap water and two different wastewater effluents from plants in US. The wastewater effluent samples were filtered through 0.22 μm filters to remove all

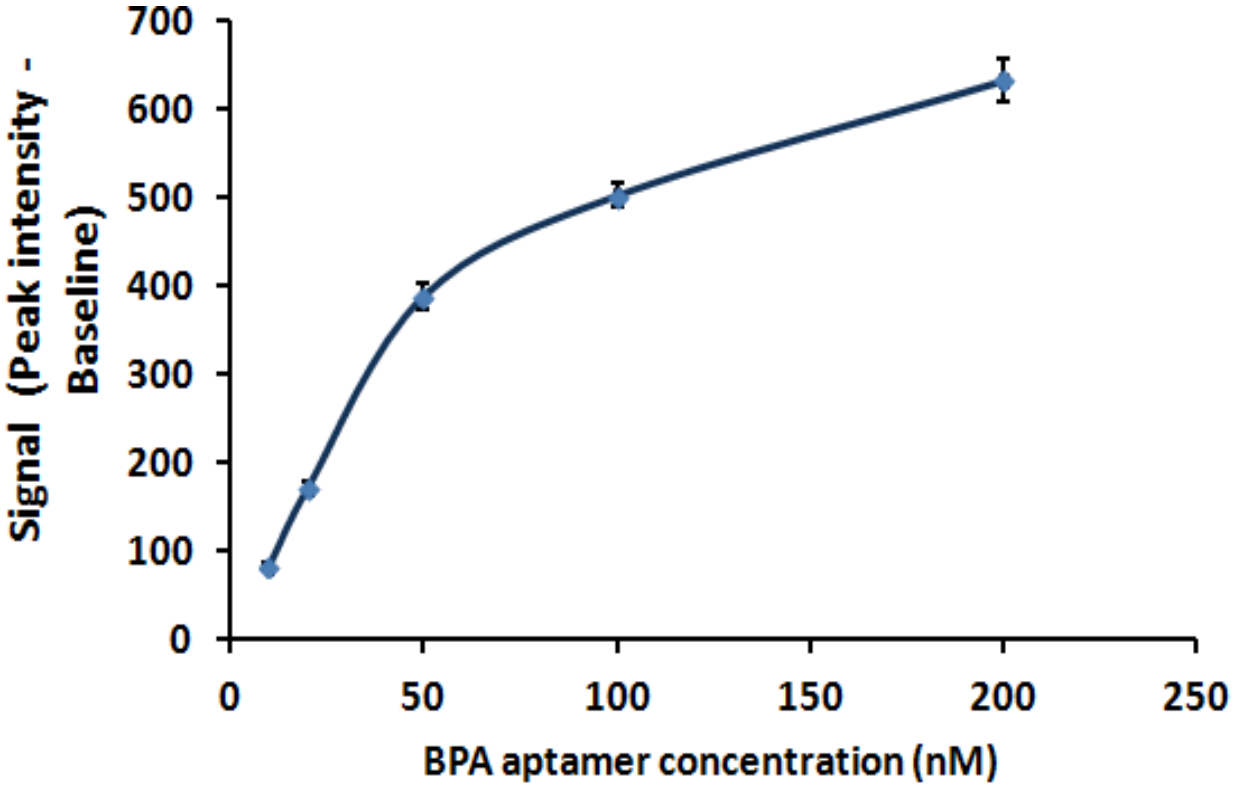
188 particulates before they were spiked with Bisphenol A. Two duplicate experiments were
189 performed for all samples. Similar analytical procedures were followed as described above.

190 **3. Results and Discussion**

191 *3.1. Optimization of the sensing conditions*

192 *3.1.1. Optimization of the fluorescent labeled Bisphenol A aptamer concentration in pre-mixing*
193 *step*

194 To optimize the aptamer concentration used in the pre-mixing step, a varying
195 concentration of fluorescent-labeled BPA-aptamer at 10, 20, 50, 100 and 200 nM were pumped
196 to the sample cell and the fluorescent intensity was observed. As shown in Figure 4, the signal at
197 100 nM aptamer was near the signal saturation level for the evanescent wave aptamer-based
198 biosensor. A compromise between fluorescent intensity (higher aptamer concentration) and cost
199 of aptamers led to the selection of 100 nM fluorescent labeled DNA being applied in all the
200 following experiments.



201

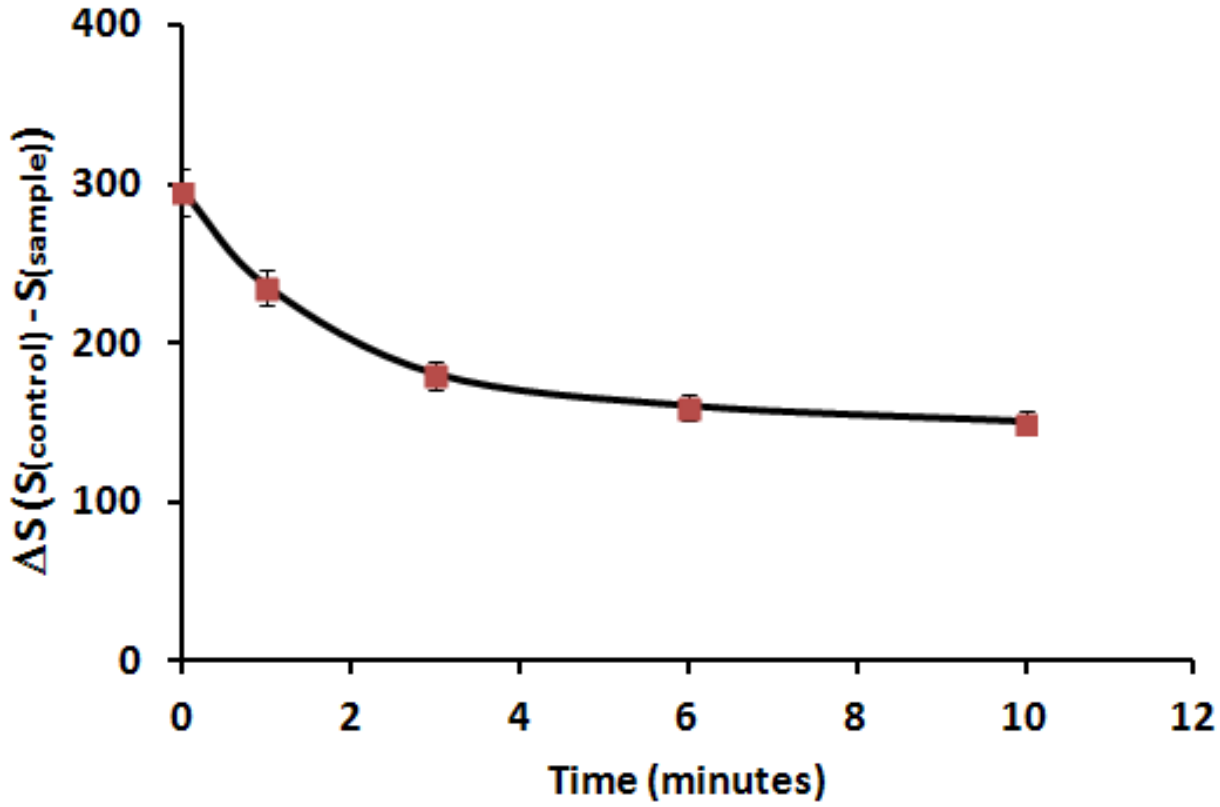
202 **Fig. 4** Optimization of the concentration of fluorescent labeled BPA-aptamer used for pre-
203 mixing with BPA-containing sample. Signal Intensity is the difference between fluorescent
204 intensity of signal peak and signal base-line. Data value is the average of two independent
205 experimental results.

206

207 *3.1.2. Incubation time optimization in the pre-mixing step*

208 Several incubation time lengths (1, 3, 6 and 10 minutes) for the pre-mixing of Bisphenol
209 A (10 nM) and fluorescence labeled aptamer (100 nM) were evaluated (Figure 5 and Figure S1).
210 Prolonged incubation time of the Bisphenol A with aptamer led to decrease in the sensor signal
211 but approaching a plateau level after 6 minutes. Therefore, we chose to use 6 minutes incubation
212 time for all the subsequent analysis.

213



214

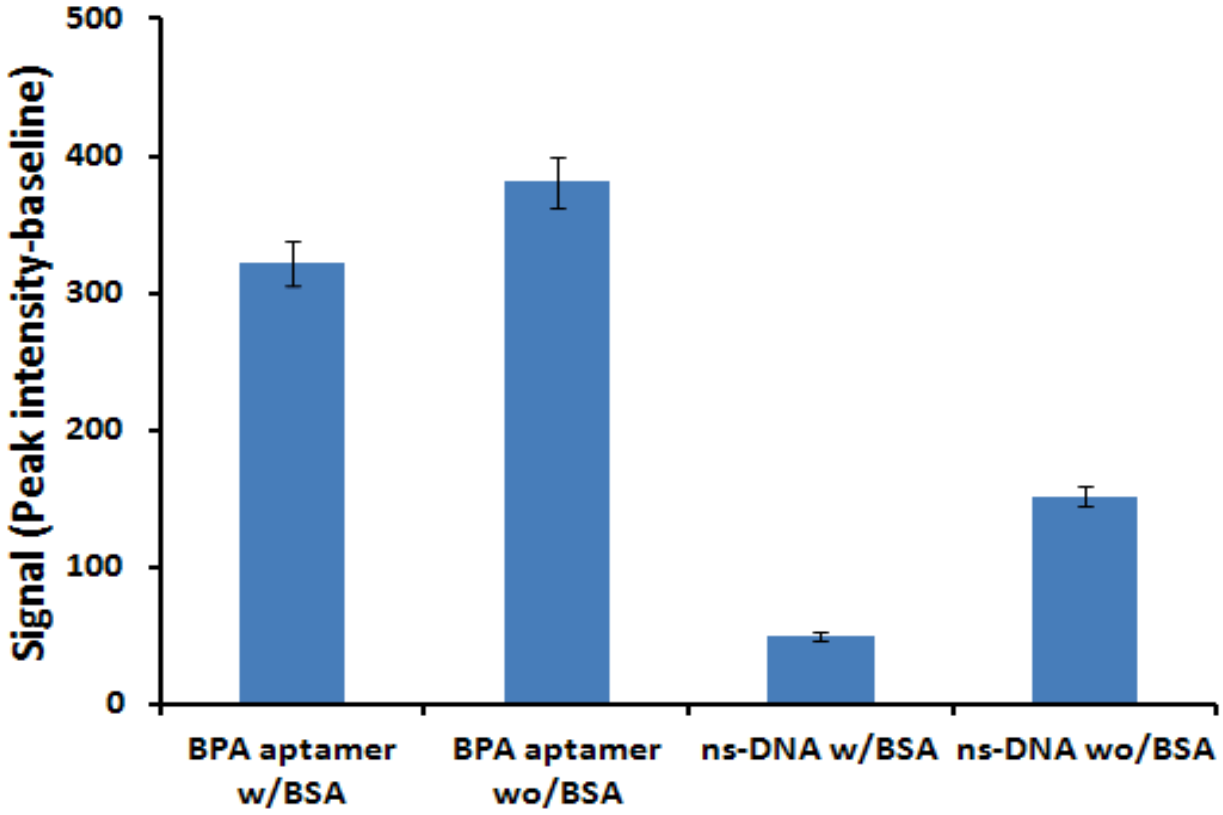
215 **Fig.5** Experimental optimization of the pre-mixing time length for pre-mixing of Bisphenol A
216 (10 nM) and its specific aptamer (100 nM). Signal intensity is the difference between
217 fluorescent intensity of signal peak and signal base-line. Data value is the average of two
218 independent experimental results.

219

220 *3.1.3. Blocking non-specific binding using BSA*

221 This experiment was performed to confirm that the observed fluorescence signal was
222 from hybridization between Bisphenol A-recognizing aptamer and its complementary DNA
223 sequence (probe-DNA) immobilized on the sensor surface, rather than the signal from non-
224 specific binding of aptamer onto the sensor surface. To confirm the specific binding of BPA-
225 aptamer with probe-DNA on sensor, fluorescently labeled non-specific DNA (represents other
226 DNA rather than the specific aptamer) was tested in comparison with BPA- aptamer. For

evaluating the effectiveness of non-specific binding sites blocking with BSA, sensing with or without BSA blocking step were performance and compared. Several control experiments were performed and the results are shown in Figure 6 and Figure S2. With BSA pre-blocking step, fluorescently labeled BPA-aptamer induced much higher (6 times) Signal (S) than that of non-specific DNA (nsDNA at 100 nM) with a signal-to-noise ratio (the ratio of the maximum fluorescent intensity to the base line) of over 5.25 obtained (Figure S2). Without the BSA–pre-injection for non-specific sites blocking, the ratio of the Signal (S) of BPA aptamer to that of non-specific DNA control was greatly diminished (by 3 times). These results confirmed the specific biding of BPA-aptamer with probe DNA with the aid of non-specific binding blocking by BSA. Another important conclusion, beside that BSA was effective for blocking non-specific binding, was that BSA treatment didn't interfere with the interaction between the aptamer and the immobilized probe-DNA on the surface.



240 **Fig. 6** Comparison of sensor responses for fluorescent labeled BPA-aptamer versus non-specific
241 DNA control, with and without BSA pre-injection for non-specific binding sites blocking.
242 Fluorescent labeled BPA-aptamer and nsDNA were applied at 100 nM. (w/BSA: experiment
243 with BSA injection; w/o BSA: experiment without BSA injection; BPA aptamer, experiment
244 with BPA-recognizig DNA apramer; nsDNA; experiment with nonspecific DNA).

245

246 *3.2. Dose-response measurements of the sensor*

247 Figure 7 shows the exemplary fluorescence intensities during a typical test cycle for
248 different amount of Bisphenol A using the optical sensor developed herein, including the BSA
249 treatment for blocking non-specific sites. The increase in the Bisphenol A concentrations in the
250 sample and known aptamer mixture led to proportional decrease in residual free aptamer,
251 therefore the fluorescence signal.

252 Figure 8 shows the calibration curve for Bisphenol A, which ΔS values were calculated
253 by subtracting the sample Signal (S) of each standard point from the blank sample containing no
254 Bisphenol A. The signal differences (ΔS) were fitted to a 4-parameter logistic equation as
255 follows:

$$y = \frac{A_1 - A_2}{1 + ([Ac]/[Ac_0])^p} + A_2 \quad (1)$$

256

257 where, [*Ac*] is the analyte concentration; A_1, A_2 are the upper and lower asymptote
258 (background signal) to the dose response curve; [Ac_0] is the analyte concentration at inflection;
259 and *p* is the slope at the inflection point (Long et al. 2010).The error bars in the figure correspond
260 to the standard deviations of the data points in triplicate experiments, with coefficient of variance
261 (CV) of all the data points being within 5-9%.

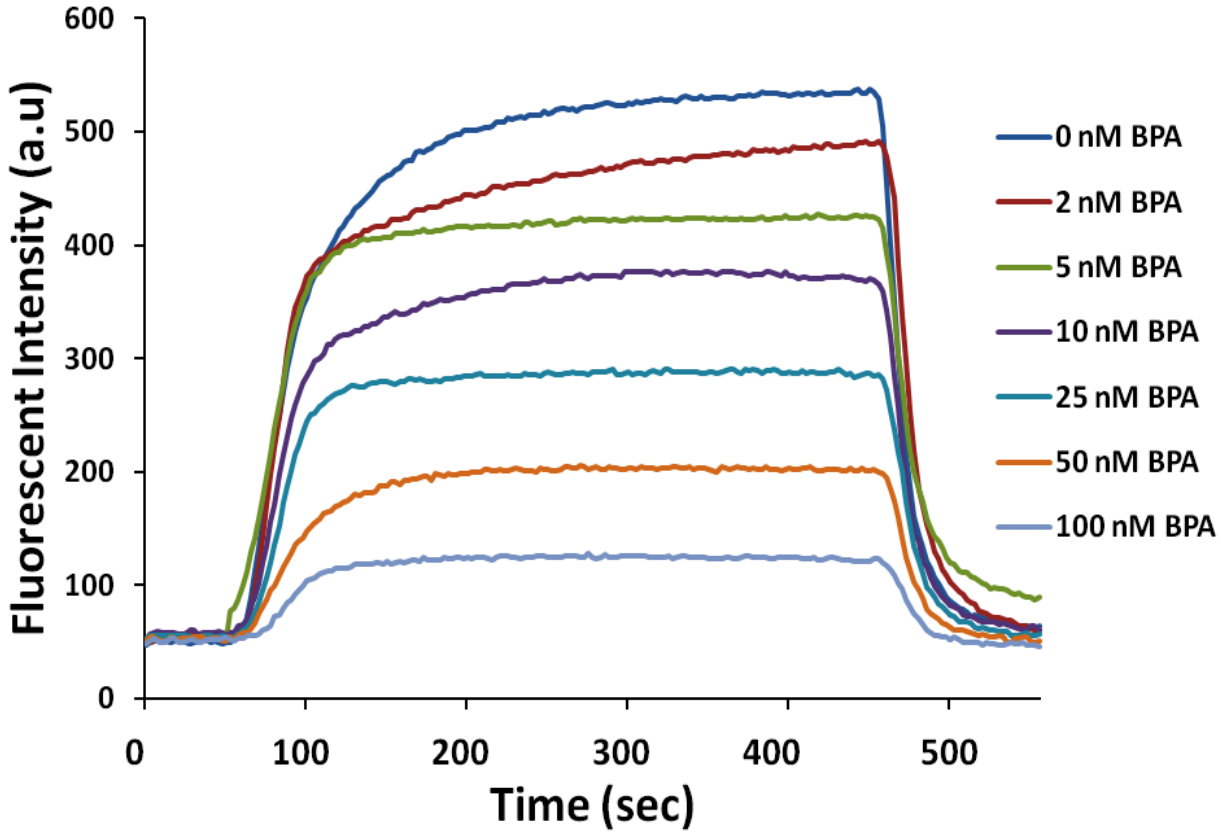


Fig. 7 The fluorescence intensity responses during a typical test cycle for different amount of Bisphenol A using the optical sensor system, including the BSA treatment for blocking non-specific sites.

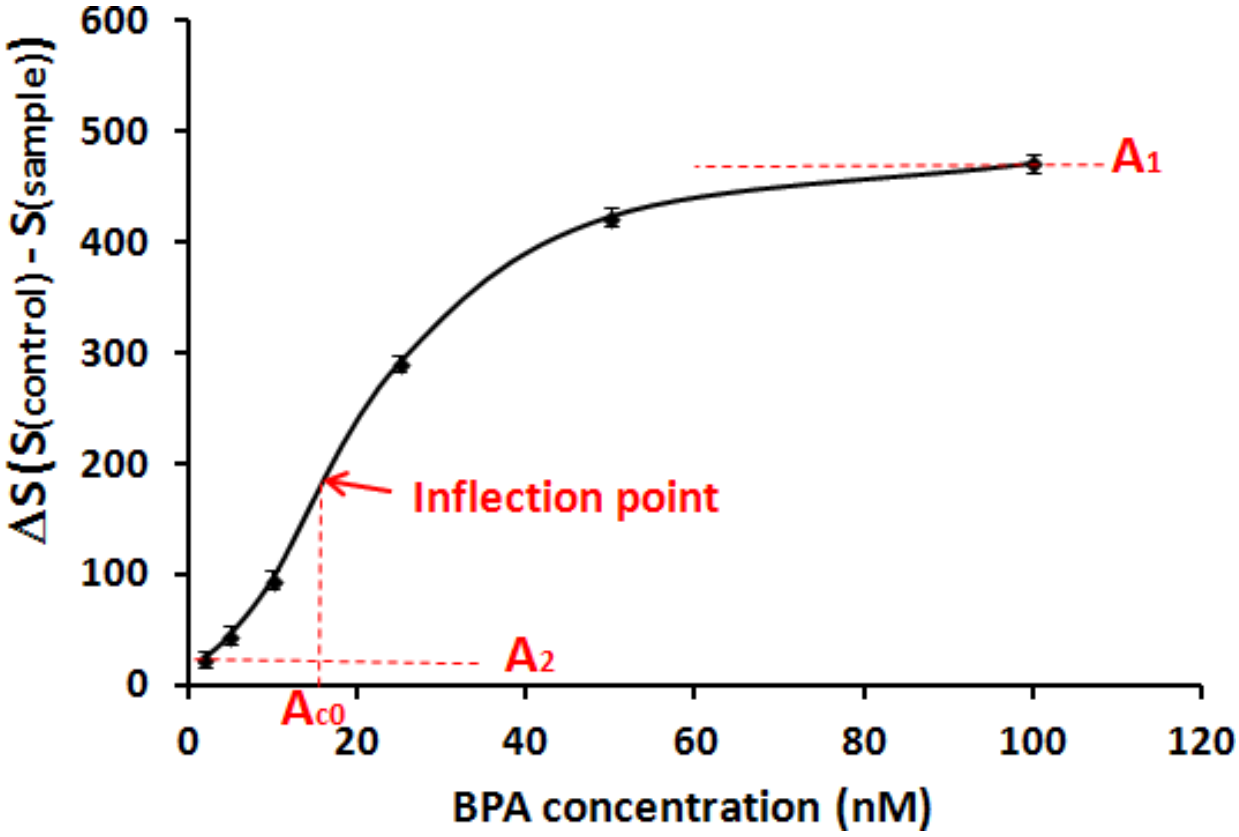


Fig. 8 The calibration plot for determination of Bisphenol A concentration using the aptamer-based fiber optic biosensor system. ΔS is the Signal (S) difference between control (blank) and sample application. Each data value is the average of three independent experimental results.

According to the figure 7, the signal to noise ratio (the ratio of the maximum fluorescent signal to the baseline) was varying from 9.8 to 2.4, which should be as high as possible, and preferably >3 in order to generate a reasonable signals[25]. Therefore, up to 50 nM BPA concentration (S/N of 3.9) we have reliable results. Additionally, the S/N of 100 nM (highest detection point of the dose response curve), which was 2.4, can be improved with using higher concentration of fluorescent-labeled BPA aptamer.

The detection limit was determined as approximately 1.86 nM (0.45 ng mL^{-1}) based on average standard deviation of measurements (σ) and slope of dose-response (S) fitting curve as

$3\sigma/S$[26]. The detection limit we obtained initially is comparable to those reported in the impedimetric detection of Bisphenol A using BPA specific polyclonal antibody which has the detection limit 0.3 ± 0.07 ng/ml (1.24 nM) [27] and the electrochemical Bisphenol A detection based on N-doped grapheme sheets which has the detection limit 5.0 nM (1.2 ng/ml)[28]. The detection limit is also comparable to chemical analysis with standard liquid chromatography detection results of BPA which are also given in Table S1 for reference and comparison. Considering the range of Bisphenol A concentrations (low μg/l level) detected in natural waters[3, 4], our novel sensor can be applied for detection of Bisphenol A in natural or wastewater samples. In addition, compared to the sensors mentioned above, the sensor developed here is simpler and faster (less than 10 min, including measurement and regeneration). In addition, the portable platform also allows for potential on-site or real time measurements.

3.3. Selectivity of the sensor

Selectivity of the sensor was assessed by analysis of other environmental pollutants such as, Estriol and 17β-Estradiol and other chemicals including phenol group such as 2, 4-dichlorophenol, Bromophenol blue, Phenol and Phenol red, all at 100 nM. The results (Figure 9) showed the signals for each chemical subtracted from control (blank experiment with PBS). According to the results the developed biosensor system has high specificity toward Bisphenol A over other EDCs and such other phenolic compounds.

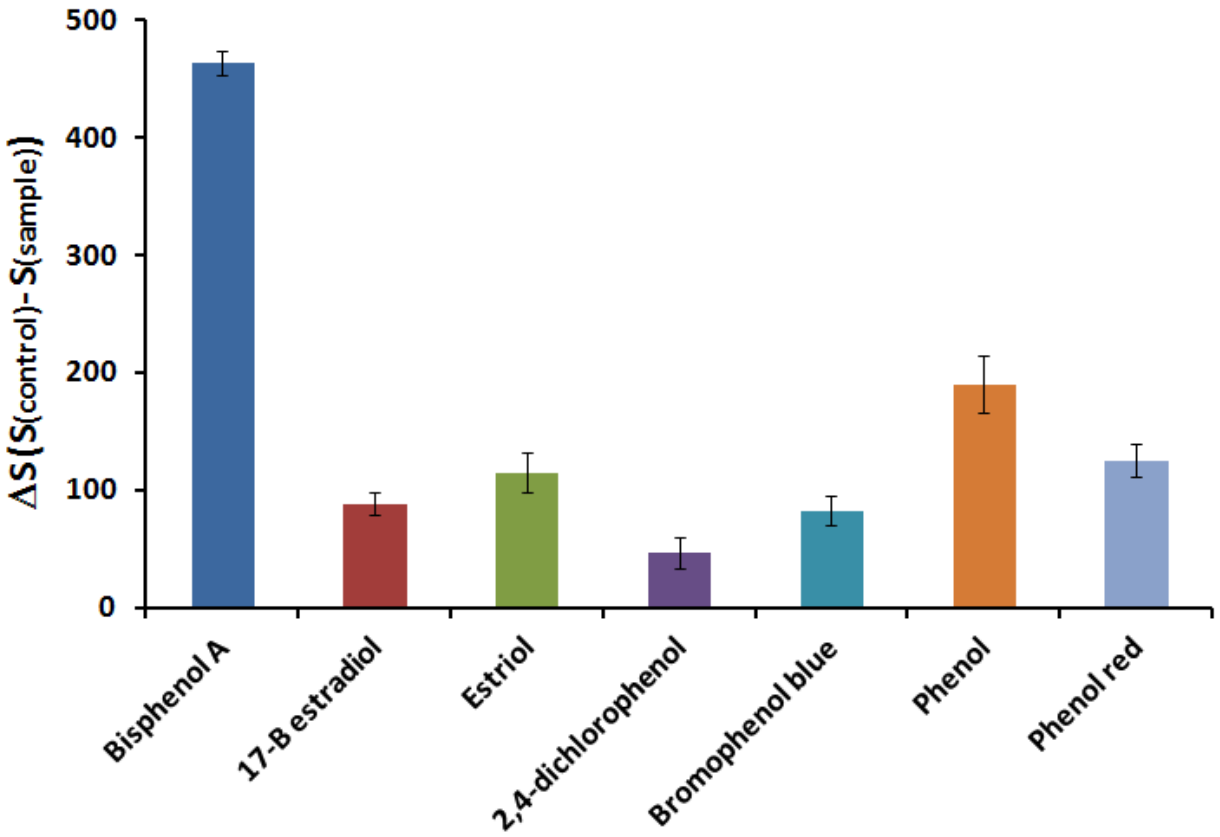


Fig. 9 Sensor specificity assessment via comparison of sensor signals of Bisphenol A, with other chemicals including phenol groups and as well as such other EDCs. ΔS is the Signal (S) difference between control (blank) and sample application. All chemicals are at 100 nM level, and each data value is the average of two independent experimental results.

3.4. Regeneration and Sensor Stability

The regeneration performance of the sensing systems is important for practical implementation of biosensors[29].Therefore, in the present system, the stability and reusability of the DNA probe covalently immobilized to the sensing surface was evaluated over a large number (>100 assay over 30 days during this study) of assays.

The stability of the proposed sensor system was evaluated by performing three daily measurements over 30 days of continuous analysis and a decrease in the average maximum signal response in the absence of analyte was less than 10% for fluorescent labeled BPA-aptamer

(figure S3). This slight drop in fluorescence signal did not affect the DNA biosensor's specific response: all measurements were normalized with respect to the blank signal at the beginning of the daily analysis to correct for the system signal shifts in the blank and sample measurements.

3.5. Analysis of spiked wastewater treatment effluent samples

To evaluate the potential matrix effect of real environmental water sample on the sensor performance, we analyzed spiked samples that contained different concentrations of Bisphenol A (10 nM, 25 nM and 100 nM) in two different wastewater effluents from different wastewater treatment plants in US. The results were summarized in Table 1. The recovery of all measured samples was between 91 and 110 %, and the parallel tests showed that the relativity coefficient was within 1.5-5.4 %, (n = 2). These results indicated that the possible interference from the different background composition of water samples on aptamer based fiber optic biosensor analysis was negligible. The developed biosensor system can be successfully applied to Bisphenol A analysis in real environmental water samples.

Table 1 here

4. Conclusion

In conclusion, we have developed a portable and easy-to-use aptamer-based evanescent wave optical biosensor for rapid and selective detection of Bisphenol A in environmental water samples. The sensing process can be completed in less than 10 min, with a detection limit of 1.86 nM (0.45 ng mL^{-1}). The stability of the covalently immobilized probe DNA on the sensor surface and the effective surface regeneration procedures allow over hundred assay cycles without any significant loss of sensor's performance. The performance of the biosensor evaluated in spiked wastewater samples showed good recovery, precision and accuracy, indicating that it was not

susceptible to water matrix interferences even without the need of complicate sample pre-treatments. All these results illustrated that the biosensor developed here could be readily extended toward the on-site monitoring of the other trace small molecular pollutants in environmental matrices with the employment of different probes modified by other analyte conjugates and fluorescence- labelled aptamers.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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Table 1. Detection results of Bisphenol A-spiked wastewater samples

Sample source	Treatment process and effluent	Bisphenol A added to the samples (nM)	Bisphenol A detected by sensor (nM)	Coefficient of variation (CV) %	Recovery %
Plant A	Membrane Bioreactor effluent	10	10.55	4.2	105.5
		25	27.2	3.6	108.8
		100	100.9	1.5	100.9
Plant B	Tertiary multi-stage filtration effluent	10	9.17	4.1	91.7
		25	27.6	3.1	110.4
		100	93.9	5.4	93.9