

Polychlorinated Biphenyl Electrochemical Aptasensor Based on a Diamond–Gold Nanocomposite to Realize a Sub-Femtomolar Detection Limit

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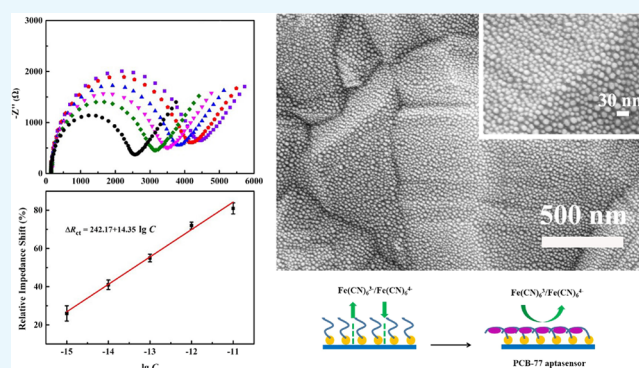


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ABSTRACT: Polychlorinated biphenyls (PCBs) with high toxicity, low lethal dose, and bioaccumulation have been inhibited for application in wide fields, and a highly efficient trace detection is thus greatly desirable. In this study, we produce dense Au-nanoparticles by twice sputtering and twice annealing (T-Au-NPs) on boron-doped diamond (BDD). The successful formation of T-Au-NPs/BDD nanocomposites was confirmed by scanning electron microscopy, X-ray diffraction, Raman spectroscopy, and X-ray photoelectron spectroscopy analysis. Based on T-Au-NPs/BDD, an electronic biosensor with aptamers is fabricated to detect trace polychlorinated biphenyl-77 (PCB-77) by electrochemical impedance. A good linear relationship in the range of femtomolar to micromolar and significantly low detection limit of sub-femtomolar level (0.32 fM) are realized based on the biosensor. The emphasis of this research lies in the key role of the diamond substrate in the biosensor. It is demonstrated that the biosensor has excellent sensitivity, specificity, stability, and recyclability, which are favorable for detecting the trace PCB-77 molecule. It is attributed to the important effect presented by the BDD substrate and the synergistic influence of T-Au-NPs combined with aptamers.



1. INTRODUCTION

Persistent organic pollutants would generally lead to genotoxicity and tumor promotion, which seriously threaten the health of the ecosystem and humans.¹ Among them, the polychlorinated biphenyls (PCBs) have high toxicity, low lethal dose, and bioaccumulation, and they have been inhibited comprehensively.² Unfortunately, there are PCBs residues in food, water, and air,³ and they are highly accumulative and gradually enriched through the food chain. The Food and Drug Administration recommends that the amount of PCBs should not exceed 3 μM in food.⁴ They are hydrophobic with low water solubility, while they have high solubility in most organic oils and fats. With the enrichment of the food chain, the PCB content in the organism could be 10^7 – 10^8 times that of the surrounding environment. Therefore, the release of most pollutants is an accumulative process, and the lower detection limit is helpful to detect the trace pollutant for early prevention and/or intervention. Therefore, it is urgent to achieve the highly sensitive and selective detection of PCBs. Thus far, several typical techniques, such as gas chromatography–mass spectrometry,⁵ liquid chromatography,⁶ and immunoassay–liquid chromatography,⁷ have been developed for the quantitative detection of PCBs with detection limits of several picomolars. However, the complex pretreatment programs and

expensive and time-consuming systems make such methods unsuitable for on-site detection limiting.⁸

The electrochemical method offers a relatively simple, low-cost, and sensitive implementation process to realize a low detection limit in a wide dynamic range.⁹ The PCBs are generally monitored by electrochemical impedance spectroscopy (EIS) analysis owing to chemical inertness and insulating properties of PCBs for electrochemical activity.¹⁰ EIS is a very sensitive technique to make response information about the interface,¹¹ while the detection limit of carbon nanotube/pyrenecyclodextrin at the μM level¹² could not satisfy the demand of environmental science. Introducing aptamers into the electrode surface is an effective strategy to enhance the specificity and sensitivity.¹³ Aptamers are single-strand DNA or RNA with high target affinities, unique conformations, and detailed three-dimensional structures,¹⁴ providing a good alternative approach to sensitively and selectively identify target molecules by changing their structures to enhance the

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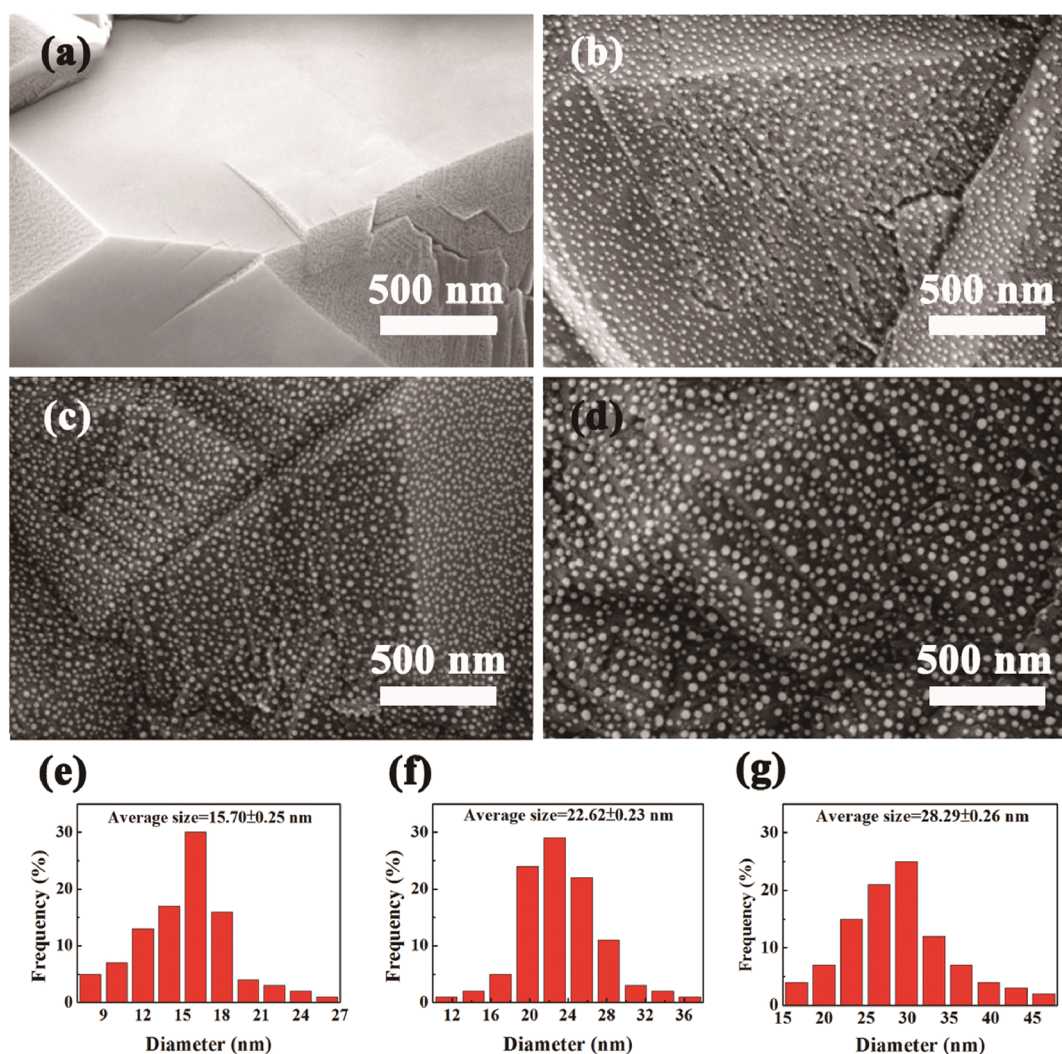


Figure 1. SEM images of (a) polycrystalline BDD and (b–d) Au-NPs/BDD. The gold deposit on polycrystalline BDD substrate with sputtering times of the Au film of (b) 10, (c) 20, and (d) 30 s and annealing treatment at 800 °C for 1 min. Panels (e), (f), and (g) are bar graphs of Au-NP size distribution of panels (b), (c), and (d), respectively.

detection limit.^{15–17} It was reported that the aptamers added in carbon nanotubes could importantly increase the detection limit to nM.¹⁸ Also, Au-based biosensors with aptamers could realize the detection limit to pM¹³ since Au electrodes had the advantages of high electrical conductivity, chemical stability, and easy attachment to aptamers.^{19,20} Au-nanoparticles (Au-NPs) with large specific surface area would provide more adsorption sites for aptamers by forming Au–S bonds.^{21–24} Carbon nanomaterials are key components of transducers in biosensors due to their intrinsic chemical and mechanical properties.²⁵ Boron-doped diamond (BDD) is an excellent carbon material for electrochemical examinations²⁶ because of its chemical inertness, wide potential window, low background current, and good electrochemical stability.^{27–29}

Recently, many groups investigated the Au-NPs on BDD electrodes by different ways (electrodeposition,³⁰ microemulsion technique,³¹ sputtering-annealing treatment). In the microemulsion technique and electrochemical deposition methods, the agglomeration of Au-NPs often occurs, which will affect the stability of diamond electrode performance. In addition, the application of these schemes is not convenient because of expensive raw materials, environmental unfriendliness, and difficult size control. However, the technology of

sputtering and annealing is simple; the density and size of nanoparticles are easy to control, and the obtained particles do not have agglomeration, which has the advantages of simple, fast, and low cost.

Based on above consideration, in this paper, we fabricate a BDD electrode coated with Au-NPs by twice sputtering and twice annealing (T-Au-NPs). The distance between T-Au-NPs reaches the nanometer level uniformly. Aptamers are demonstrated on the T-Au-NPs/BDD to test the trace polychlorinated biphenyl-77 (PCB-77) molecule. A detection limit of as low as 0.32 fM is realized by EIS. It is demonstrated that the linear detection range of the aptasensor could work on an extensive examination scale from the femtomolar to micromolar by diluting PCB-77 concentrations several orders of magnitude for the secondary measurements. The sensitivity, specificity, stability, and recyclability of aptasensors show a positively significant improvement.

2. RESULTS AND DISCUSSION

2.1. Characterization of the Au-NPs/BDD Electrode.

As seen from the SEM images, Figure 1a shows the polycrystalline BDD film without depositing a Au film, and the diamond film has a flat surface and a prominent crystal

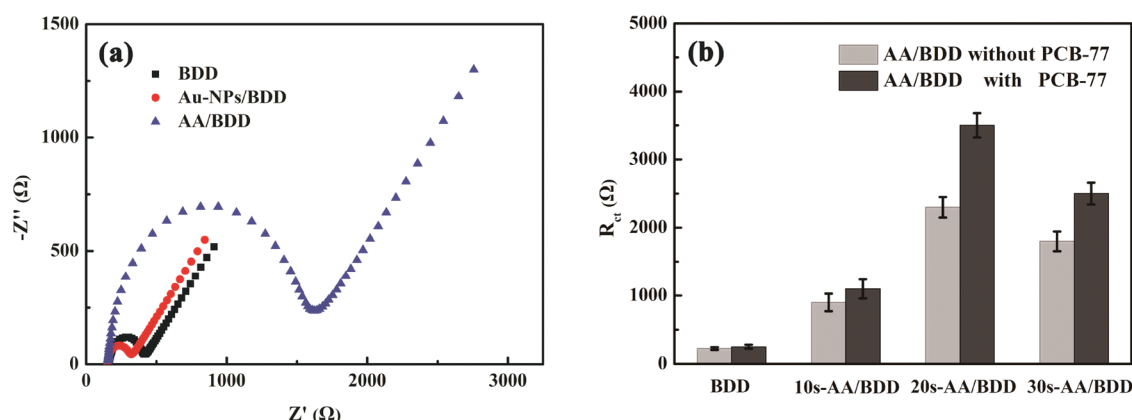


Figure 2. (a) Impedance spectra (Nyquist plots) of BDD, Au-NPs/BDD, and AA/BDD electrodes based on a sputtering time of 20 s tested in solution containing $5 \text{ mmol L}^{-1} \text{ Fe(CN)}_6^{3-/4-}$ and $0.1 \text{ mol L}^{-1} \text{ KCl}$. (b) Bar graph of impedance change of the electrodes based on different sputtering times before and after incubation with a concentration of $10^{-11} \text{ mol L}^{-1} \text{ PCB-77}$.

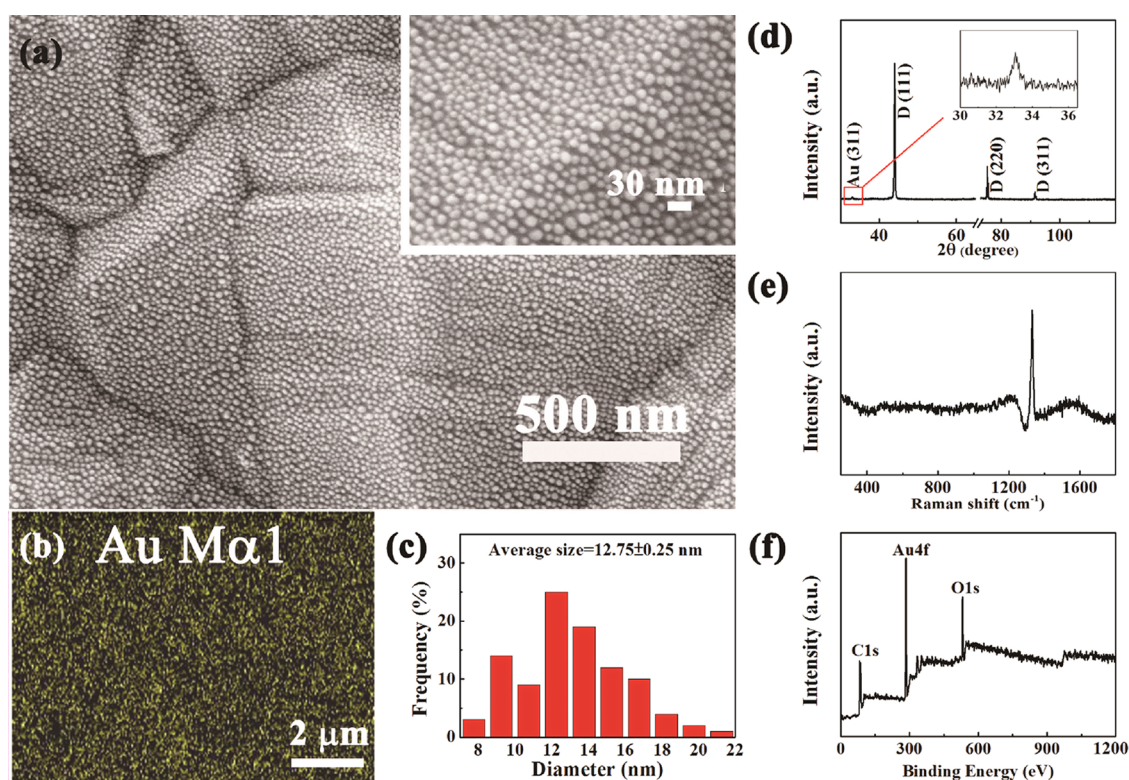


Figure 3. SEM image of T-Au-NPs/BDD after two-step treatment of sputtering and annealing with total sputtering time (20 s) and total annealing time (1 min). The inset image is obtained at high magnification. (b) EDS mapping of the Au element. (c) Bar graph of particle size distribution in panel (a). (d) XRD and (e) Raman spectrum of T-Au-NPs/BDD. (f) XPS survey scan of the T-Au-NPs/BDD.

shape with an annealing time of 1 min. Figure 1b–d shows the Au-NPs/BDD films after sputtering a gold film of different thickness and same annealing. The sputtering times of the gold film were (b) 10, (c) 20, and (d) 30 s. The annealing time of the samples in Figure 1 was 1 min (800 °C). As sputtering time increases, the content of gold atoms increases gradually. Using software NanoMeasurer1.2, the size of nanoparticles can be obtained and calculated. The average sizes of Au-NPs in Figure 1b–d are about 15.7, 22.6, and 28.3 nm. After sputtering and annealing, the mean densities are 4.8×10^{10} , 7.6×10^{10} , and $3.52 \times 10^{10} \text{ Au-NPs/cm}^2$, respectively. It can be seen from Figure 1 that the size and density of Au-NPs are related to the sputtering time. A longer sputtering time

increases the average particle size of Au-NPs. When the temperature decreases, the gold particles solidify and form Au-NPs with a certain size distribution on the surface of BDD. This process makes the thick gold film sputtered more likely to form larger particles and lower density of particles. The rate of the Au-NP area is 0.30:1.00:0.73 with sputtering times of 10, 20, and 30 s, respectively. Thus, a longer sputtering time with a thicker gold film does not increase the surface area of the Au-NPs. The surface area of Au-NPs formed by an appropriate sputtering time (20 s) is the largest.

2.2. Characterization of Aptamers/Au-NPs/BDD (AA/BDD) Electrodes. Nyquist plots of BDD, Au-NPs/BDD, and AA/BDD with a sputtering time of 20 s are presented in Figure

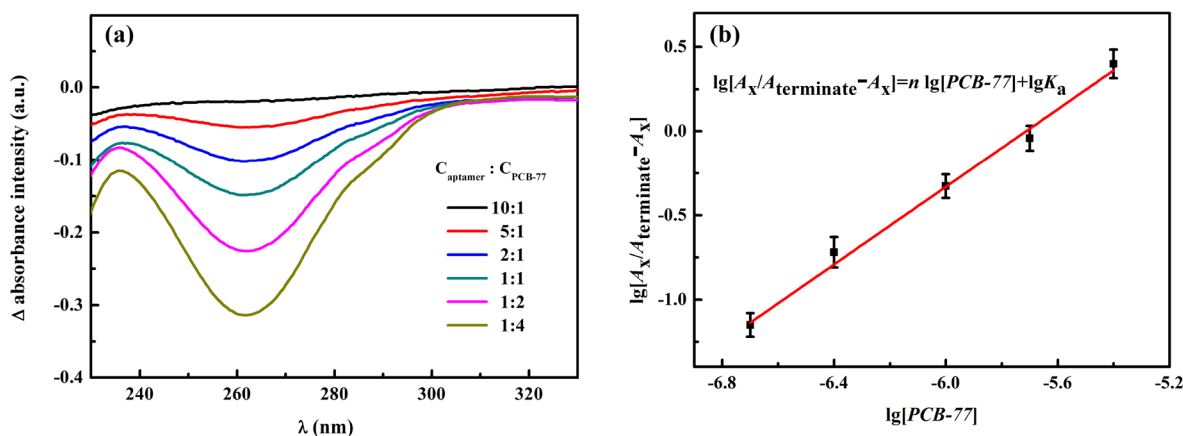


Figure 4. (a) UV spectra of different proportions of aptamer with PCB-77. The concentration of aptamer solution is $2 \mu\text{mol L}^{-1}$. (b) Linear fitting to calculate the corresponding association constant (K_a) reflecting the affinity of PCB-77 on aptamers.

2a. The charge transfer resistance of the BDD electrode is 387Ω . After Au-NP deposition, the charge transfer resistance of the Au-NPs/BDD electrode drops to 189Ω , which would facilitate electron transfer denoting the improving conductivity. After modification of aptamers on the Au-NPs/BDD electrode, the impedance increases to 1715Ω , which is attributed to the electrostatic repulsion between the negatively charged aptamers and the $\text{Fe}(\text{CN})_6^{3-/4-}$ redox probe, making it difficult for $\text{Fe}(\text{CN})_6^{3-/4-}$ to reach the surface of AA/BDD.³² These results agree well with cyclic voltammetry (CV) spectra (Figure S1). For the AA/BDD electrodes with sputtering times of 10, 20, and 30 s immersed in solution with $1.0 \times 10^{-11} \text{ M}$ PCB-77, the impedance enhanced compared to those without PCB-77 (Figure 2b). The rate of the electrode impedance change with sputtering times of 10, 20, and 30 s before and after incubation with a concentration of PCB-77 is 0.43:1:0.74, which is incongruous with that of Au-NP area. The electrodes might improve the sensitivity of electrochemical detection of target molecules by enhancing the detection area.³³ It suggests that the AA/BDD electrode with a sputtering time of 20 s has the highest sensitivity of detecting PCB-77 for the largest relative impedance shift because the sensor obtained with a sputtering time of 20 s has more surface area of Au-NPs to modify more aptamers.

2.3. Characterization of T-Au-NPs/BDD. In order to further increase the surface area of Au-NPs, we propose an approach to get Au-NPs/BDD by a twice sputtering and twice annealing process. The BDD film was coated with a thin Au layer by sputtering the Au target for 10 s, and following a heat treatment at 800°C in air for 30 s, the Au-NPs appeared on BDD film. Sputtering on the former Au-NPs/BDD and an annealing process under the same conditions were performed to produce a second Au nanolayer. Evidently, the T-Au-NPs have a higher Au-NP density of $\sim 2.5 \times 10^{11}/\text{cm}^2$ on BDD (Figure 3a). According to the elemental mapping using an energy-dispersive spectrometer (EDS), the BDD surface is uniformly covered by T-Au-NPs (Figure 3b). However, the average size of the T-Au-NPs decreased to $\sim 12.8 \text{ nm}$ (Figure 3c). More uniformly distributed nanoparticles are obtained by this method, and the agglomeration of Au-NPs hardly occurs.³⁴ Compared to the Au-NPs/BDD by single sputtering and annealing with the same total sputtering time (20 s) and total annealing time (1 min), the area of T-Au-NPs increased slightly by 5.5%, which had more sites for the absorption of aptamers. From the XRD results of T-Au-NPs/BDD in Figure

3d, the BDD film consists of micrometer grains with the typical (111), (110), and (311) surfaces, and Au-NPs are mainly (311) grains. The BDD film characterized by Raman spectroscopy (Figure 3e) shows a typical Fano effect related asymmetrical and downshift feature of a diamond peak at 1330 cm^{-1} .³⁵ The XPS survey scan of the T-Au-NPs/BDD (Figure 3f) shows that the T-Au-NPs are elementary substances offering sites for aptamers. The O1s peak comes from the oxygen termination of the annealed BDD surface in air.

2.4. Importance of BDD. For the AA/BDD electrodes based on sputtering times of 10, 20, and 30 s (Figure 2b) and Aptamers/T-Au-NPs/BDD (ATA/BDD) electrode, a relative impedance shift in solution with $1.0 \times 10^{-11} \text{ M}$ PCB-77 could effectively enhance by 22, 52, 36, and 88% compared to those without PCB-77, respectively. The ATA/BDD biosensor has excellent sensitivity. Meanwhile, the excellent sensitivity on ATA/BDD could not be caused entirely by a slightly increased area of T-Au-NPs providing more sites for aptamers. The high sensitivity of the ATA/BDD sensor might be due to the key factor (BDD substrate). We hypothesize that a conformational change in the aptamer upon specific binding of PCB-77 may alter the channel of $\text{Fe}(\text{CN})_6^{3-/4-}$ reaching the surface of BDD. Before capturing PCB-77, $\text{Fe}(\text{CN})_6^{3-/4-}$ could reach the surface of ATA/BDD freely with stretching aptamers. After capturing PCB-77, the aptamers present a compact tetrahedral structure to block $\text{Fe}(\text{CN})_6^{3-/4-}$ from contacting the Au surface. When the distance of the T-Au-NPs is close to the size of the compact tetrahedral aptamer, the channel of $\text{Fe}(\text{CN})_6^{3-/4-}$ reaching the surface of BDD is closed. The BDD substrate has good stability and high resistance to fouling, which can reduce the adsorption of nonspecific substances in the detection process. Thus, the ATA/BDD sensor shows excellent sensitivity because the impedance increases sharply because the channel to the Au-NPs and the channel to the BDD could be blocked. By comparison, the AA/BDD sensor is not sensitive enough because only the channel to the Au-NPs could be blocked and the partial channel to the BDD could not be blocked when the distance between the Au-NPs is much larger than the compact aptamer structure (Figure S2).

2.5. Strong Affinity between Aptamers and PCB-77.

The strong affinity between aptamers and PCB-77 is important to realize high sensitivity and improve the detection limit, which is confirmed by UV spectral measurement. As shown in Figure 4a, PCB-77 causes a significant increasing effect of the adsorption band for the PCB-77 aptamer centered at 262 nm

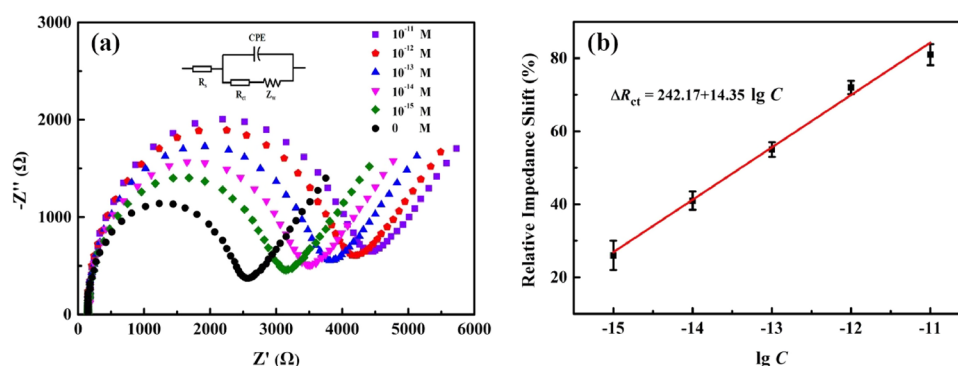


Figure 5. (a) Impedance spectra (Nyquist plots) of the ATA/BDD sensor incubated with varying concentrations of PCB-77. The inset of panel (a) is the equivalent circuit of text. (b) The calibration plot of the relative impedance shift as a function of PCB-77 concentration.

Table 1. Relevant Published Various Methods of PCB Detection

method	linear range (M)	detection limit (M)	reference
gas chromatography–mass spectrometry	6.8×10^{-11} to 8.6×10^{-7}	9.2×10^{-12}	5
liquid chromatography–mass spectrometry		1.6×10^{-10}	37
surface-enhanced Raman spectroscopy aptamer sensor	3.3×10^{-8} to 1.0×10^{-6}	3.3×10^{-8}	38
electrochemical aptamer-carbon nanotube biosensor	1.9×10^{-7} to 8.8×10^{-6}	1.2×10^{-8}	18
electrochemical aptamer/Au biosensor	6.8×10^{-10} to 6.8×10^{-7}	3.4×10^{-11}	13
electrochemical oxide graphene/ β -cyclodextrin polymer	1.0×10^{-12} to 1.0×10^{-5}	5.0×10^{-13}	39
electrochemical β -cyclodextrin/400 nm-diameter gold electrode	1.0×10^{-15} to 1.6×10^{-14}	2.1×10^{-16}	40
electrochemical ATA/BDD biosensor	1.0×10^{-15} to 1.0×10^{-11}	3.2×10^{-16}	this work

with increasing PCB-77 concentration in aptamer solution. Figure 4b reveals the linear relationship based on the equation ($\lg[A_x/A_{\text{terminate}} - A_x] = n \lg[\text{PCB-77}] + \lg K_a$), where A_x and $A_{\text{terminate}}$ are the intensities of the spectra after adding $x \text{ mol L}^{-1}$ PCB-77 and excess PCB-77 into $2 \mu\text{mol L}^{-1}$ aptamer solution, n is the comprehensive coordination number, $[\text{PCB-77}]$ is the concentration of PCB-77, and K_a is the corresponding association constant. The fitted n and K_a are 1.145 and $3.47 \times 10^6 \text{ M}^{-1}$, respectively. Such a large value of K_a reflects a high affinity of PCB-77 on aptamers.

2.6. ATA/BDD for the Sub-Femtomolar Detection Limit of PCB-77. The impedance spectra of PCB-77 with different concentrations are shown in Figure 5a, and the inset presents the corresponding equivalent circuit. Evidently, the impedance increases with the increase of concentration of PCB-77. All impedance spectra are fitted with detailed fitting data (Table S1). The peak current in CV curves tested in $\text{Fe}(\text{CN})_6^{3-/4-}$ solution nonlinearly decreases with the increase of PCB-77 concentration ranging from 1.0×10^{-15} to $1.0 \times 10^{-11} \text{ M}$ (Figure S3), corresponding to the EIS spectra in Figure 5a. As plotted in Figure 5b, the relative impedance linearly increases with PCB-77 concentration in the region of 1.0×10^{-15} to $1.0 \times 10^{-11} \text{ M}$. Determined by equation $\Delta R_{\text{ct}} = R_0 + A \lg C$, where C is the PCB-77 concentration, the fitting data include 242.17Ω for R_0 , 14.35 for A , and a correlation coefficient (R^2) of 0.993. The detection limit of the AA/BDD electrode reaches a sub-femtomolar magnitude of 0.32 fM , which is calculated based on the response of three times the standard deviation of blank samples on the aptasensor.³⁶ Further, the achieved detection limit is 3 orders superior to most of the previous reports as shown in Table 1.^{5,13,18,37–39} Noted elsewhere,⁴⁰ a single Au nanoneedle (400 nm in diameter) modulated by β -cyclodextrin was proposed as the electrode to realize a detection limit of 0.21 fM , while its linear detection was in a narrow range of 2–16 fM. Comparatively, the ATA/

BDD sensor offers a rigid and recyclable diamond-based electrode with a wide linearity range and low background noise signals, implying that the electrode in this work is more favorable for practical applications.

In this work, the ATA/BDD aptasensor presents high-performance electrochemical properties, which is attributed to the following factors. (i) The BDD substrate has a wide potential window, good stability, and high resistance to fouling, which can reduce the adsorption of nonspecific substances in the detection process.⁴¹ The sensor is sensitive enough because not only the channel to the Au-NPs could be blocked but also the channel to the BDD could be blocked. The BDD is the optimal material as a substrate for detecting PCB-77 with limitation as low as 10^{-16} M . (ii) T-Au-NPs can promote electron transfer and increase the superficial area to have more sites for the absorption of aptamers. Appropriate density of T-Au-NPs is helpful for compact tetrahedral aptamers to block the channel to BDD. (iii) The high affinity and specificity of aptamers for PCB-77 further realize high sensitivity and improve the detection limit.⁴² The superior stability and sensitivity of the ATA/BDD electrode are due to the synergistic action of the above factors.

2.7. Specificity and Stability of the ATA/BDD Aptasensor. The high-performance specificity is the most unique characteristic of the ATA/BDD aptasensor, which is examined in the 50-fold solutions containing PCB-28, PCB-52, PCB-81, PCB-126, lindane, coronene, pyrene, and hexachlorobenzene, in which $1.0 \times 10^{-13} \text{ M}$ PCB-77 is added (Figure S4). The selected additional persistent organic pollutants have similar structures or functional groups to PCB-77. The impedance for pure PCB-77 is set as 100%, and the relative impedance fluctuation of each mixture ranges between -2.9 and 5.8% . Moreover, the relative impedance shift of a series of PCBs is also examined, such as PCB-28, PCB-52, PCB-81, and PCB-126 probes. Evidently, in contrast to the result of PCB-

77, all these PCB samples have nonspecificity to the aptasensors at the varying concentrations (Figure S5), implying the excellent specificity of the ATA/BDD sensor. The aptasensor is also tested for 10 days under the same test conditions, as shown in Figure S6. For 10^{-13} M PCB-77, the daily change in the relative response is in the region of -2.4 – 4.5% compared to the first day with 100%, which reveals the stability of the ATA/BDD aptasensor within 10 days.

2.8. Linear Detection Range (LDR). For expanding the LDR beyond the region of 10^{-15} to 10^{-11} M, the following strategy is adopted. When the concentration of PCB-77 is higher than 10^{-11} M (e.g., 10^{-10} – 10^{-6} M), the impedance signals of the tested PCB-77 solutions approach the calibrated data of 10^{-11} M (Figure S7a). These samples with the higher PCB-77 concentrations are then diluted by one or several orders of magnitude for the secondary measurements, showing a linear relationship of relative impedance shift and PCB-77 concentrations (Figure S7b). The real concentrations of the undiluted samples are then calculated from the secondarily measured PCB-77 concentration by multiplying the dilution factor when the PCB-77 concentrations are in the region of 10^{-15} – 10^{-11} M. The PCB-77 with a higher concentration thus can be accurately detected. It is demonstrated that the LDR of ATA/BDD aptasensors could work on an extensive examination scale from the femtomolar to micromolar region.

2.9. Detecting PCB-77 Trace in Real Samples by the ATA/BDD Aptasensor. It is of great significance to investigate the performance of the aptasensor in practical applications. The aptasensor is applied to water samples collected from a natural water source with and without addition of PCB-77. Trace amounts of PCB-77 are added to the water, and the recoveries of the sensor are in the range of 96–106% with relative standard deviations less than 5.3% (Table 2). These results further confirm the high sensitivity, accuracy, and wide range of the aptasensor, which has great potential in trace detection of PCBs with low concentration.

Table 2. Recoveries of PCB-77 with Varying Concentrations Added in Lake Water Samples

lake water	added PCB-77 (M)	detected (M)	recovery (%)	RSD (%)
1	0	undetectable		
2	1×10^{-15}	0.98×10^{-15}	98%	3.4
3	1×10^{-13}	0.96×10^{-13}	96%	3.8
4	1×10^{-11}	1.05×10^{-11}	105%	4.7
5	1×10^{-9}	1.01×10^{-9}	101%	4.2
6	1×10^{-7}	1.06×10^{-7}	106%	5.3

2.10. Recyclability of the Aptasensors. The recyclability of the sensing electrode is another important advantage of aptasensors.⁴³ The electrochemical desorption method is adopted to break the gold–thiol bond and remove all aptamers from the T-Au-NPs/BDD surface after each detection. The EIS and CV tests of T-Au-NPs/BDD and regenerated T-Au-NPs/BDD after desorption (Figure 6) prove that all components are detached from the surface of T-Au-NPs/BDD. Compared with T-Au-NPs/BDD, the regenerated T-Au-NPs/BDD has the same impedance spectrum and peak current. For PCB-77 detection, the recyclable aptasensor by modifying aptamers still retains 95% of its original response, confirming the recyclability of the sensor without significant loss of response.

3. CONCLUSIONS

In this study, we design and fabricate an aptasensor based on BDD films coated with dense T-Au-NPs and aptamers by forming Au–S bonds for detecting organic pollutants. The sensor exhibits good linearity from the femtomolar to micromolar and a low detection limit of sub-femtomolar magnitude (0.32 fM) for the PCB-77 molecule, which is superior to other sensors, suggesting the successful realizing of trace detection of PCB-77. Moreover, the high selectivity, specificity, stability, and recyclability of the aptasensor are found. The high performance is attributed to the synergistic effect of BDD films, T-Au-NPs, and aptamers as follows: the BDD substrate exhibits low background signals; the dense T-Au-NPs evidently increase the absorption sites for aptamers and the channel to the BDD could be blocked when the distance between the T-Au-NPs is close to the compact aptamer structure; the high affinity of PCB-77 on aptamers could realize high sensitivity and improve the detection limit. This work is helpful to construct a diamond-based sensor to detect various trace substances with low concentrations in wide fields.

4. METHODS

4.1. Preparation of Au-NPs/BDD and T-Au-NPs/BDD Electrodes. The polycrystalline BDD films were fabricated on a p-type Si substrate by microwave plasma chemical vapor deposition (CVD) at 2.45 GHz.⁴⁴ The surface of the Si substrate was cleaned and seeded with nanodiamond powders for the following CVD diamond film growth.³⁵ During the CVD deposition process, the doping of boron was carried out under hydrogen flow (2.5 sccm) with trimethylborate, and the flow rate of the reaction gases was $\text{H}_2:\text{CH}_4 = 150:2$ (sccm). After deposition for 12 h, the thickness of BDD films was 15 μm . In the formation process of the Au-NPs/BDD structure, the BDD film was coated with a thin Au layer by sputtering the Au target for 10, 20, 30, and 40 s. Following a heat treatment at 800 $^\circ\text{C}$ in air for 1 min, an annealing process of the Au film occurred, and the Au-NPs appeared on the BDD film. The BDD film was coated with a thin Au layer by sputtering the Au target for 10 s, and following a heat treatment at 800 $^\circ\text{C}$ in air for 30 s, the Au-NPs appeared on the BDD film. Sputtering on the former Au-NPs/BDD and an annealing process under the same conditions were performed to produce a second Au nanolayer. Evidently, the T-Au-NPs have a higher Au-NP density on BDD.

4.2. Preparation of ATA/BDD. The 38-mer single-strand DNA aptamers for PCB-77 were synthesized by Sangon Biotech, China. The structure of the thiol-terminated aptamer probes (obtained by SELEX)⁴² was

5'-SH-(CH₂)₆-AAGCGCGCGAGACTACGTTTGTAGG-GATACCGATGTCG-3'. Aptamers were grafted on the T-Au-NPs/BDD by forming Au–S bonds and subsequently incubated at 37 $^\circ\text{C}$ for 5 h. The obtained ATA/BDD was washed and cleaned with Tris-HCl buffer and ultrapure water to remove the unhybridized aptamers. The PCB-77 aptasensor showed the specific adsorption of PCB-77 for detection. The design and manufacturing processes of the PCB-77 aptasensor is schematically illustrated in Scheme 1. The preparation of the AA/BDD scheme is presented in Figure S2. The sensors were then stored in a refrigerator at 4 $^\circ\text{C}$.

4.3. Electrochemical Measurements and Detection of PCB-77. PCB-77 was from the Laboratory of the Government

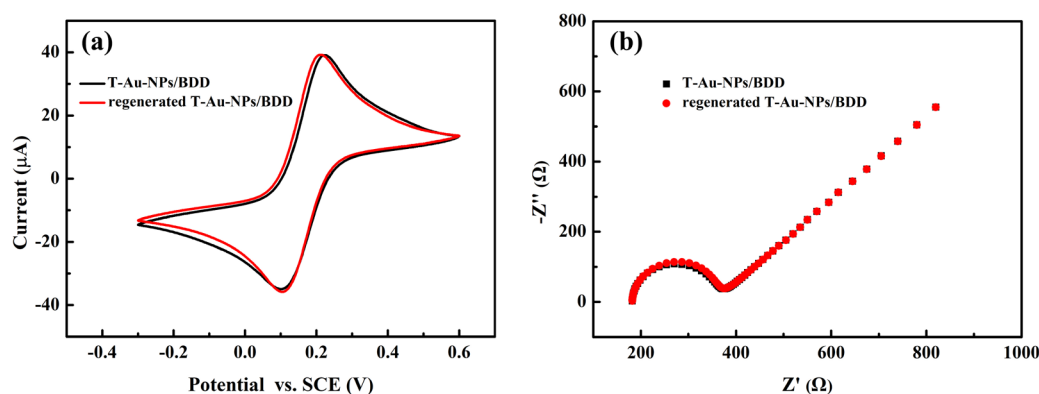
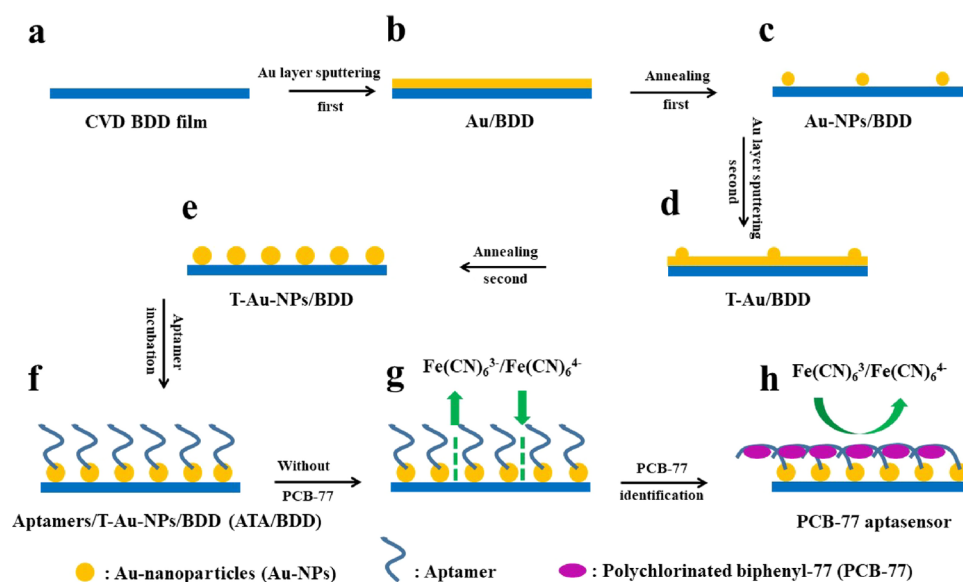


Figure 6. (a) CV and (b) EIS of T-Au-NPs/BDD and the regenerated T-Au-NPs/BDD in 5 mmol L⁻¹ Fe(CN)₆^{3-/4-} solution containing 0.1 mol L⁻¹ KCl (scan rate: 50 mV s⁻¹).

Scheme 1. Schematic Illustration of the Fabrication Procedure for PCB-77 Aptasensors



Chemist Labor Gesellschaft mit beschränkter Haftung. PCB-77 probes with varying concentrations were dissolved in a binding buffer (100 mM Tris-HCl at pH = 8.0 with 200 mM NaCl, 25 mM KCl, 10 mM MgCl₂). The DNA aptamer was dissolved in ultrapure water without any enzymes. The electrochemical experiments were carried out in an electrochemical cell containing 5 mM Fe(CN)₆^{3-/4-} and 0.1 M KCl as the electrolyte. The amplitude of the sinusoidal interference voltage was 5 mV. In the detection of PCB-77, the aptasensor was immersed in binding buffer with different standard concentrations of PCB-77 for 15 min and then washed with binding buffer to remove unbound PCB-77. The EIS tests were performed in the electrolyte solution in a frequency region of 10⁻¹–10⁵ Hz. The relative impedance shift ΔR_{ct} is defined as $\Delta R_{ct} = (R_{ct} - R_{ct0})/R_{ct0}$, where R_{ct0} is the initial impedance without PCB-77 and R_{ct} is the impedance of the ATA/BDD aptasensor after treatment with certain concentrations of PCB-77.

4.4. Determination of PCB-77 in Real Samples. The PCB-77 solution was diluted with anhydrous methanol into a stock solution (10 μM). The ATA/BDD aptasensors were applied to examine trace amounts of PCB-77 in water, which was a natural water source. The examined samples were filtered through a membrane followed by the addition of Tris-HCl

buffer solution. Then, the obtained liquid sample was spiked with certain amounts of PCB-77 standard solution, and the natural sample without added PCB-77 was used as the control group. Trace PCB-77 was detected by the ATA/BDD aptasensor.

4.5. Characterization and Electrochemical Measurements. The morphologies, structures, and vibrational characters of the electrodes were characterized by scanning electron microscopy (SEM, JSM-6480LV), X-ray diffraction (XRD, Rigaku D/MAX-RA), and Raman spectroscopy (Renishaw in Via Raman microscope equipped with 514 nm laser excitation), respectively. The affinity between aptamers and PCB-77 was confirmed by ultraviolet spectroscopy measurement (UV, UV-vis 1700). All electrochemical measurements were performed with an electrochemical workstation (CHI 760E). The aptasensor was based on the BDD electrode, platinum foil, and saturated calomel electrode as the working electrode, counter electrode, and reference electrode, respectively.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.0c02846>.

Schematic illustrations of the fabrication procedure for AA/BDD; CV curves taken from BDD, Au-NPs/BDD, and AA/BDD; CV curves of the ATA/BDD sensor incubated with varying concentrations of PCB-77; bar graph of relative impedance responses of PCB-77 by the ATA/BDD aptasensor incubated with different interferents; relative impedance shifts as a function of concentration (C) for PCB-77, PCB-28, PCB-52, PCB-81, and PCB-126; bar graph of the relative impedance responses of the ATA/BDD aptasensor from 1st to 10th day; impedance spectra of the ATA/BDD sensor incubated with varying concentrations of 10^{-11} – 10^{-6} M PCB-77; R(Q(RW)) circuit modeling results of the ATA/BDD sensor (PDF)

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Notes

The authors declare no competing financial interest.

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