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RESEARCH PAPER

Highly sensitive aptamer based on electrochemiluminescence biosensor for label-free detection of bisphenol A

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Abstract Bisphenol A (BPA), a typical endocrine disruptor, is widely used as a key monomer in the packaging industry. Residual monomer can transfer from the package material to the food and thereby pose a risk to the health of the consumer, so determination of BPA migration is highly important for food safety control. In this study, a simple but sensitive electrochemiluminescence (ECL) biosensor, which combines the characteristics of high selectivity of an aptamer and high sensitivity of ECL, has been developed to detect BPA from package materials. The aptamer was immobilized on a gold electrode surface through Au–S interaction. The aptamer was then hybridized with complementary DNA (CDNA) to form double-stranded DNA (dsDNA). $Ru(phen)_3^{2+}$ can intercalate into the grooves of dsDNA and acts as an ECL indicator; high ECL intensity can therefore be detected from the electrode surface. In the presence of BPA, which can competitively bind with the aptamer owing to their high affinity, $Ru(phen)_3^{2+}$ is released from the electrode surface and the ECL of the system is decreased. The decreasing ECL signal has a linear relationship with BPA in the range of 0.1–100 pM with a detection limit of 0.076 pM. The developed biosensor has been applied to detect migration of BPA from different categories of canned drink with satisfactory results.

Keywords Bisphenol A · Migration · Aptamer · Electrochemiluminescence · $Ru(phen)_3^{2+}$

Introduction

Bisphenol A (BPA), also called 4,4′-dihydroxydiphenylpropane, is widely used as the base chemical in the manufacture of polycarbonate plastics and the resin lining of food and beverage cans [1, 2]. BPA can leach into the food and water, increasing susceptibility to mammary and prostate cancer, and it may be associated with infertility in women [3, 4]. In vitro experiments show that concentrations of BPA as low as 1 pM can disrupt the endocrine functions of human endocrine cells [5]. Many countries have banned the use of BPA in baby bottles and limited its concentration in food to less than 3 mg/kg [6].

Currently, the most widely applied methods for BPA detection rely on GC–MS [7, 8] and LC–MS [9, 10]. These two methods are the gold standards for BPA detection, but the process of extraction and purification of samples is expensive and subject to strict requirements, which have hindered the wider application of these chromatographic approaches. Other strategies have been adopted for BPA detection, including fluorescence spectrometry [11], photometry [12], and ELISA [13]. Although the ELISA method has been widely applied for BPA with high specificity and without the need for complex sample treatment, its operation is easily affected by site conditions as it relies heavily on the enzymatic activity.

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Even though photometry is operationally simple, it is susceptible to complex substrates and requires high sample purity. Therefore new alternatives for BPA determination are in high demand.

Aptamers are single-stranded oligonucleotides that are capable of binding specifically to proteins or other small molecules. Upon binding to the ligand, the aptamers fold into spatially stable structures, such as a pseudoknot, G-quaternary spiral, hairpin, etc., that are suitable for ligand binding by complementary pairing, electrostatic interaction, van der Waals force, hydrogen bonding, and stacking. Aptamers are a potential replacement probe for antibodies, which are used for rapid and on-site testing of metal ions [14], organic molecules [15], peptides [16], proteins [17], and even whole cells [18] in the field of analytical science. Many highly selective aptamer-based biosensors for BPA detection have been developed. For example, Zhu et al. [19] developed a sensitive fluorescence sensor for BPA detection based on a graphene oxide (GO) fluorescently modified anti-BPA aptamer (FAM), in which the presence of BPA can switch the conformation of FAM-ssDNA to prevent the adsorption of GO, resulting in fluorescence recovery in the sensing system.

Electrochemical (EC) detectors detect targets by measuring changes in current across an electrode pair. EC analysis has the advantages of simplicity, fast response, high sensitivity, and so on. In order to improve the sensitivity of the EC sensor, a signal amplification strategy is usually adopted. For example, Liu et al. [20] demonstrated a sensitive aptasensor for BPA based on horseradish peroxidase (HRP)-catalyzed signal amplification and direct electrochemical detection of BPA. The presence of BPA resulted in aptamer/CDNA duplex dissociation, and the CDNA linked to avidin-HRP-AuNP was released from the senor interface, which resulted in "signal-on" of the BPA signal and "signal-off" of the avidin-HRP-AuNP-catalyzed signal. Electrochemiluminescence (ECL), which combines the characteristics of electrochemistry and chemiluminescence, has also been applied to the detection of BPA. Guo et al. [21] proposed a sensitive ECL sensor for the detection of BPA based on L-cysteine-functionalized multiwalled carbon nanotubes/gold nanocomposites modified glassy carbon electrode. The presence of BPA led to the quenching effect on peroxydisulfate solution. Both of the aforementioned methods were proposed as sensors for BPA, but the amount of modification is more difficult to control.

In this study, we integrate the high affinity and specificity of an aptamer and the high sensitivity of EC detection and chemiluminescent techniques. The proposed ECL biosensor has been applied to detect migration of BPA into drink from different categories of canned beverage with satisfactory results. The proposed simplified analytical procedures are an attractive strategy for detecting small molecules.

Experimental section

Instruments

The ECL intensity detection system consisted of a CHI660D electrochemical workstation (Chenhua Instruments, Shanghai, China) and a BPCL ultra weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China). A conventional three-electrode configuration was employed for all experiments, in which a gold electrode was employed as working electrode, a platinum auxiliary electrode, and a Ag/AgCl (3 mol/L KCl) reference electrode.

Reagents and materials

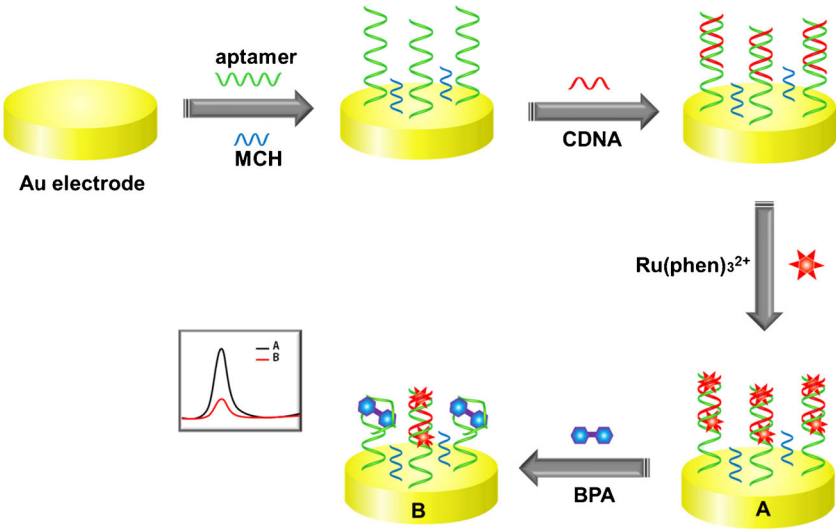
All nucleotide sequences were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China) and their sequences are shown below [21]:

Complementary DNA (CDNA): 5′-TGG TGC GAA CCC GTG ATG CGC TGG GCC ATA-3′.
BPA aptamer: 5′-SH-CCG GTG GGT GGT CAG GTG GGA TAG CGT TCC GCG TAT GGC CCA GCG CAT CAC GGG TTC GCA CCA-3′.

Dichlorotris(1,10-phenanthroline)ruthenium(II) hydrate ($Ru(phen)_3^{2+}Cl_2 \cdot H_2O$), 6-mercaptohexanol (MCH), and tripropylamine (TPA) were purchased from Sigma Aldrich Chemical Co. Ltd. (St. Louis, MO, USA). Bisphenol A (BPA) (Aladdin), ascorbic acid, phenol, 4-nitrophenol, and hydroquinone (HQ) (Sinopharm) were purchased and used without further purification. Other chemicals were of analytical reagent grade. The ultrapure water was produced with an ultrapure water purification system (Milli-Q, Millipore, resistance 18. 2 MΩ).

Electrode modification and ECL biosensor fabrication

The fabrication of the ECL biosensor is based on a literature protocol [22]. Briefly, the gold electrode was polished with 0.3 and 0.05 μm alumina powder for 5 min each to obtain a mirror surface. The gold electrode was then sonicated in ethanol and deionized water for 3 min each. The electrode then gave a stable redox peak by cyclic voltammetry in 0.5 M sulfuric acid solution. The electrode was placed in buffer containing thiolated aptamers at 37 °C in darkness for 2 h, resulting in Au–S interaction on the surface of the gold electrode, and then immersed in 1 mM MCH for 0.5 h to block unoccupied sites. The aptamer modified electrode was then immersed in a buffer solution containing 1 μM CDNA, 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM $MgCl_2$, and 10 mM KCl and kept for 3 h at 37 °C to make the aptamer hybridize with CDNA. Finally, the gold electrode was soaked



Scheme 1 Working principle of the aptamer-based ECL biosensor for BPA

in PBS buffer for 0.5 h to remove nonspecifically bound adsorbent.

The prepared electrode was immersed in 1 mM $Ru(phen)_3^{2+}$ solution at 37 °C for 8 h so that $Ru(phen)_3^{2+}$ molecules intercalate into the dsDNA grooves. After that, the electrode was also immersed in a buffer solution to remove nonspecifically bound adsorbent.

BPA determination

The prepared electrode was then immersed in a solution containing various concentrations of BPA at 37 °C for more than 7 h. After the electrode was extensively washed with buffer solution, the ECL signal of the regenerated biosensor was recorded while being scanned linearly from 0.4 to 1.4 V in the 0.2 M PBS buffer containing 5 mM TPA, with a scan rate of 100 mV/s.

Each measurement was repeated three times and the average values were calculated. The error bar shows the standard deviation of the three repeated measurements.

Sample preparation

The Yili milk, Binglu mineralized water, and Minute Maid samples were purchased from a local supermarket (Fujiang, China). The preparation procedures followed the methods of Deiminiat et al. [23]. In brief, the mineral water was mixed with the same volume of Tris-HCl buffer. Milk and Minute Maid samples were centrifuged for 15 min to remove suspended particles, and then the supernatant of the solution was diluted fourfold with Tris-HCl. After 15 min sonication and 10 min shaking, the resulting mixture was centrifuged for 10 min. Finally, the prepared samples were spiked with certain amounts of BPA for further detection.

Results and discussion

Principle of proposed ECL biosensor

The principle of the proposed ECL biosensor is shown in Scheme 1. A thiolated aptamer of BPA is modified on the gold

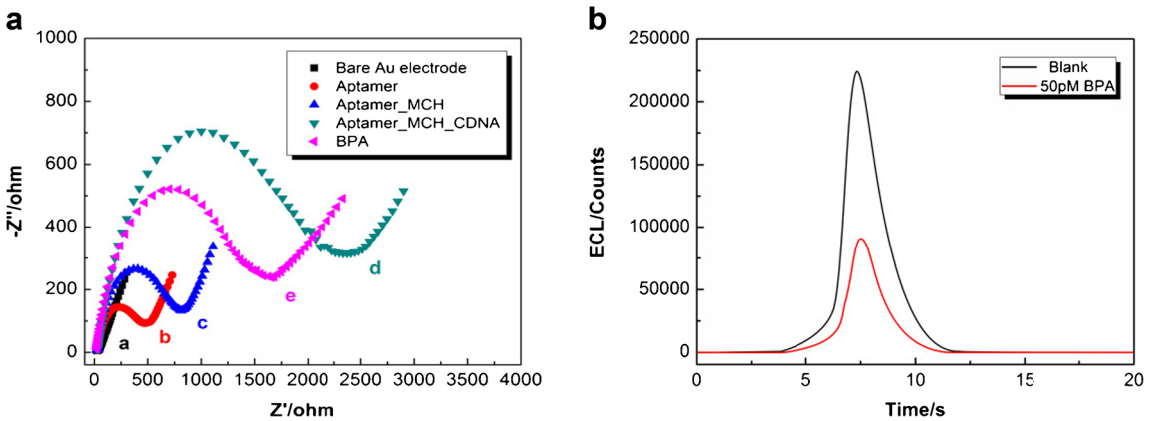


Fig. 1 (**a**) EIS signals corresponding to different electrodes. (**b**) ECL signals of the developed sensing system in the presence and absence of 50 pM BPA

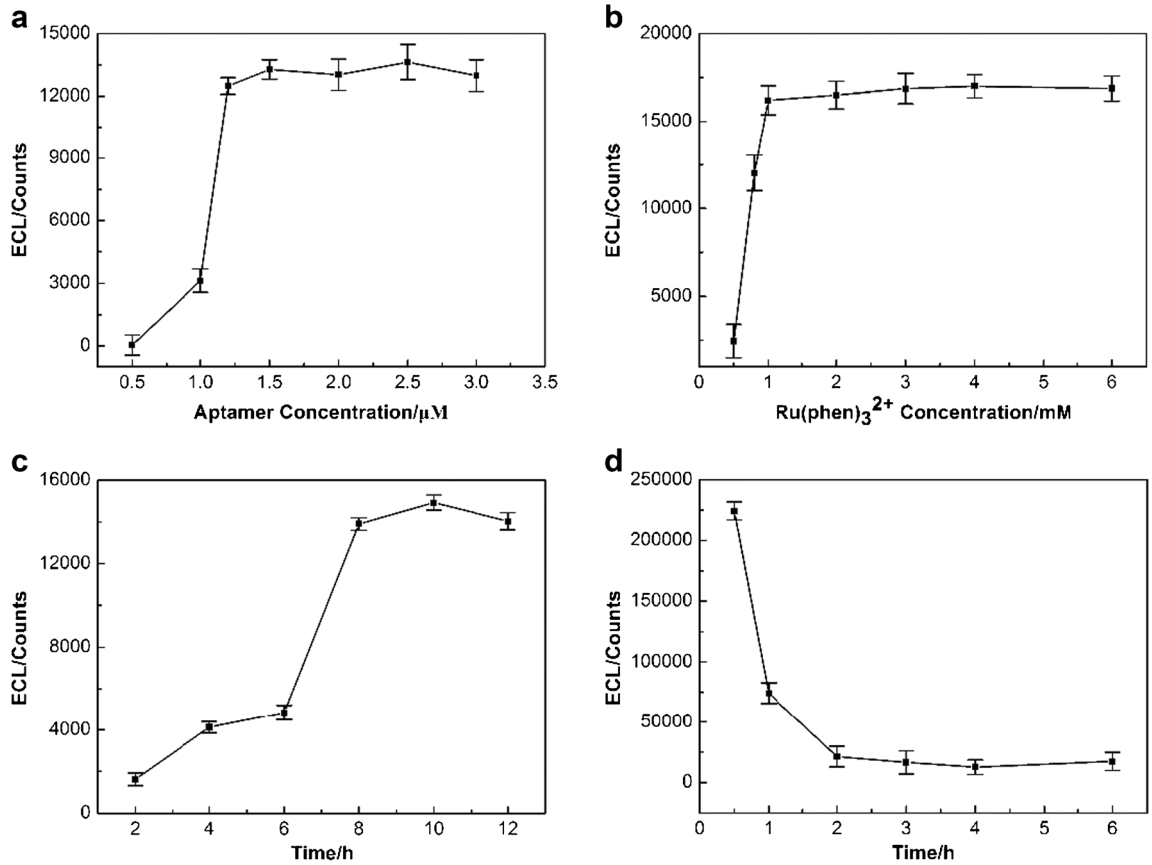


Fig. 2 Effect of (**a**) concentration of aptamer, (**b**) concentration of $Ru(phen)_3^{2+}$, (**c**) $Ru(phen)_3^{2+}$ incubation time, and (**d**) BPA incubation time on the ECL intensity with 10 nM BPA

electrode surface through Au–S interaction and then hybridizes with CDNA. $Ru(phen)_3^{2+}$ intercalates into the dsDNA grooves and acts as ECL indicator. The ECL signal from the intercalated $Ru(phen)_3^{2+}$ using TPA as a coreactant was recorded as the basic value for BPA determination. In the presence of BPA, the aptamer immobilized on the electrode surface specifically binds with BPA; this changes the aptamer's structure and releases the CDNA from the electrode, so the $Ru(phen)_3^{2+}$ intercalated in the dsDNA can be released from the electrode surface. The ECL signal of the system therefore becomes weak. The decreased ECL intensity has a direct relationship with the BPA concentration and a sensitive biosensor for BPA can thus be developed.

Characterization of modified electrodes

Electrochemical impedance spectroscopy (EIS) was applied to monitor the electrode modification procedures. As shown in

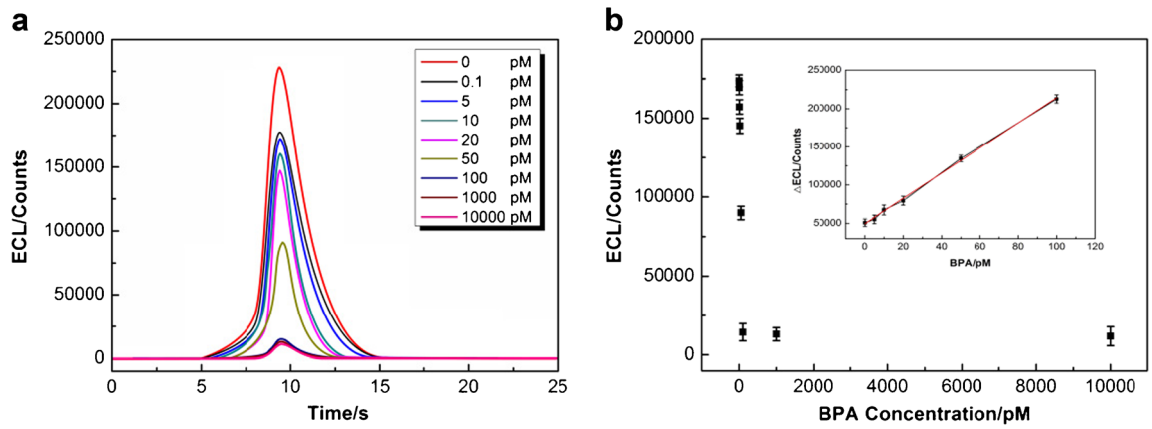


Fig. 3 (**a**) ECL response at different BPA concentrations. (**b**) Relationship between ΔECL and the BPA concentrations. The inset shows the calibration curve between the BPA concentrations and ΔECL. The errors bars show the standard deviation of three replicate determinations

Fig. 1a, the bare Au electrode (curve a) shows a very small semicircular domain, which indicated that the electroactive ions are transported easily on the electrode surface. As a result of the electrostatic repulsion between the negatively charged phosphate backbone of the aptamer and the anionic $[Fe(CN)_6]^{3-/4-}$ ions, the impedance increased to about 500 Ω after modification of the aptamer (curve b). Blocking of other non-binding sites on the electrode by MCH prevented the transport of electroactive ions on the electrode surface causing the impedance to become about 800 Ω (curve c). Hybridization of the aptamer with CDNA on the electrode further increased the impedance (2500 Ω, curve d), which indicates that the three-dimensional double-stranded phosphate backbone inhibits oxidation of anionic $[Fe(CN)_6]^{3-/4-}$ ions. However, after reaction with BPA (50 pM), the impedance decreased to 1700 Ω (curve e) because grooves of dsDNA disappeared. Figure 1b shows the ECL signal in the presence and absence of BPA (50 pM). In the absence of BPA, a strong ECL signal was detected, but in the presence of BPA, only a weak ECL signal was recorded. These results confirmed the proof of principle of our proposed ECL biosensor.

Optimization of reaction conditions

The parameters which affect the performance of the biosensor were optimized. The aptamer plays a critical role in the modification of the gold electrode, so its concentration was optimized first. As shown in Fig. 2a, the ECL intensity increased with concentration of aptamer from 0.5 μM to 1.5 μM and then reached saturation above 1.5 μM. So 1.5 μM of aptamer was chosen as the best condition. The ECL intensity increased gradually with increasing concentration of $Ru(phen)_3^{2+}$ from 0.5 mM to 1.0 mM and then reached a plateau without much more increase thereafter (Fig. 2b). As shown in Fig. 2c, the ECL signal increased with increasing culture time of $Ru(phen)_3^{2+}$ from 2 h to 8 h and then reached saturation after 8 h. So 1.0 mM $Ru(phen)_3^{2+}$ and 8 h of intercalation time were chosen in the following study.

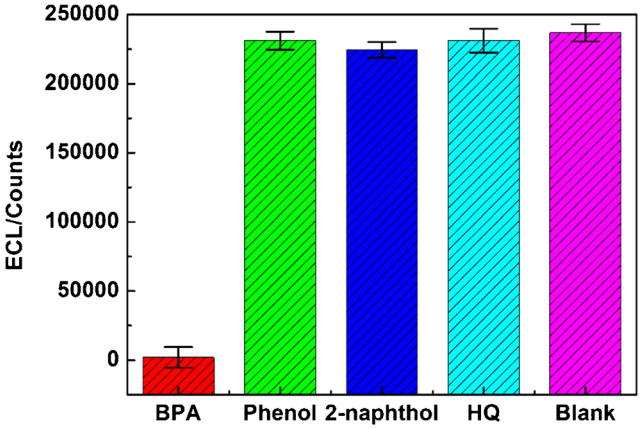


Fig. 4 Target selectivity of the developed ECL biosensor

Since the reaction time of BPA and aptamer directly affected the results of detection, it is essential to optimize the reaction time with BPA. As shown in Fig. 2d, the ECL signal decreased gradually with increasing of BPA reaction time from 1 h to 3 h, but the ECL intensities showed no substantial differences from 3 h to 6 h. Hence, 3 h was selected as the least BPA reaction time in the following study.

Performance of ECL biosensor

As shown in Fig. 3a, the ECL intensity decreased gradually with increasing of BPA concentration in the range of 0.1 pM to 100 pM. Figure 3b shows the relationship between the decreasing ECL intensity (ΔECL, i.e., the difference between ECL intensity in the presence and absence of BPA) and the concentration of BPA; there is a linear relationship between ΔECL and the BPA concentration from 0.1 pM to 100 pM. The linear equation can be expressed as

$$\Delta ECL = 49332.5 + 1649.1 * C.\ R = 0.998$$

where C represents the concentration of BPA and R is the correlation coefficient. The detection limit (LOD) was calculated to be 0.076 pM on the basis of the response of three times the standard deviation of the blank samples measured with the

Table 1 Comparison of some electrochemical aptasensors for BPA detection

Reference	Methods	Medium	Analytical range	LOD
Ding et al. [25]	Potentiometric	Buffer	0.1–100 nM	80 pM
Pan et al. [26]	DPV	Buffer	2.5 nM–3.0 μM	1 nM
Guo et al. [21]	ECL	Buffer	0.25–100 μM	83 nM
Liu et al. [20]	DPV	Buffer	1 pM–1 nM	0.41 pM
Derikvandi et al. [27]	DPV	Buffer	1–400 nM	210 pM
Deiminiat et al. [23]	SWV	Buffer	0.1–10 nM	50 pM
Beiranvand and Azadbakht [28]	DPV	Not mentioned	1–20 nM	300 pM
This work	ECL	Deionized water	50–600 nM 0.1–100 pM	76 fM

DPV differential pulse voltammetry, *ECL* electrochemiluminescence, *SWV* square wave voltammetry

Table 2 Determination and recoveries of BPA in drink transferred from different categories of canned beverage ($n = 3$)

Samples	Added (pM)	Detected (pM)	Recovery (%)	RSD (%)
Yili milk	0	Not detected	–	–
	5	5.24	105	5.8
	10	9.66	96	6.2
Binglu mineralized water	0	Not detected	–	–
	5	4.82	96	6.0
	10	9.86	98	4.1
Minute Maid	0	Not detected	–	–
	5	4.96	99	8.0
	10	10.37	104	8.6

ECL biosensor, which is in accordance with those of previous methods [24]. As is shown in Table 1, our fabricated sensor enables more sensitive detection limits than most other previously reported electrochemical methods for BPA in solution.

The specificity of the proposed biosensor was studied by using Na^+, K^+, Mg^{2+}, Cl^-, SO_4^{2-}, NO_3^-, ascorbic acid (AA), phenol, 4-nitrophenol, and hydroquinone (HQ) as interferences. The ECL intensities from AA, phenol, 4-nitrophenol, and HQ, which were present at 100 times the concentration (1 μM) of the target (10 nM BPA), showed no obvious variations compared with that from the control system without target BPA; whereas, BPA induced a very large reduction in ECL. Moreover, the addition of the 1 mM of $NaNO_3$, KCl, or $MgSO_4$ into the BPA solution, separately, afforded ECL intensities that were similar to that from the solution with only BPA added. The corresponding results are shown in Fig. 4. These results suggested that the interference caused by these substances could be neglected, demonstrating that the proposed biosensor has good selectivity for the target in the presence of the aforementioned interferencs.

The stability of the system was tested by storing the modified electrodes at 4–8 °C for 7 days. Only a 5.4% change in the ECL intensity was observed compared with fresh electrodes, displaying the good stability of the developed aptasensor. The relative standard deviation was 7.2% by comparing the ECL intensity from three different ECL aptasensors for the same concentration of BPA, demonstrating that this method has good reproducibility.

Real sample analysis

To investigate the application of the proposed biosensor in real samples analysis, we studied the concentration of BPA in drink from different categories of canned beverages. Since the drink samples on the market are all free of BPA, the obtained drinks were spiked with a known amount of the BPA standard solution to determine the recovery of BPA. The results are shown in Table 2; the standard addition recoveries were in the range of 96–105%. Therefore, the developed sensor might be initially applied for the determination of BPA in drink transferred from the can.

It should be noted that compared with the traditional LC–MS/MS method for the detection of bisphenol A in drink transferred from cans, the proposed biosensor is not very stable; sometimes even a large deviation was noted, which is closely related to the aptasensor. Therefore, it is crucial to control temperature and balance the buffering system in the detection process. To this end, the ECL biosensor is suitable for the development of protocols that have a period of validity, instead of standard alternatives.

As shown in Table 3, although the sample studied in our protocol is not very complex and the detection limit is not the lowest, the proposed biosensor is simplified compared with other methods while maintaining a certain low detection limit.

Conclusion

A highly specific and sensitive ECL biosensor in which $Ru(phen)_3^{2+}$ intercalates into grooves of ds-DNA and BPA hybridizes with its aptamer with high affinity has been fabricated for the detection of BPA. This ECL platform exhibits several attractive features: (a) The detection limit for BPA in

Table 3 Comparison of some other methods for BPA detection mentioned in this paper

Reference	Method	Medium	Analytical range	LOD	Experimental procedures
Selvaraj et al. [7]	GC–MS	Water sample from river	5–200 ng/mL	1.5 ng/mL	Complex
Cappiello et al. [8]	GC–MS	Liver matrix and brain matrix	0.4–8000 ng/g	0.2 ng/g	Complex
Noonan et al. [9]	LC–MS	Canned food (soup, meat, vegetables, fish, pasta)	2.6–730 ng/g	2 ng/g	Complex
Becerra and Odermatt [10]	LC–MS	Paper samples	0–100 mg/kg	0.35 mg/kg	Complex
Ji et al. [11]	Fluorescence spectrometry	Milk	1–10 pg/L	3.4 pg/L	Complex
Mei et al. [12]	Photometry	Tap water	0.01–100 ng/mL	0.049 ng/mL	Complex
Lei et al. [13]	ELISA	Human urine	0.01–1000 ng/mL	0.03 ng/mL	Complex
This work	ECL	Deionized water	0.1–100 pM	76 fM	Simple

solution is as low as 0.076 pM; (b) it requires simplified experimental procedures without complex chemical markers or biomarkers; (c) allows extensive scope for alternative by replacing the corresponding aptamer to allow detection of other small molecules. Furthermore, the ECL biosensor was applied to detect BPA in canned drink with satisfactory results.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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