

Article

Development of Polydopamine–Chitosan-Modified Electrochemical Immunosensor for Sensitive Detection of 7,12-Dimethylbenzo[a]anthracene in Seawater

Huili Hao ¹ , Chengjun Qiu ^{1,2,*}, Wei Qu ^{3,*} , Yuan Zhuang ¹, Zizi Zhao ¹ , Haozheng Liu ¹, Wenhao Wang ¹, Jiahua Su ¹  and Wei Tao ¹ 

¹ College of Mechanical and Marine Engineering, Beibu Gulf University, Qinzhou 535011, China; haohuili0022@163.com (H.H.); zhuangyuan@bbgu.edu.cn (Y.Z.); sissyzizi@163.com (Z.Z.); 19538351206@163.com (H.L.); 17629837306@163.com (W.W.); lavaloon@163.com (J.S.); 13332493888@163.com (W.T.)

² College of Computer Science, Shandong Xiehe University, Jinan 250109, China

³ College of Electronic and Information Engineering, Beibu Gulf University, Qinzhou 535011, China

* Correspondence: qiuchengjun@bbgu.edu.cn (C.Q.); quwei@bbgu.edu.cn (W.Q.)

Abstract

7,12-Dimethylbenzo[a]anthracene (DMBA-7,12), a highly toxic and environmentally persistent polycyclic aromatic hydrocarbon (PAH), poses significant threats to marine biodiversity and human health due to its bioaccumulation through the food chain. Conventional chromatographic methods, while achieving comparable detection limits, are hindered by the need for expensive instrumentation and prolonged analysis times, rendering them unsuitable for rapid on-site monitoring of DMBA-7,12 in marine environments. Therefore, the development of novel, efficient detection techniques is imperative. In this study, we have successfully developed an electrochemical immunosensor based on a polydopamine (PDA)–chitosan (CTs) composite interface to overcome existing technical limitations. PDA provides a robust scaffold for antibody immobilization due to its strong adhesive properties, while CTs enhances signal amplification and biocompatibility. The synergistic integration of these materials combines the high efficiency of electrochemical detection with the specificity of antigen–antibody recognition, enabling precise qualitative and quantitative analysis of the target analyte through monitoring changes in the electrochemical properties at the electrode surface. By systematically optimizing key experimental parameters, including buffer pH, probe concentration, and antibody loading, we have constructed the first electrochemical immunosensor for detecting DMBA-7,12 in seawater. The sensor achieved a detection limit as low as 0.42 ng/mL. In spiked seawater samples, the recovery rates ranged from 95.53% to 99.44%, with relative standard deviations (RSDs) $\leq 4.6\%$, demonstrating excellent accuracy and reliability. This innovative approach offers a cost-effective and efficient solution for the in situ rapid monitoring of trace carcinogens in marine environments, potentially advancing the field of marine pollutant detection technologies.

Keywords: polydopamine; chitosan; 7,12-Dimethylbenzo[a]anthracene; electrochemical immunosensor; carcinogens; marine environment



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1. Introduction

The ocean plays a pivotal role in Earth's life support system, maintaining global climate balance through its substantial carbon sequestration capabilities [1]. It also supports rich

biodiversity, serving as a crucial ecological barrier for coastal regions [2]. Furthermore, the ocean accommodates extensive human activities, underpinning the development of the marine economy and fulfilling diverse societal needs [3]. However, the surge in maritime trade and energy exploitation has led to unprecedented pollution challenges [4].

Polycyclic aromatic hydrocarbons (PAHs) are highly toxic [5], bioaccumulative [6], and environmentally persistent [7] organic pollutants. They primarily originate from oil spills [8], incomplete combustion of fossil fuels [9], and industrial emissions [10], posing significant threats to global marine ecosystems. Among them, 7,12-dimethylbenzo[a]anthracene (DMBA-7,12), an alkylated PAH, exhibits high hydrophobicity [11] and chemical stability [12]. In the marine environment, it readily adsorbs to sediments and accumulates through the food chain [13]. The International Agency for Research on Cancer (IARC) has classified DMBA-7,12 as a potential carcinogen based on animal experimental evidence [14]. Long-term exposure to this substance increases the risk of malignant tumors in humans, such as breast cancer and skin cancer [15]. DMBA-7,12 induces CYP1A1 enzyme activity through the aryl hydrocarbon receptor pathway, leading to the formation of DNA adducts and chromosomal aberrations [16]. This not only threatens the genetic diversity of marine organisms but also enters the human body through the food chain. Furthermore, the persistent presence of DMBA-7,12 in sediments disrupts benthic ecosystem functions [17], affecting key ecological processes such as the marine carbon cycle, thereby posing a dual threat to both the environment and human health. Therefore, developing green, precise, and efficient detection technologies for DMBA-7,12 is of significant practical importance for safeguarding both marine ecological environments and human health.

Currently, research on DMBA-7,12 detection is limited, with chromatography being the primary method. H.-P. Nirmaier et al. [18] utilized high-performance liquid chromatography (HPLC) with amperometric detection (AD) to identify six PAHs, including DMBA-7,12, listed in the German drinking water standards, achieving detection limits between 1.01 ng/mL and 12.45 ng/mL. Amperometric detection demonstrated 5 to 10 times higher sensitivity compared to UV detection techniques. V. Pino et al. [19] employed the nonionic surfactant polyoxyethylene-10-lauryl ether to extract 14 PAHs, including DMBA-7,12, from seawater samples. Through concentration, HPLC separation, and fluorescence detection with wavelength programming, they achieved the detection of 14 PAHs, with detection limits ranging from 0.1 ng/mL to 0.15 ng/mL. Meng Qiao et al. [20] developed a method based on gas chromatography (GC) for the simultaneous detection of 16 PAHs, including DMBA-7,12, in complex matrices, with detection limits ranging from 2×10^{-5} ng/mL to 7.4×10^{-3} ng/mL. Chromatographic methods exhibit good selectivity and high analytical precision in practical applications [21]. However, the separation process in chromatographic columns is time-consuming [22]. To achieve optimal separation, strict requirements exist for conditions such as the column type [23], composition of the mobile phase, and flow rate [24], resulting in relatively long analysis times for individual samples. Due to the high demands of the detection instruments on the samples, they must undergo complex pretreatment processes to avoid contamination of electrodes and clogging of chromatographic columns, which significantly increases the workload and time costs of the analysis [25]. Moreover, HPLC instruments are expensive and require professional knowledge for operation and maintenance.

Electrochemical immunoassays combine the specificity [26] of immunoreactions with the high sensitivity of electrochemical detection [27]. This technique involves immobilizing antibodies on the electrode surface, where specific binding with target antigens induces changes in electrochemical properties. These changes are detected to qualitatively [28] or quantitatively [29] analyze the target substance. Due to their high specificity, operational convenience, and ability to detect multiple targets simultaneously, electrochemical

immunoassays are widely used in clinical diagnostics [30], environmental monitoring [31], and food safety testing [32].

In this study, polydopamine (PDA) was innovatively employed as the scaffold for Anti-DMBA-7,12. PDA's strong adhesive properties [33] transform inert electrodes into functional ones rich in various functional groups, enhancing the adsorption of electroactive species [34] and increasing the number of substances participating in electrochemical reactions [35]. Chitosan (CTs) is introduced as a signal amplification material. The amino groups on CTs covalently and electrostatically bond with PDA's functional groups [36], constructing a sensing interface that promotes electron transfer and accelerates redox reactions. In this study, a redox probe composed of potassium ferricyanide ($K_3Fe(CN)_6$) and potassium ferrocyanide ($K_4Fe(CN)_6$) was employed. Owing to its reversible single-electron transfer reaction, the resulting current signal variations can intuitively reflect changes in electron transfer efficiency caused by immunoreactions on the electrode surface. This provides a stable electrochemical baseline for the quantitative detection of the target analyte, making it highly suitable for precise analytical applications. Targeting DMBA-7,12 as the analyte, Anti-DMBA-7,12 is immobilized on the PDA-CTs composite interface, efficiently capturing DMBA-7,12 from seawater with the aid of a coupling agent. Using cyclic voltammetry (CV) and differential pulse voltammetry (DPV), an electrochemical immunosensor for DMBA-7,12 detection in seawater is successfully developed.

2. Materials and Methods

2.1. Reagents

Polydopamine (PDA) nanoparticles (particle size: 100 nm) were purchased from Xi'an Rui Xi Biotechnology Co., Ltd. (Xi'an, China). Chitosan (CTs) (high viscosity, >400 mPa·s) was obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Potassium hexacyanoferrate ($K_3Fe(CN)_6$; $\geq 99.95\%$ metals basis) and phosphate-buffered saline (PBS; containing 0.01 mol KCl) were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA) was obtained from the Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences (Chengdu, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC; 99%) and N-Hydroxysuccinimide (NHS; 99%) were purchased from Beijing Bailingwei Technology Co., Ltd. (Beijing, China). The 7,12-Dimethylbenz[a]anthracene antibody (Anti-DMBA-7,12) was purchased from Santa Cruz Biotechnology. (Shanghai, China). 7,12-Dimethylbenz[a]anthracene (DMBA-7,12; 98.5%) was obtained from Beijing Bailigwei Technology Co., Ltd. (Beijing, China).

2.2. Instruments

The CHI660E electrochemical analyzer (Shanghai Chenhua Instrument Company, Shanghai, China); Quattro S scanning electron microscope (Thermo Fisher Scientific, Guangzhou, China); LC-101-0B electric blast drying oven, FA124 external calibration electronic balance, LC-LX-H165A high-speed centrifuge, DF-101S magnetic stirrer, and LC-LX-H165A ultrasonic cleaner (Shanghai Lichen Instrument Technology Co., Ltd., Shanghai, China); and PLUSE2-5TH ultrapure water system (Nanjing EPU Technology Co., Ltd., Nanjing, China) were used in this study.

2.3. Procedures

2.3.1. Solution Preparation

Based on preliminary experiments and data analysis, it was determined that the optimal experimental results are achieved when the mass ratio of polydopamine (PDA) to chitosan (CTs) is 2:3. Therefore, subsequent experiments will strictly adhere to this

ratio for solution preparation to ensure consistency of experimental conditions and reliability of the results. Dissolve 0.02 g of PDA nanoparticles and 0.03 g of CTs powder separately in 25 mL of a 1% acetic acid solution, and then sonicate until thoroughly mixed to obtain a 0.8 mg/mL PDA acetic acid solution (PDA-Ac solution) and a 1.2 mg/mL chitosan acetic acid solution (CTs-Ac solution). Dissolve 1.5 g of bovine serum albumin (BSA) powder in 50 mL of ultrapure water to obtain a 3% (*w/v*) BSA solution. Dissolve 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (99%) and N-hydroxysuccinimide (NHS) in ultrapure water at a mass ratio of 1:1 to obtain an EDC/NHS solution. Mix a 0.5 mol/L $[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ solution with a 0.5 mol/L PBS buffer (pH = 7) in a 1:1 volume ratio to obtain $[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ -PBS buffer electrolyte ($[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ -PBS BE). Store all prepared solutions sealed at 4–6 °C.

2.3.2. Sensor Construction

Polish the glassy carbon electrode (GCE) with Al_2O_3 powder of different particle sizes (1.0, 0.3, and 0.5 μm) until the surface is clean. Rinse the electrode surface with absolute ethanol and ultrapure water, then dry it at room temperature. Place the dried GCE in 0.5 mol/L H_2SO_4 and perform several cyclic voltammetry scans until the cyclic voltammogram stabilizes. After activation, rinse the electrode with ultrapure water and dry it at room temperature for use.

Next, apply 5 μL of PDA-Ac solution dropwise onto the GCE surface and dry it in a 25 °C air-drying oven until completely dry. Then, drop 5 μL of the EDC/NHS solution onto the surface and leave it at 4–6 °C for 1.5 h to activate the carboxyl groups, facilitating efficient coupling reactions. Apply 5 μL of CTs-Ac solution dropwise onto the surface and dry it at 25 °C to obtain PDA/CTs/GCE.

Apply 5 μL of Anti-DMBA-7,12 dropwise onto the PDA/CTs/GCE and then incubate it in a 25 °C air-drying oven for 1.5 h. Subsequently, apply 5 μL of BSA solution dropwise onto the dried electrode surface and incubate it at room temperature for 0.5 h to stabilize the Anti-DMBA-7,12 activity and block nonspecific binding sites on the electrode surface. The final obtained immunosensor is PDA/CTs/Anti-DMBA-7,12/BSA/GCE.

2.3.3. Immunoassay

The immunoassay process is based on a three-electrode system (working electrode: 3 mm diameter glassy carbon electrode; auxiliary electrode: platinum wire; reference electrode: silver chloride electrode). The buffer electrolyte used in the experiment is $[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ -PBS BE.

The PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor was used as the working electrode for CV and electrochemical impedance spectroscopy (EIS) to characterize the electrochemical performance of the prepared immunosensor. DPV was used for the detection of DMBA-7,12 at different concentration gradients. In the detection system, $[\text{Fe}(\text{CN})_6]^{3-}$ and $[\text{Fe}(\text{CN})_6]^{4-}$ serve as a redox probe pair, and they undergo a reversible one-electron transfer reaction. By analyzing the linear relationship between the DMBA-7,12 concentration and the corresponding peak current (I_p), the detection line of the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor was established. The performance of the sensor was then verified in actual seawater environments.

2.3.4. Seawater Samples Preparation

Maowei Sea, located in Qinzhou City, Guangxi Zhuang Autonomous Region, China, is a coastal region with a common estuarine runoff from the Qinjiang and Maolingjiang rivers. Its unique geographical location and hydrological conditions make its ecological environment highly susceptible to terrestrial pollution. In this study, four representative sampling sites (covering the estuary S1, aquaculture area S2, navigation channel S3, and a

relatively clean control area S4) were selected in Maowei Sea for the systematic collection of surface seawater samples. To facilitate the complete precipitation of impurities in the sample, the samples were left to settle for one week after collection, followed by filtration using a 0.45 μm microporous membrane for solid–liquid separation. UV irradiation (254 nm, 30 min) was then applied for microbial inactivation and organic impurity degradation, and the pH of the water samples was precisely adjusted to 7.0 ± 0.1 using NaOH solution, completing the pretreatment process.

3. Optimization and Results

3.1. Sensor Characterization

3.1.1. Morphological Characterization

The Sigma HD scanning electron microscope (SEM) provides high-resolution imaging to clearly display the microscopic morphological characteristics of the PDA, CTs, and PDA/CTs modification layers on the GCE surface at different magnifications. Figure 1 shows the micrographs of PDA, CTs, and PDA/CTs at magnifications of 20 kx and 100 kx.

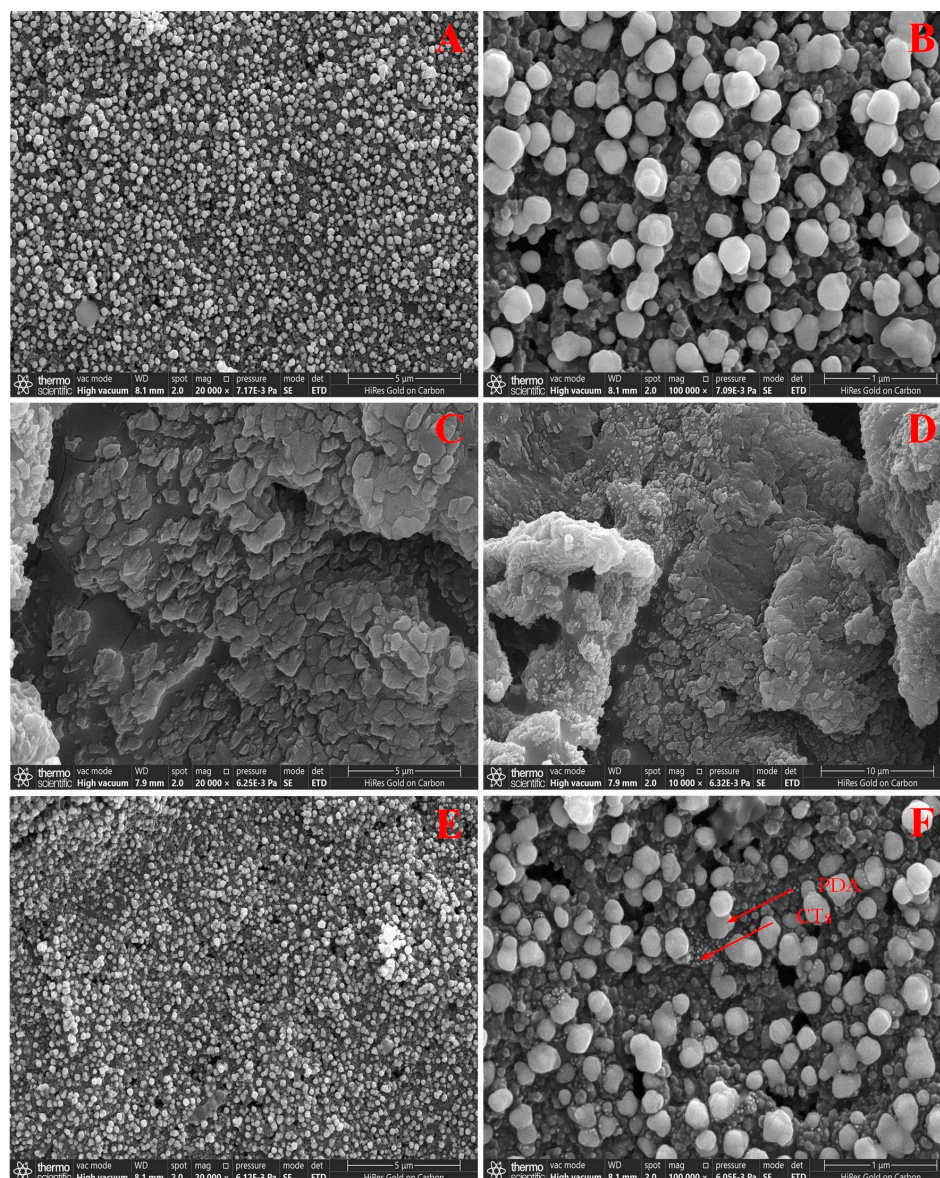


Figure 1. SEM images. 20 kx: (A) PDA; (C) CTs; (E) PDA/CTs; 100 kx: (B) PDA; (D) CTs; (F) PDA/CTs.

PDA is a polymer formed by the self-polymerization of dopamine monomers under basic conditions [37]. Figure 1A,B present the microscopic characteristics of PDA at magnifications of 20 kx and 100 kx, respectively. The surface shows a specific porous structure and a three-dimensional network system. The structure contains numerous catechol and amine groups [38], which can firmly adhere to the glassy carbon electrode surface through coordination, hydrogen bonding [39], and covalent bonding, increasing the roughness and active sites of the electrode surface, thus enhancing the adsorption capacity and electron transfer efficiency for target substances.

CTs is a linear polysaccharide formed by β -(1,4)-2-amino-2-deoxy-D-glucose units connected by glycosidic bonds [40]. Figure 1C,D display the microscopic features of CTs at 20 kx and 100 kx magnifications. It shows a specific three-dimensional lamellar structure, with subtle surface textures visible under higher magnification. The molecular chain of CTs contains numerous free amine (—NH_2) and hydroxyl (—OH) groups [41], which can covalently bond and physically adsorb with Anti-DMBA-7,12, stably fixing it on the electrode surface for the specific detection of DMBA-7,12.

Figure 1E,F present the microscopic characteristics of the PDA/CTs composite nanomaterial modification film at magnifications of 20 kx and 100 kx, respectively. At 20 kx magnification, the overall coverage of the PDA/CTs composite nanomaterial modification film on the GCE surface can be observed at a macro level. The surface exhibits a certain degree of roughness, and some localized aggregation phenomena are visible, which may result from uneven intermolecular forces during the self-assembly or preparation process of PDA and CTs. This leads to the accumulation of materials in certain regions, forming small protrusions or cluster-like structures. When the magnification is increased to 100 kx, the interwoven nanonetwork structure of PDA and CTs can be more clearly observed. The nanometer-scale pores on the composite film surface provide more channels and spaces for the adsorption and diffusion of the target substances. Additionally, the presence of nanoparticle-like or fiber-like structures on the composite film surface further increases the surface area and the number of active sites. These fine structural features significantly enhance the ability of the modification film to bind with biomolecules and improve the electrochemical performance of the electrode.

3.1.2. Performance Characterization

The electrochemical immuno-performance of different modification layers was characterized using CV and EIS in a $[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ -PBS BE environment.

CV is a technique where a linear potential scan is applied between the working electrode and the auxiliary electrode, leading to oxidation–reduction reactions at the electrode surface, allowing for detection. Figure 2A presents a comparison of the cyclic voltammograms for different modification layers. When the GCE was tested, a pair of stable and symmetrical oxidation–reduction peaks was obtained, with a peak potential difference of approximately 62.35 mV, which is about 5.68% different from the theoretical peak potential difference of 59 mV, as predicted by the Nernst equation. This value is far smaller than the conventional 20% deviation, indicating that the electrode reaction is highly reversible, and the electron transfer efficiency at the electrode surface is high, suggesting strong catalytic activity for the oxidation–reduction reaction and good reproducibility and stability of the electrode. After modification with PDA-Ac solution, the peak current significantly increased. This is because PDA has strong adhesive properties, allowing it to firmly attach to the GCE surface, transforming it from a relatively inert surface to one rich in functional groups. This promotes the adsorption of electroactive substances on the electrode surface and increases the number of materials participating in electrochemical reactions. Additionally, PDA itself has good conductivity, providing more efficient pathways for

electron transfer, thus improving the electrode's electron transfer ability [42]. After further modification with CTs-Ac solution, the peak current continued to increase, with an approximate 112.2% increase. This is due to the interaction between the amine groups on the CTs molecules and the carbonyl groups on the PDA surface, forming hydrogen bonds [43]. The more stable composite structure formed between the two not only increases the number of active sites on the electrode surface but also optimizes the charge distribution on the electrode. The molecular chains of PDA interlace with the polysaccharide chains of CTs, optimizing the electrode's pore structure, which is favorable for the diffusion of electroactive substances in the solution and increases the contact area between electroactive substances and the electrode surface. When Anti-DMBA-7,12 and BSA were sequentially drop-coated onto the PDA/CTs/GCE surface, the cyclic voltammetric peak currents decreased by 38.6% and 23.2%, respectively. This is because Anti-DMBA-7,12 is not a good conductor of electricity, and its adsorption on the electrode surface disrupts the existing conductive pathways, increasing the distance for electroactive substances to reach the electrode surface. The BSA coverage occupies more active sites, reducing the available area for electrochemical reactions. Thus, the peak current decreases after the application of these two substances, impeding the progress of the electrochemical reaction.

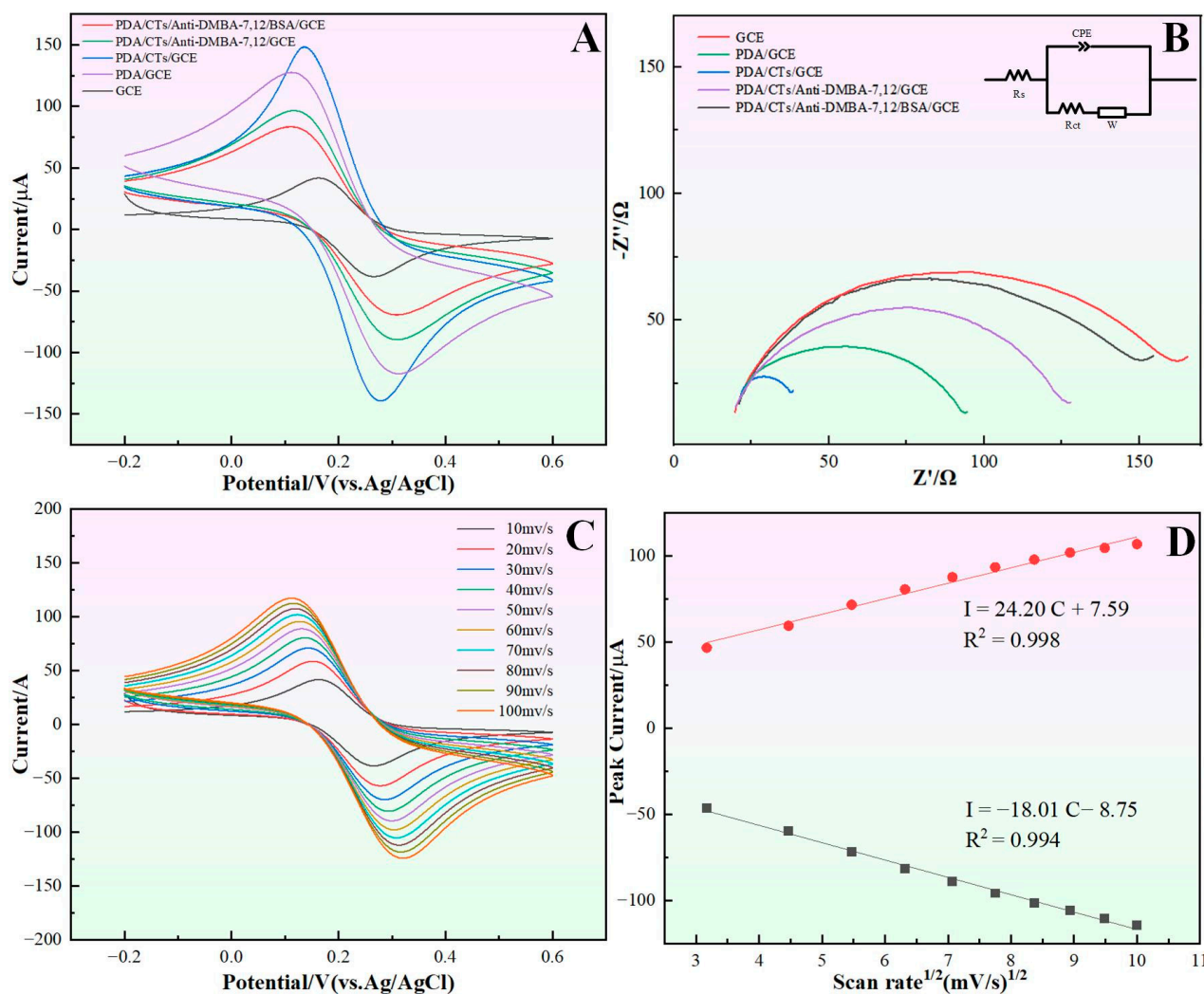


Figure 2. (A,B) are the CV curves and Nyquist curves of five different electrodes, (C,D) are the different scan rate curves and corresponding fitting curves of PDA/CTs/Anti-DMBA-7,12/BSA/GCE.

We used the Randles–Sevcik equation for surface area calculation:

$$I_p = 2.69 \times 10^5 A D^{\frac{1}{2}} n^{\frac{3}{2}} v^{\frac{1}{2}} c \quad (1)$$

where I_p is the peak current (μA), A is the effective surface area of the electrode (cm^2), D is the diffusion coefficient (cm^2/s), n is the number of electrons transferred in the electrochemical reaction, v is the potential scan rate (V/s), and c is the concentration of potassium ferrocyanide (mmol/L).

The effective areas of the GCE, PDA/GCE, and PDA/CTs/GCE electrodes were calculated to be 16.35 cm^2 , 43.57 cm^2 , and 54.63 cm^2 , respectively. This shows that, under the synergistic effect of PDA and CTs, the effective surface area of the electrochemical immunosensor is significantly increased.

EIS is a technique where a small-amplitude sinusoidal AC voltage signal is applied to the electrochemical system, and the impedance response of the system at different frequencies is measured [44]. EIS is typically presented in the form of a complex-plane impedance plot (Nyquist plot). The semicircular part of the Nyquist plot is generally related to the charge transfer process. The diameter of the semicircle represents the charge transfer resistance and reflects the ease or difficulty of charge transfer during electrochemical reactions at the electrode surface. A larger semicircle diameter indicates greater resistance to charge transfer. The linear part of the plot is typically associated with the diffusion process of substances and appears in the low-frequency region [45]. Figure 2B displays the Nyquist plots of electrodes with different modification layers. The inset illustrates the equivalent circuit model employed to fit the EIS data. Among them, the frequency range is 0.1 Hz–100 kHz, the amplitude is 5 mV, and there is open circuit potential. From the figure, it is clear that the Nyquist plot for the GCE has the largest semicircle radius, indicating that the GCE encounters the greatest resistance during the charge transfer process. The resistance decreases in the following order: PDA/CTs/Anti-DMBA-7,12/BSA/GCE, PDA/CTs/Anti-DMBA-7,12/GCE, and PDA/GCE. The PDA/CTs/GCE modification shows the largest semicircle diameter. To further validate the accuracy of our results, we conducted a comparative analysis of the solution resistance (R_s) and charge transfer resistance (R_{ct}) obtained from the fitted equivalent circuits, as presented in Table 1. The ranking of R_{ct} values across the five different electrodes aligns with the previously discussed trends, and the variations in R_s values also follow the anticipated patterns. These consistent observations from multiple parameters provide robust evidence supporting the reliability and accuracy of our findings. This result is consistent with the cyclic voltammetry (CV) measurements, confirming the accuracy of the results and the signal amplification effect of PDA and CTs. It further demonstrates the successful fixation of the modification layers on the electrode surface, providing a solid foundation for subsequent performance analysis and practical applications.

Table 1. Randles equivalent circuit parameters for five different electrodes.

Electrodes	R_s (Ω)	R_{ct} (Ω)
GCE	21.11 ± 1.12	149.23 ± 1.01
PDA/GCE	19.77 ± 0.95	71.38 ± 5.71
PDA/CTs/GCE	21.35 ± 1.22	21.02 ± 2.52
PDA/CTs/Anti-DMBA-7,12/GCE	19.86 ± 0.74	109.95 ± 8.12
PDA/CTs/Anti-DMBA-7,12/BSA/GCE	19.37 ± 0.83	130.34 ± 9.33

CV was conducted at a range of scan rates. Figure 2C presents the cyclic voltammograms of PDA/CTs/Anti-DMBA-7,12/BSA/GCE at scan rates ranging from 10 mV/s to 100 mV/s. As observed, the cyclic voltammograms exhibit symmetry, with a small difference between the anodic and cathodic peak potentials, ranging from approximately 50 mV to 100 mV. The peak potential shift is minimal with varying scan rates, indicating the electrode's strong reversibility. Further analysis of the peak current versus scan rate reveals a linear relationship between the peak current and the square root of the scan rate, as shown in Figure 2D. This indicates that the electrochemical process of the PDA/CTs/Anti-DMBA-7,12/BSA/GCE is primarily diffusion-controlled, demonstrating robust stability and reproducibility.

From the above analysis, it can be concluded that the PDA/CTs/Anti-DMBA-7,12/BSA/GCE exhibits excellent electrochemical performance. The strong reversibility significantly reduces energy loss during redox reactions, facilitating efficient electrochemical processes. The stability and reproducibility afforded by the diffusion-controlled process ensure a reliable detection basis for practical applications. This excellent electrochemical performance suggests that the electrode has the potential to serve as the core component of a precise and reliable seawater-based DMBA-7,12 immunosensor.

3.2. Experimental Conditions Optimization

3.2.1. Buffer pH Optimization

To investigate the effect of different buffer electrolytes on the differential pulse detection performance of the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor, six buffer electrolytes were prepared by mixing 0.5 mol/L PBS with pH values ranging from 6.0 to 8.5 with 0.5 mol/L $[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ solution at a 1:1 volume ratio. Differential pulse detection experiments were performed on the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor using these six different buffer systems, and the results are shown in Figure 3A. Standard deviation values in the range of 0.59–1.34% were obtained, as shown in the right image of Figure 3A ($n = 3$). The peak current reached its minimum value when the pH was set to 7.

From a molecular biology perspective, at pH 7, the surface-bound Anti-DMBA-7,12 and other biomolecules such as BSA are in their most stable structural and active states. Anti-DMBA-7,12 can bind to the target DMBA-7,12 in a highly specific manner with its optimal conformation, while BSA efficiently blocks non-specific binding sites, significantly reducing non-specific adsorption. This leads to the highest specificity and sensitivity for the recognition and detection of the target analyte. From an electrochemical standpoint, at neutral pH, the redox reaction rate of the $[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ couple is the fastest [46]. Under these conditions, the number of ions participating in the reaction is relatively stable and abundant, ensuring rapid attainment of electrochemical equilibrium, a stable current response, and effective control of the background current. After a comprehensive analysis, pH 7 PBS buffer was selected as the experimental buffer for this study.

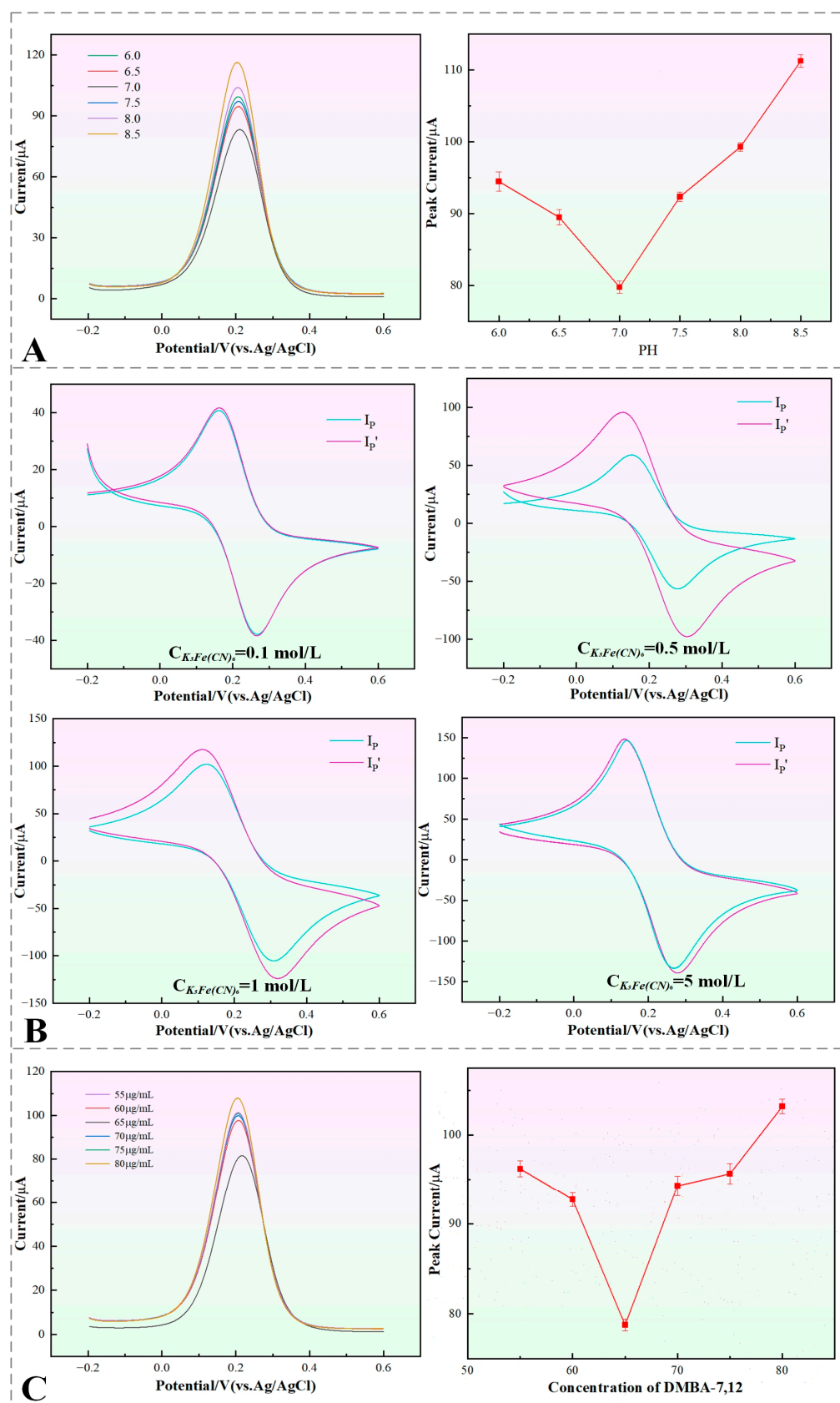


Figure 3. (A) DPV for different pH buffer solutions and the trend line for peak current variation. (B) CV curves before and after immune response at different probe concentrations. (C) DPV curves and trend line of the immunosensor using different antibody concentrations.

3.2.2. Probe Concentration Optimization

To determine the optimal probe concentration for subsequent sensor performance analysis, an investigation was conducted on the effect of different $[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ solution concentrations on the performance of the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor. Precise amounts of 10 mL of $[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ solutions with concentrations of 0.1 mol/L, 0.5 mol/L, 1 mol/L, and 5 mol/L were mixed with an equal volume of 0.5 mol/L PBS buffer solution at pH 7 to prepare four different buffer electrolytes with varying probe concentrations. These four buffer electrolytes were used to perform CV on the immunosensor both before and after immunization, and the results are shown in Figure 3B. The calculation and analysis revealed that the largest percentage changes in the oxidation and reduction peak currents (81.82% and 89.66%, respectively) occurred when using a 0.5 mol/L $[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ solution mixed with an equal volume of 0.5 mol/L PBS buffer solution (pH 7).

From the perspective of sensor operation, utilizing a 0.5 mol/L $[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ solution as the redox probe resulted in the most significant changes in peak current, indicating its superior capability to amplify electrochemical signal variations induced by antigen–antibody interactions, thereby enhancing detection sensitivity. Electrochemically, this concentration balances electron transfer efficiency and diffusion kinetics, mitigating issues such as insufficient signal at lower concentrations or elevated background interference at higher concentrations. Additionally, a 0.5 mol/L concentration offers practical advantages in terms of reagent cost and operational convenience. Based on comprehensive analysis, 0.5 mol/L $[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ was selected as the optimal probe concentration for subsequent sensor performance evaluations.

3.2.3. Antibody Concentration Optimization

To optimize the performance of the immunosensor, a systematic screening of the Anti-DMBA-7,12 concentration was carried out. A series of Anti-DMBA-7,12 solutions with concentrations ranging from 55 $\mu\text{g}/\text{mL}$ to 80 $\mu\text{g}/\text{mL}$ were prepared. A 5 μL aliquot of each Anti-DMBA-7,12 solution was drop-cast onto the surface of the PDA/CTs composite-modified layer, resulting in six immunosensors with different antibody concentrations. These immunosensors were then immersed in $[\text{Fe}(\text{CN})_6]^{3-}/^{4-}$ -PBS BE and tested using DPV, with the results shown in Figure 3C. Standard deviation values in the range of 0.68~1.13% were obtained as shown in Figure 3C's right image ($n = 3$). The analysis revealed that the immunosensor prepared with 65 $\mu\text{g}/\text{mL}$ Anti-DMBA-7,12 exhibited the lowest peak current.

From the perspective of surface chemistry and immunoreaction principles, the PDA/CTs composite-modified layer has a limited number of antibody immobilization sites. When the antibody concentration is low, the antibody molecules, due to their chemical functional groups, can effectively and uniformly bind to these immobilization sites via chemical bonding and physical adsorption mechanisms, forming a stable and ordered immunorecognition interface. However, when the antibody concentration exceeds the saturation adsorption amount, excess free antibodies in the solution cannot effectively participate in the immunosensor reaction. Instead, they accumulate near the electrode surface, forming an interference layer that hinders the electron transfer process and negatively affects the detection signal, ultimately leading to a reduction in the measured peak current. Based on the comprehensive analysis, a concentration of 65 $\mu\text{g}/\text{mL}$ Anti-DMBA-7,12 was selected as the optimal antibody concentration for fabricating the immunosensor, aiming to achieve high sensitivity and specificity in the detection of DMBA-7,12 in future practical applications.

3.3. Sensor Performance Analysis

3.3.1. Performance Detection

In the performance analysis of the immunosensor, the limit of detection (LOD) is a key indicator of its ability to detect trace amounts of the target analyte [47]. In this study, DPV was employed to detect trace amounts of DMBA-7,12. This method superimposes a small-amplitude pulse voltage (typically 50–100 mV) on the direct current (DC) scanning voltage, and measures the current difference before and after the pulse. By analyzing the difference between the Faradaic current and the charging current, along with the variations in peak current and peak potential, it allows for the quantitative analysis and characterization of the target analyte [48].

When the Anti-DMBA-7,12 fixed on the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor surface specifically binds with DMBA-7,12, an immunocomplex is formed on the electrode surface. As the concentration of DMBA-7,12 in the solution increases, more DMBA-7,12 molecules will bind to the Anti-DMBA-7,12 immobilized on the electrode surface, causing the thickness of the biological film on the electrode to increase. From an electrochemical kinetics perspective, this spatial hindrance significantly impedes the diffusion of the $[\text{Fe}(\text{CN})_6]^{3-}/4-$ electroactive species to the electrode surface, reducing their effective concentration involved in the electrode surface redox reactions. During the DPV detection process, the peak current is closely related to the amount of electroactive species participating in the redox reaction on the electrode surface and the electron transfer rate. Therefore, the peak current in DPV is negatively correlated with the concentration of DMBA-7,12 in the solution.

Based on the analysis, the limit of detection (LOD) for the electrochemical immunosensor can be derived using the following formula:

$$\text{LOD} = \frac{3SD}{K} \quad (2)$$

where SD is the standard deviation and k is the slope of the calibration curve.

In this study, the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor was used as the working electrode in a three-electrode system (with a saturated Ag/AgCl reference electrode and a platinum wire counter electrode) to measure eight sets of DMBA-7,12 standard solutions with concentrations ranging from 0.5 ng/mL to 100 ng/mL. The LOD was calculated to be 0.42 ng/mL. The calibration curve, shown in Figure 4, exhibits a good linear relationship ($R^2 = 0.991$) within the 0.5 to 100 ng/mL concentration range, indicating that the immunosensor has high sensitivity and reliability for detecting trace DMBA-7,12.

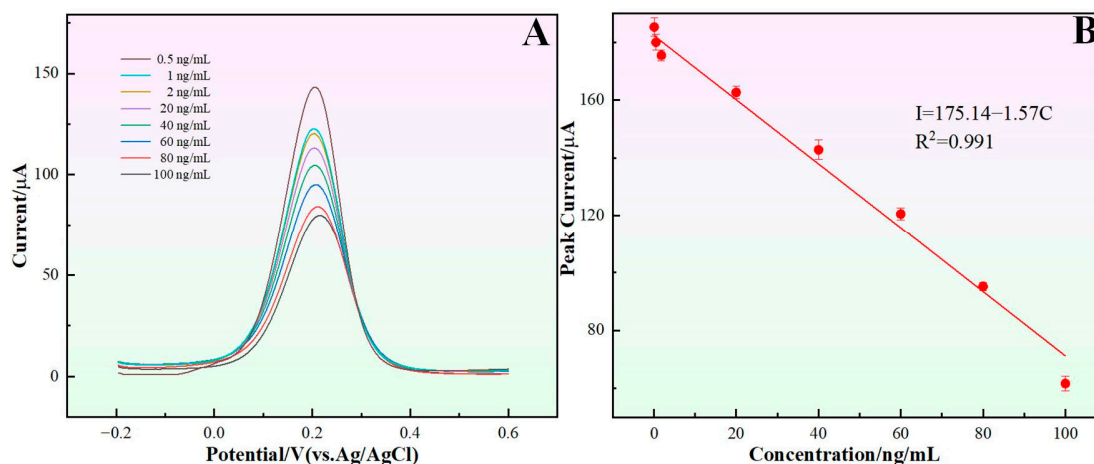


Figure 4. Differential pulse voltammetry curves (A) and the fitting curve (B) for different concentrations of DMBA-7,12.

Table 2 presents a comparative analysis of the detection limits and linear ranges for DMBA-7,12 using various analytical methods. The results indicate that high-performance liquid chromatography coupled with fluorescence detection (HPLC-FD) achieves a slightly lower detection limit compared to the electrochemical immunosensor developed in this study. This enhanced sensitivity can be attributed to the superior separation capabilities of HPLC combined with the high sensitivity of fluorescence detection, effectively minimizing interference from impurities.

Table 2. Performance comparison of various methods for detecting DMBA-7,12.

Detection Method	Linear Range (ng/mL)	LOD (ng/mL)	References
HPLC-AD	0.1–80	1.01	[18]
HPLC-FD	1.0–20	0.15	[19]
GC	0.31–29.6	0.74	[20]
PDA/CTs/Anti-DMBA-7,12/BSA/GCE	0.5–100	0.42	—

However, despite the marginally lower detection limit of HPLC-FD, the electrochemical immunosensor offers significant advantages in practical applications. HPLC-FD is often associated with high equipment costs and complex operational procedures, and it requires highly skilled personnel, leading to increased analysis time and operational expenses. In contrast, the electrochemical immunosensor is characterized by its simplicity, cost-effectiveness, and ease of operation, making it suitable for in situ and real-time monitoring. These attributes not only reduce the overall cost but also expand the applicability of the sensor in various environmental settings. Therefore, considering factors such as cost, operational convenience, and application flexibility, the electrochemical immunosensor stands out as a more practical and efficient choice for the detection of DMBA-7,12.

3.3.2. Stability and Anti-Interference Performance

To assess the stability of the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor, measurements were taken every 6 days over a period of 31 days in a 45 ng/mL DMBA-7,12 solution. After each measurement, the electrodes were dried at 25 °C and subsequently stored at 4 °C to maintain their stability and performance for future analyses. The results are shown in Figure 5A. Compared to the results from day 1, the changes in peak current on day 7, 13, 19, 25, and 31 were 0.74%, 1.46%, 2.11%, 3.54%, and 4.74%, respectively, with all changes being less than 5%. These results demonstrate that the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor exhibits excellent stability.

To verify the anti-interference performance of the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor, 10-fold concentrated interference substances, including phenanthrene (PhE), benzo[a]pyrene (BaP), benzo[a]anthracene (BaA), and naphthalene[a]pyrene (NaP), were added to the 45 ng/mL DMBA-7,12 solution. Differential pulse voltammetry was then performed to assess the interference effects. The peak current results are shown in Figure 5B. Compared to the first group without added interference substances, the percentage changes in peak current after adding the interference substances were 3.09%, 0.38%, 2.06%, and 1.14%, all of which were less than 5%. Since Anti-DMBA-7,12 specifically binds only to DMBA-7,12, this highly specific antigen–antibody interaction significantly enhances the anti-interference capability of the sensor. These results indicate that the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor exhibits excellent anti-interference performance.

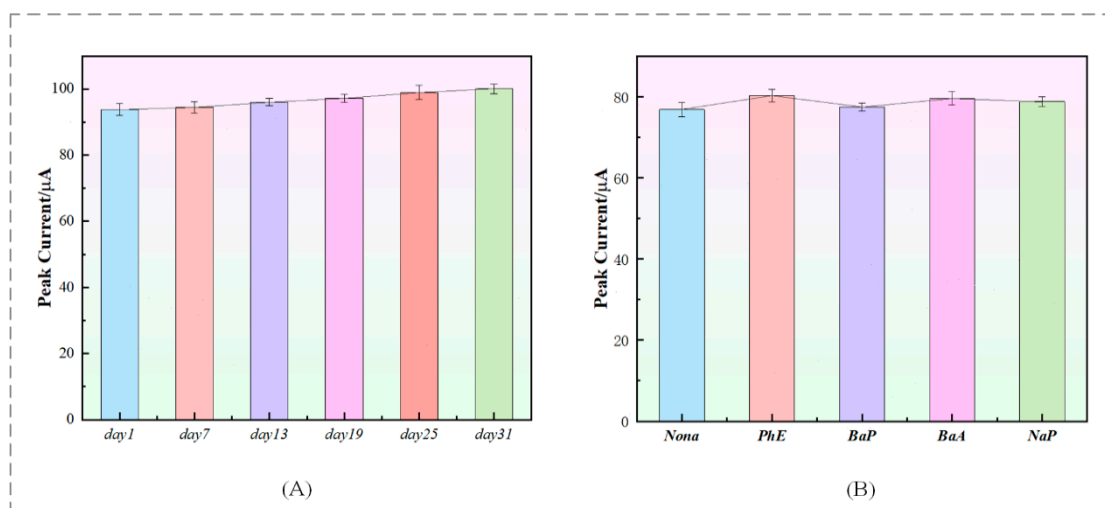


Figure 5. (A) Stability of the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor over 31 days. (B) Peak current changes in the presence of various interfering substances at a concentration of 45 ng/mL DMBA-7,12.

3.3.3. Practical Application

The PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor was used to detect the DMBA-7,12 concentrations in the pretreated seawater samples. To verify the accuracy of the detection, a multipoint calibration experiment was employed for quantitative analysis. The experimental results are presented in Table 3. The recovery rates of the spiked samples ranged from 95.53% to 99.80%, with an RSD of $\leq 4.6\%$. These results strongly demonstrate that the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor exhibits excellent detection performance in complex seawater matrices, enabling the precise determination of trace DMBA-7,12. It provides reliable technical support for on-site rapid detection of carcinogenic substances in marine environments, offering significant practical application value and promotion potential.

Table 3. Actual seawater detection analysis using the PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor.

Experiment	Added (ng/mL)	Site	Found (ng/mL)	Recovery %	RSD %
1	10	S1	9.98	99.80	0.01
		S2	9.75	97.50	0.02
		S3	9.84	98.40	0.25
		S4	9.85	98.50	0.11
2	50	S1	47.98	95.96	1.44
		S2	48.32	96.64	1.21
		S3	48.11	96.22	1.30
		S4	49.55	99.10	0.33
3	100	S1	98.15	98.15	1.71
		S2	99.44	99.44	0.94
		S3	97.02	97.02	2.32
		S4	95.53	95.53	4.60

4. Conclusions

In this study, based on the synergistic enhancement mechanism of PDA and CTs, a PDA/CTs/Anti-DMBA-7,12/BSA/GCE immunosensor was successfully developed. PDA serves as the core scaffold material for antibody immobilization, where its catechol groups

form stable chemical bonds with Anti-DMBA-7,12 via covalent bonding. Additionally, the three-dimensional network structure formed during the self-polymerization process effectively improves the antibody immobilization efficiency and interface stability. The introduction of CTs as a functional enhancement layer significantly increases the specific surface area for antibody loading due to its porous nanostructure. The quaternary ammonium cationic groups of CTs also neutralize the negative charge on the electrode surface, effectively suppressing nonspecific adsorption.

Through systematic performance characterization, the immunosensor achieved a detection limit of 0.42 ng/mL for DMBA-7,12. In actual seawater sample analysis, the spiked recovery rates ranged from 95.53% to 99.44%, with an RSD of $\leq 4.6\%$, demonstrating excellent detection accuracy and reproducibility. Compared with traditional chromatographic methods, this electrochemical immunosensor offers significant advantages in terms of detection cost, analysis time, and portability. It provides a cost-effective technological solution for the on-site rapid detection of trace polycyclic aromatic hydrocarbons in marine environments, making it a valuable tool in marine ecological monitoring.

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