

Article

Detection of Trace Fluoranthene in Marine Environments Using a PANI/Nano-Fe₃O₄-Based Immunosensor

Xiaochun Han ^{1,2}, Xuan Wang ³, Runze Liu ¹, Junjie Yin ¹, Zhiqiang Ai ¹, Ruiyuan Xue ¹, Qixue Liao ¹ and Huili Hao ^{1,2,*} 

¹ Maritime College, Beibu Gulf University, Qinzhou 535011, China

² China-ASEAN Smart Port Integrated Hub Technology Innovation College, Beibu Gulf University, Qinzhou 535011, China

³ College of Chemical Engineering, East China University of Science and Technology, Shanghai 200237, China

* Correspondence: haohuili0022@163.com

Abstract

In this study, an electrochemical immunosensor based on polyaniline/nano-Fe₃O₄ (PANI/Nano-Fe₃O₄) nanocomposite (PANI/Nano-Fe₃O₄/Anti-FLA/BSA/GCE) was developed for the highly sensitive and selective detection of trace levels of fluoranthene (FLA) in marine environments. Fluoranthene antibodies (Anti-FLA) were covalently immobilized on a glassy carbon electrode (GCE) modified with PANI/Nano-Fe₃O₄ via an EDC/NHS activation strategy, enabling specific recognition of FLA based on the antigen–antibody binding mechanism. The performance of the sensor was systematically optimized using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), linear sweep voltammetry (LSV), and differential pulse voltammetry (DPV). The results demonstrated a linear inverse relationship between peak current (*I*_p) and FLA concentration in the range of 0.5–80 ng/mL, with a regression equation of $I = -1.55C + 174.602$ ($R^2 = 0.996$). The limit of detection (LOD) was as low as 0.354 ng/mL (*S/N* = 3). In real seawater sample analysis, spiked recovery tests at three representative sites in the Maowei Sea, Guangxi, yielded recoveries of 95.44%~97.51%, with RSDs below 3%, confirming the sensor's resistance to matrix interference. The synergistic effect of the porous conductive network of PANI and the high specific surface area of Nano-Fe₃O₄ significantly amplified the electrochemical signal, while the molecular specificity of the antibody ensured targeted recognition. This sensor provides a novel and effective approach for the on-site rapid detection of polycyclic aromatic hydrocarbon (PAH) pollutants in complex marine environments, offering both high sensitivity and selectivity.

Keywords: immunosensor; polyaniline; nano-Fe₃O₄; fluoranthene; seawater detection; polycyclic aromatic hydrocarbons



Received: 30 June 2026

Revised: 17 July 2026

Accepted: 24 July 2026

Published: 3 August 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Fluoranthene (FLA), a typical four-ring polycyclic aromatic hydrocarbon (PAH), is widely distributed in the environment due to various anthropogenic activities such as industrial production, fossil fuel combustion, and transportation [1]. With the acceleration of industrialization, pollutants containing FLA have increasingly entered the marine environment through pathways such as wastewater discharge and atmospheric deposition [2]. Among these sources, emissions from ocean-going marine diesel engines fueled by heavy oil are particularly prominent and may represent a significant source of contamination in

offshore regions. Studies have shown that FLA is highly persistent in seawater and tends to bioaccumulate [3], potentially disrupting the physiological metabolism [4], reproduction, and growth of marine organisms. Moreover, FLA can be transmitted through the food chain, posing serious risks to human health. Long-term exposure has been linked to DNA damage [5], and it may cause carcinogenic, teratogenic, and mutagenic effects [6]. Therefore, developing a rapid and effective method for detecting FLA in marine environments is crucial for safeguarding both ecological balance and public health.

Since the advent of FLA detection research, the field has remained a focal point of scientific attention, primarily involving chromatographic [7–11] and spectroscopic methods [12]. As early as the 1990s, Fusheng Sun's team [13] employed reverse-phase liquid chromatography (RP-LC) coupled with ultraviolet (UV) absorption detection to simultaneously analyze 16 PAHs, including FLA, in soil samples. Under conditions of a 10 μ L injection volume and 254 nm detection wavelength, the limits of detection (LODs) for most PAHs, including FLA, ranged from 0.6 to 1.0 ng/mL, with acenaphthene (Ace) exhibiting a slightly higher LOD of 1.005 ng/mL. This pioneering work laid the foundation for the application of chromatographic techniques in FLA detection. More recently, Nathan de Souza Coelho's group [14] utilized polydimethylsiloxane (PDMS)-based in-tube extraction fibers combined with two-dimensional gas chromatography/time-of-flight mass spectrometry (GC $\times$ GC/O-TOFMS) to detect 16 PAHs, including FLA, achieving LODs between 0.5 and 1.0 ng/mL—an improvement over previous generations of techniques. In 2017, Inga Tijunelyte's team [15] developed a surface-enhanced Raman spectroscopy (SERS) nanosensor for FLA detection, achieving an LOD of 1.64 ng/mL over a concentration range of 10^{-6} to 10^{-3} g/L. In a subsequent study, Xinna Yu's group [16] focused on optimizing SERS substrates by constructing a multifunctional membrane composed of Ag-hydroxypropyl- β -cyclodextrin/reduced graphene oxide/nanocellulose (Ag-HP- β -CD/rGO/NCF), reducing the LOD of FLA to as low as 0.12 ng/mL—representing a significant advancement in detection performance. Chromatographic and spectroscopic techniques have thus established a robust foundation for trace FLA detection. In 2014, Xiangli Zheng's team [17] developed a novel electrochemical sensing platform by electrochemically polymerizing polypyrrole (PPy) and benzo[a]anthracene-7,12-dione (BaD) on a pyrolytic graphite electrode (PGE). The platform leveraged strong π - π stacking interactions between the planar-conjugated structure of BaD and the aromatic rings of benzo[k]fluoranthene (BkF), mimicking antigen-antibody specificity and spatial complementarity. This approach enabled the selective detection of BaD, achieving an LOD of approximately 0.93 ng/mL. Notably, this method addressed limitations of chromatographic and spectroscopic methods, such as high instrumentation costs and operational complexity, while offering a lower LOD. However, because the recognition mechanism relied solely on intermolecular interactions without incorporating biological recognition elements, the anti-interference capability remained limited. Despite this, the study presented a novel strategy for PAH detection.

Polyaniline (PANI), a semi-crystalline conductive polymer, provides a highly hydrophilic cavity environment that possesses excellent electrical conductivity and a well-developed porous architecture, providing a favorable microenvironment for molecular accumulation and diffusion [18]. Moreover, PANI exhibits excellent stability in aqueous dispersion systems [19], enabling the stable immobilization of nanoparticles [20] while facilitating efficient electron transfer across the electrode–electrolyte interface. Additionally, nano-sized iron oxide (Nano-Fe₃O₄) offers a large specific surface area [21], high electrical conductivity [22], and a spinel crystal structure [23], enhancing directional molecular transport and signal transduction at the sensor interface. As a nanocarrier for antibody immobilization, Nano-Fe₃O₄ provides abundant binding sites [24], enabling the ordered arrangement of surface-bound antibodies. Within the PANI/Nano-Fe₃O₄ com-

posite structure, PANI creates a favorable environment for molecular adsorption and signal release, while Nano-Fe₃O₄ offers structural support for antibody immobilization and electrode stability.

Building on the above findings, this study innovatively integrates FLA antibodies (Anti-FLA) onto a PANI/Nano-Fe₃O₄ composite substrate. By combining molecular biology, immunology, and electrochemical techniques, and utilizing the highly specific binding interaction between FLA and Anti-FLA, a highly accurate detection platform for FLA in complex marine environments was developed. The incorporation of biological recognition elements significantly enhances sensor specificity and effectively addresses the challenges of interference in environmental analysis. This approach offers a new solution for the selective and sensitive detection of environmental pollutants.

2. Instruments and Reagents

2.1. Experimental Instruments

All electrochemical experiments were performed using a CHI660E electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd., Shanghai, China). A conventional three-electrode system was employed, comprising a glassy carbon electrode (GCE, $\Phi = 3$ mm) as the working electrode, an Ag/AgCl electrode as the reference electrode, and a platinum wire as the counter electrode. Surface morphology characterization was conducted using a Quattro S scanning electron microscope (SEM, Thermo Fisher Scientific, Guangzhou, China). Ultrapure water with a resistivity of 18 M Ω ·cm was prepared using a PLUSE2-5TH ultrapure water system (EPED Technology Co., Ltd., Nanjing, China). Additional instruments, including a thermostatic drying oven, ultrasonic cleaner, and magnetic stirrer, were supplied by Shanghai Lichen Instrument Technology Co., Ltd. (Shanghai, China).

2.2. Experimental Reagents

Nano-Fe₃O₄ (99.5%, powder, 20 nm) was purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). PANI (98.0%, powder), phosphate-buffered saline (PBS), FLA (98.0%) and potassium ferricyanide (K₃Fe(CN)₆, $\geq 99.95\%$ metals basis) were obtained from Macklin Biochemical Co., Ltd. (Shanghai, China). Anti-FLA antibodies were acquired from Santa Cruz Biotechnology (Shanghai, China), and bovine serum albumin (BSA) was sourced from Chengdu Organic Chemicals Co., Ltd., Chinese Academy of Sciences. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 99%) and N-hydroxysuccinimide (NHS, 99%) were purchased from Beijing Bailingwei Technology Co., Ltd. (Beijing, China).

3. Construction of the Immunosensor

3.1. Fabrication of the PANI/Nano-Fe₃O₄ Composite Electrode

A total of 15 mg of PANI was dissolved in 10 mL of ultrapure water and magnetically stirred for 1.5 h until fully dispersed to obtain a 1.5 mg/mL PANI aqueous solution. Separately, 20 mg of Nano-Fe₃O₄ was dispersed in 10 mL of 75% anhydrous ethanol (*v/v*, ethanol:water = 3:1) and stirred magnetically for approximately 2.5 h to obtain a homogeneous suspension, resulting in a 2 mg/mL Nano-Fe₃O₄ dispersion. Both solutions were stored at 2–6 °C for later use. The GCE was polished sequentially with 0.3 μ m and 0.05 μ m alumina (Al₂O₃) powders, rinsed with ultrapure water, and dried in an oven at 25 °C. The electrode was then immersed in 0.5 M H₂SO₄ solution and subjected to 10–15 cycles of CV at a scan rate of 50 mV/s, over a potential window of −0.2 to 1.6 V. Stable CV curves (with a relative standard deviation of peak current and peak potential < 5%, and peak-to-peak separation $\Delta E_p \leq 80$ mV) indicated successful activation of the electrode

surface by homogenizing active sites [25]. The electrode was then rinsed with ultrapure water to neutral pH and dried for further use. Next, 5 μL of the PANI aqueous solution was vertically drop-cast onto the activated GCE surface and dried at 25 $^{\circ}\text{C}$ for 2 h. Subsequently, 6 μL of EDC/NHS mixed solution ($v/v = 1:1$, each at 0.1 M) was applied and dried at room temperature for 2 h to promote physical adsorption. Finally, 5 μL of the Nano- Fe_3O_4 dispersion was drop-cast and dried at 25 $^{\circ}\text{C}$ for 3 h to yield the PANI/Nano- Fe_3O_4 /GCE composite electrode. This stepwise modification strategy enabled the synergistic assembly of the porous PANI network and conductive Nano- Fe_3O_4 particles, forming a high-surface-area, high-electron-transfer-efficiency interface platform suitable for subsequent antibody immobilization.

3.2. Construction of the Immunosensor Platform

A total of 10 μL of Anti-FLA solution was drop-cast onto the surface of the PANI/Nano- Fe_3O_4 /GCE composite electrode and incubated at 2–6 $^{\circ}\text{C}$ for 2 h. Covalent immobilization of the antibody onto the electrode surface was achieved via the EDC/NHS activation mechanism. After incubation, the electrode was dried at room temperature (20–25 $^{\circ}\text{C}$) for 2 h to allow complete solvent evaporation. Subsequently, 6 μL of 3% BSA solution was applied to the electrode surface and left to incubate at 2–6 $^{\circ}\text{C}$ for 2 h to block nonspecific binding sites. The electrode was then dried at room temperature for 1 h, resulting in the fabrication of the PANI/Nano- Fe_3O_4 /Anti-FLA/BSA immunosensor. The final device was stored at 2–6 $^{\circ}\text{C}$ for future use. This protocol enables oriented antibody immobilization via covalent bonding and uses BSA blocking to prevent nonspecific adsorption, thereby ensuring high specificity of the sensor interface.

3.3. Basic Analytical Procedure for FLA Detection

The fabricated immunosensor was immersed in a PBS buffer solution (pH = 7.0) containing 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ and 0.1 M KCl. Its performance and surface modifications were characterized using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV). Additionally, the microstructure of the modified electrode was investigated using scanning electron microscopy (SEM). After systematically optimizing experimental parameters via single-factor experiments, DPV was used to record the peak current (I_p) responses to varying FLA concentrations. A quantitative calibration curve was then established based on the relationship between I_p and FLA concentration. This detection strategy is based on the specific antigen–antibody immunoreaction. Formation of the immune complex layer on the electrode surface increases interfacial resistance and layer thickness with rising FLA concentrations, which hinders electron transfer. Consequently, the peak current exhibits a negative correlation with analyte concentration.

4. Analysis and Detection

4.1. Characterization of the Immunosensor

4.1.1. Morphological Characterization of the Immunosensor

Scanning electron microscopy (SEM) enables visualization of microstructural details on sample surfaces through the detection of secondary electrons generated by interactions between a high-energy electron beam and the sample [26]. Figure 1 presents the microstructure of the composite substrate used in the immunosensor. As shown in Figure 1A, PANI exhibits a layered, porous morphology, with stacked sheets forming abundant nanoscale pores. This architecture provides a high surface area favorable for molecular adsorption and signal transduction. Figure 1B reveals that Nano- Fe_3O_4 appears as spherical particles with minor aggregation; its rigid core structure offers physical anchoring sites for anti-

body immobilization. Figure 1C shows the distinct morphology of the PANI/Nano-Fe₃O₄ composite, characterized by spherical nanoparticles embedded within the porous polymer matrix. Some Nano-Fe₃O₄ particles are tightly integrated with the PANI framework, forming continuous electron conduction pathways, while others appear to rest atop the polymer surface. This composite structure, supported by both physical interlocking and interfacial contact, significantly enhances the mechanical stability of the electrode interface. At higher magnification (100KX), the interface between the nanoparticles and polymer matrix exhibits an “anchored–embedded” configuration, with rough nanoparticle surfaces providing abundant sites for subsequent covalent antibody conjugation.

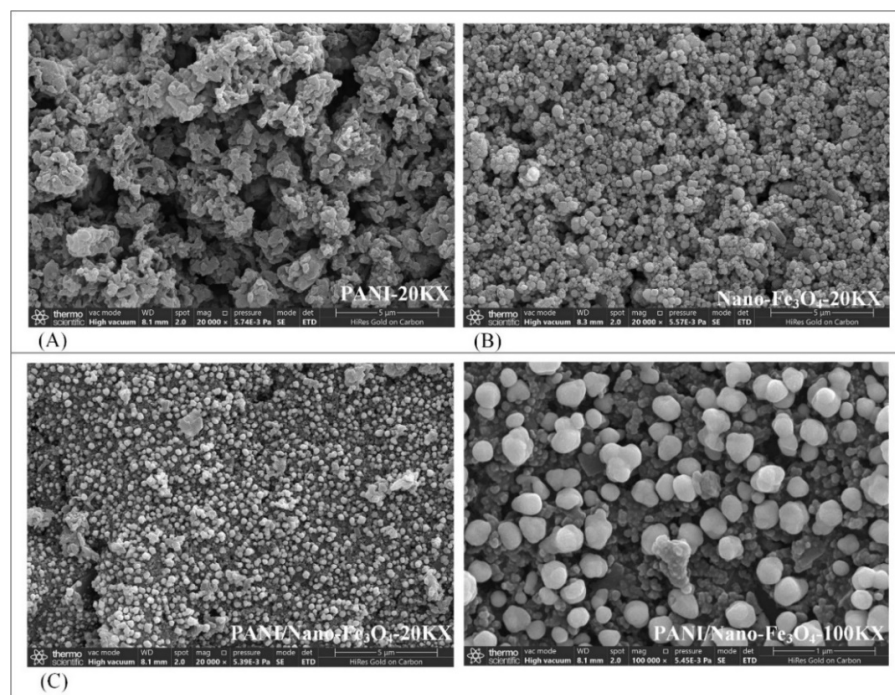


Figure 1. Microstructural morphology of the immunosensor composite substrate.

4.1.2. Electrochemical Characterization of the Immunosensor

EIS is a technique that applies alternating current signals at different frequencies to evaluate the interfacial impedance characteristics between the electrode and the electrolyte. This method allows for the investigation of electrode kinetics, interfacial structure, and diffusion behavior [27]. In this study, EIS was employed to analyze the charge transfer resistance (R_{ct}) of various electrodes in PBS buffer (pH = 7.0) containing 5 mM K₃Fe(CN)₆ and 0.1 M KCl. Figure 2A displays the Nyquist plots for five electrode types: GCE, PANI/GCE, PANI/Nano-Fe₃O₄/GCE, PANI/Nano-Fe₃O₄/Anti-FLA/GCE, and PANI/Nano-Fe₃O₄/Anti-FLA/BSA/GCE. All electrodes exhibit a semicircular region in the high-frequency domain and a linear region in the low-frequency domain. The diameters of the semicircles in the high-frequency region decrease in the following order: GCE > PANI/Nano-Fe₃O₄/Anti-FLA/BSA/GCE > PANI/Nano-Fe₃O₄/Anti-FLA/GCE > PANI/GCE > PANI/Nano-Fe₃O₄/GCE. This trend is attributed to the long-range π -conjugated system formed by alternating benzenoid and quinoid rings within PANI molecules, which grants intrinsic conductivity and facilitates efficient intramolecular electron transport. The embedding of Nano-Fe₃O₄ into the porous PANI matrix results in the formation of an interpenetrating PANI–Nano-Fe₃O₄ conductive network. This network enhances charge transfer by both physical reinforcement and interfacial electronic coupling. In contrast, Anti-FLA and BSA, being insulating proteins, form a resistive layer upon adsorption on the electrode surface, thereby reducing the electrode’s overall conductivity.

These results confirm the successful modification of the electrode with functional materials and demonstrate how their intrinsic properties influence the electrochemical behavior via interfacial interactions. The experimental impedance data were fitted using the Randles equivalent circuit (inset of Figure 2A), which includes the following components: R_{ct} (charge transfer resistance), R_s (solution resistance), Z_w (Warburg impedance), and Q (constant phase element of the electric double layer) [28]. The extracted R_{ct} values were: GCE (158.32 Ω), PANI/Nano- Fe_3O_4 /Anti-FLA/BSA/GCE (135.14 Ω), PANI/Nano- Fe_3O_4 /Anti-FLA/GCE (83.66 Ω), PANI/GCE (70.37 Ω), and PANI/Nano- Fe_3O_4 /GCE (25.82 Ω). This trend is consistent with the changes in semicircle diameter observed in the Nyquist plots. The relatively higher R_{ct} value observed for the GCE can be attributed to the reuse of the electrode following polishing with Al_2O_3 . Although the electrode was thoroughly rinsed and ultrasonically cleaned prior to reuse, trace amounts of residual alumina particles or adsorbed impurities may have remained on the electrode surface, thereby slightly increasing the interfacial charge-transfer resistance. Nevertheless, the primary focus is not the absolute R_{ct} value of the bare electrode, but rather the pronounced decrease in R_{ct} after surface modification. Therefore, the relative change in R_{ct} , rather than its absolute value, provides the more meaningful indicator of the successful electrode modification. Reinforcing the assertion that the synergistic effects of PANI's conjugated structure and Nano- Fe_3O_4 's conductive framework significantly reduce charge transfer resistance, while the insulating characteristics of Anti-FLA and BSA increase it. These findings further validate the functional role of surface modifications in tailoring the electrode's electrochemical response.

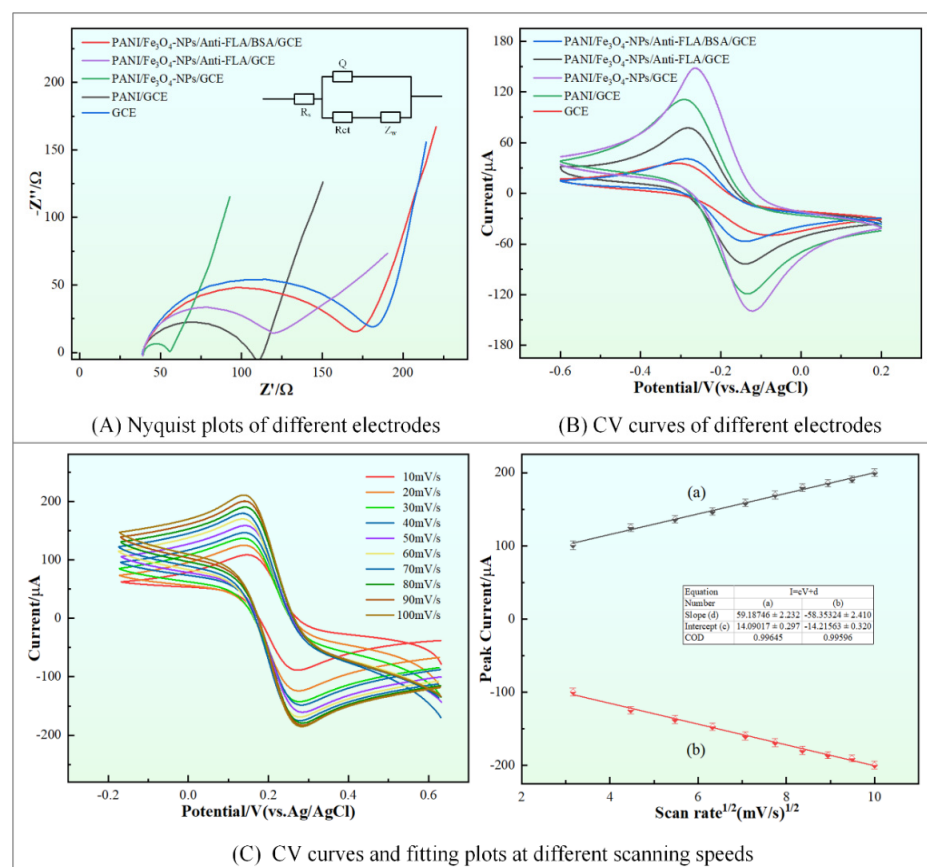


Figure 2. Electrochemical characterization of electrode interfaces: (A) Nyquist plots of different modified electrodes; (B) cyclic voltammetry curves; (C) CV curves at different scan rates and the corresponding fitting plots of peak current versus square root of scan rate.

CV is an electrochemical method wherein a linearly varying voltage is applied between a working and reference electrode, and the resulting current is recorded to study the kinetics of redox reactions, adsorption behavior, and interfacial electron transfer processes [29]. To verify the accuracy of EIS results, CV scans were performed on the five aforementioned electrodes in PBS (pH = 7.0) containing 5 mM $K_3Fe(CN)_6$ and 0.1 M KCl. Figure 2B presents the corresponding CV curves. The oxidation peak current (I_{pa}), reduction peak current (I_{pc}), and the average absolute peak current ($|I_p|$) for each electrode are summarized in Table 1. These current responses clearly indicate differences in electrochemical activity among the electrodes and corroborate the R_{ct} trends observed via EIS, thereby confirming the modulation of interfacial electron transport by the functional modification materials. The synergistic effect of the PANI/Nano- Fe_3O_4 interpenetrating network enhances conductivity, while the presence of Anti-FLA and BSA increases resistance due to their insulating properties. The agreement between CV and EIS data strongly supports the validity and reproducibility of the electrode modification strategy.

Table 1. Summary of Peak Currents from CV Curves of Different Electrodes.

Electrode	$I_{pa}/\mu A$	$I_{pc}/\mu A$	$ I_p /\mu A$
GCE	42.46	−41.81	42.14
PANI/Nano- Fe_3O_4 /Anti-FLA/BSA/GCE	52.27	−53.31	52.79
PANI/Nano- Fe_3O_4 /Anti-FLA/GCE	87.78	−88.39	88.09
PANI/GCE	118.87	−119.08	118.98
PANI/Nano- Fe_3O_4 /GCE	166.32	−165.85	166.09

The Randles–Sevcik equation [30] was employed to quantitatively estimate the effective electroactive surface area (A) of the electrodes, based on the linear relationship between peak current and the square root of the scan rate in diffusion-controlled processes:

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

where I_p is the peak current (μA , using the average of $|I_{pa}|$ and $|I_{pc}|$), A is the effective surface area (cm^2), D is the diffusion coefficient (cm^2/s , taken as $7.6 \times 10^{-6} cm^2/s$), n is the number of electrons transferred ($n = 1$), v is the scan rate (V/s , $v = 0.05 V/s$), and c is the concentration of $K_3Fe(CN)_6$ (mM, $c = 5 mM$).

The calculated effective areas were: GCE ($0.07 cm^2$), PANI/GCE ($0.09 cm^2$), and PANI/Nano- Fe_3O_4 /GCE ($0.12 cm^2$). The effective area of PANI/Nano- Fe_3O_4 /GCE is four times that of the bare GCE, indicating that the synergistic combination of PANI and Nano- Fe_3O_4 significantly expands the electroactive surface. This enhancement arises from the formation of an interpenetrating conductive network, in which Nano- Fe_3O_4 particles are embedded within the porous PANI matrix. This configuration not only constructs continuous electron pathways but also increases surface roughness and the density of active binding sites, thereby improving the adsorption of $K_3Fe(CN)_6$ and boosting current response. This quantitative analysis further supports the proposed enhancement mechanism of the modified electrode interface from a mass transport perspective.

To investigate the kinetics and reversibility of the redox reaction at the electrode interface, CV measurements were conducted on the PANI/Nano- Fe_3O_4 /Anti-FLA/BSA/GCE at various scan rates in PBS containing 5 mM $K_3Fe(CN)_6$ and 0.1 M KCl (pH = 7.0). As shown in Figure 2C, both I_{pa} and I_{pc} increase linearly with the square root of the scan rate ($v^{1/2}$), and the corresponding linear fitting (Figure 2C, right) yields correlation coefficients $R^2 > 0.98$. At low scan rates, the peak-to-peak separation ($\Delta E_p = E_{pa} - E_{pc}$) approaches the theoretical value of 59 mV at 25 °C [31], and the peak current ratio ($|I_{pa}|/|I_{pc}|$) approximates

1, indicating ideal reversibility. With increasing scan rate, ΔE_p slightly increases due to solution resistance and polarization effects; however, the linearity between peak current and $v^{1/2}$ is maintained, confirming that the redox reaction remains primarily diffusion-controlled and reversible. Collectively, these results demonstrate that the PANI/Nano-Fe₃O₄/Anti-FLA/BSA/GCE electrode exhibits reversible redox behavior governed by interfacial diffusion kinetics, thereby providing a theoretical foundation for the sensor's interfacial reaction mechanism.

4.2. Optimization of Experimental Conditions

4.2.1. Optimization of Composite Substrate Concentrations via Orthogonal Design

To optimize the concentration ratio of PANI and Nano-Fe₃O₄ during the fabrication of the immunosensor, an orthogonal experimental design was employed. Concentrations of PANI were set at 1.3 mg/mL, 1.5 mg/mL, and 1.8 mg/mL, while Nano-Fe₃O₄ concentrations were set at 1.7 mg/mL, 2.0 mg/mL, and 2.3 mg/mL, generating a total of 9 test combinations. The experimental results are presented in Table 2. As shown in Table 2, the combination of 1.5 mg/mL PANI and 2.0 mg/mL Nano-Fe₃O₄ yielded the lowest peak current ($I_p = 103.226 \mu A$), indicating the optimal performance of the sensor at these concentrations. To further analyze the contribution of each material, the average peak currents for different concentrations of one material while fixing the other were calculated, and the results are summarized in Table 3. Range analysis revealed that variations in Nano-Fe₃O₄ concentration led to greater differences in peak current than those caused by changes in PANI concentration. This indicates that Nano-Fe₃O₄ has a more significant influence on the electrochemical response within the composite system. In conclusion, the optimal ratio of electrode-modifying materials was determined to be 1.5 mg/mL PANI and 2.0 mg/mL Nano-Fe₃O₄. This combination synergistically enhances interfacial electron transfer efficiency and the exposure of active sites, thereby improving the sensor's overall performance.

Table 2. Orthogonal Experimental Results for PANI and Nano-Fe₃O₄ Concentration Combinations.

Experiments	PANI (mg/mL)	Nano-Fe ₃ O ₄ (mg/mL)	Peak Current (μA)
Experiment 1	1.3	1.7	107.532
Experiment 2	1.3	2.0	114.885
Experiment 3	1.3	2.3	114.053
Experiment 4	1.5	1.7	108.259
Experiment 5	1.5	2.0	103.226
Experiment 6	1.5	2.3	116.689
Experiment 7	1.8	1.7	114.247
Experiment 8	1.8	2.0	110.637
Experiment 9	1.8	2.3	105.633

Table 3. Average Peak Current Under Varying Single Concentrations.

Average	PANI (mg/mL)	Average Peak Current (μA)	Nano-Fe ₃ O ₄ (mg/mL)	Average Peak Current (μA)
Average 1	1.3	112.157	1.7	110.346
Average 2	1.5	109.725	2.0	109.582
Average 3	1.8	110.172	2.3	112.125
Range	—	2.432	—	2.543

4.2.2. Optimization of Buffer pH

The pH of the buffer solution is a critical parameter influencing immunosensor performance. It affects the charge distribution on the antibody surface, the antigen–antibody binding efficiency, and the electron transfer pathways at the interface, thereby significantly impacting detection sensitivity. In this study, a PBS solution containing 5 mM $K_3Fe(CN)_6$ and 0.1 M KCl was used as the electrolyte, and a pH gradient from 5.5 to 8.0 (in 0.5-unit increments) was established. The DPV I_p was monitored under each condition, as shown in Figure 3A.

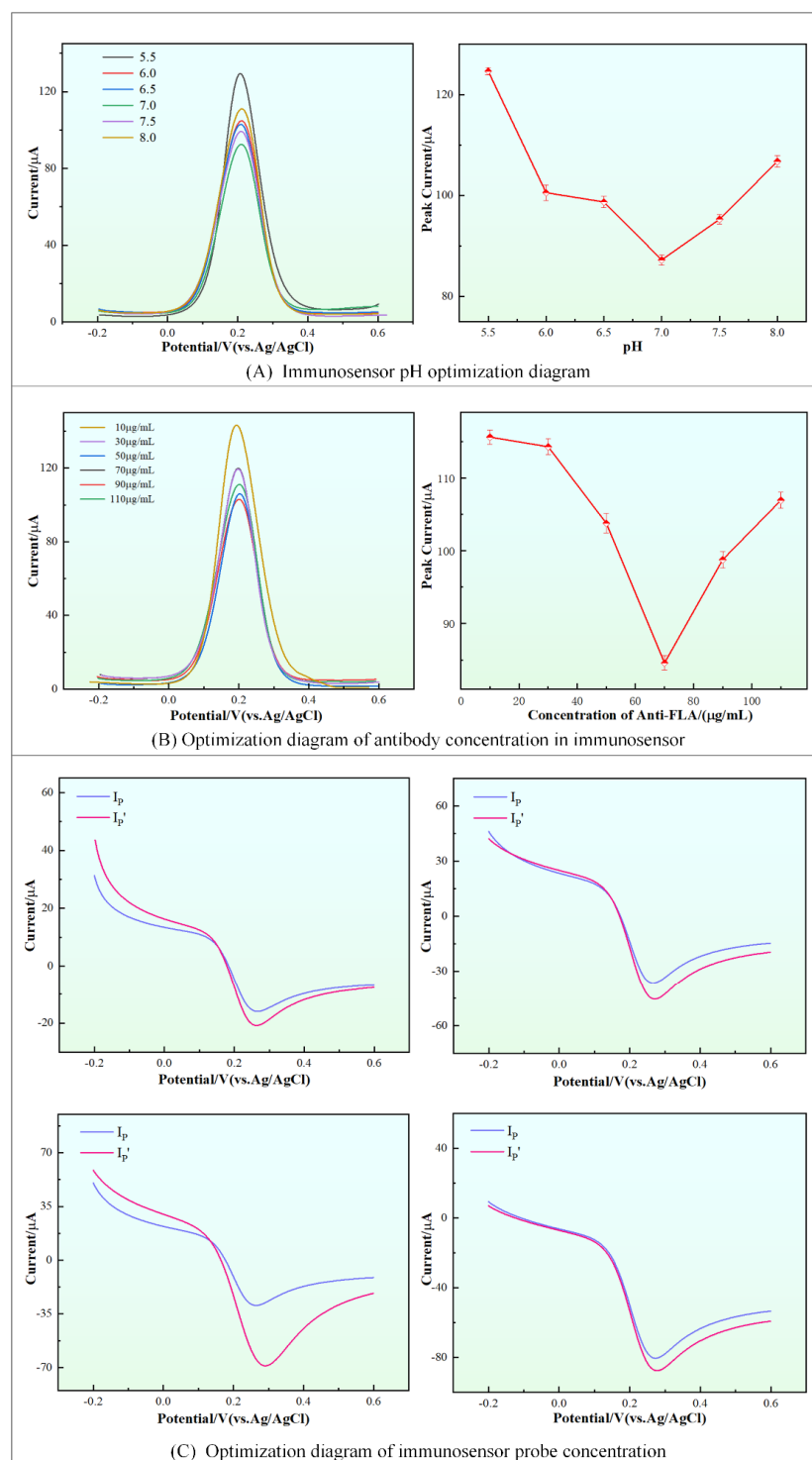


Figure 3. Multifactorial optimization of the immunosensor.

In the acidic region ($\text{pH} < 7$), increasing proton activity enhances the protonation of amine ($-\text{NH}_2$) groups on the antibody surface, forming positively charged $-\text{NH}_3^+$ moieties [32]. These positively charged groups experience electrostatic repulsion from the PANI/Nano- Fe_3O_4 composite membrane, which is also positively charged due to protonated amine groups in PANI. This repulsion reduces the directional immobilization efficiency of antibodies and introduces spatial hindrance at antigen-binding sites, significantly weakening the specific recognition between antibody and FLA. From an electrochemical perspective, this leads to decreased deposition of the immune complex on the electrode interface, reducing the coverage density of the resistive biolayer and lowering the resistance along the electron transfer pathway. As a result, the peak current (I_p) increases with decreasing pH. In the alkaline region ($\text{pH} > 7$), carboxyl ($-\text{COOH}$) groups on the antibody surface deprotonate to form negatively charged $-\text{COO}^-$ groups. Concurrently, the deprotonation of PANI's amine groups reduces their positive charge [33], and the hydroxyl groups on the surface of Nano- Fe_3O_4 (isoelectric point $\approx \text{pH}$ 6.8) deprotonate into $-\text{O}^-$, collectively increasing the negative surface charge density of the composite membrane. Due to like-charge repulsion, the adsorption efficiency of the antibody onto the electrode is further reduced. Electrochemically, this results in a decreased deposition of immune complexes on the electrode interface, reducing the coverage of the resistive biolayer and facilitating electron transfer. Consequently, I_p increases as pH rises. This trend forms a “U-shaped” response curve across the pH spectrum, confirming that at $\text{pH} = 7.0$, the antibody–antigen binding efficiency is maximized, resulting in the highest interfacial resistance. Therefore, the sensor's recognition capability reaches its peak at this pH, providing a critical basis for optimizing immunosensor performance.

4.2.3. Optimization of Antibody Concentration

Antibody concentration is a critical parameter that governs both the specific recognition efficiency and signal response intensity of immunosensors. By dynamically modulating the density of effective binding sites and the degree of steric hindrance at the electrode interface, antibody concentration significantly influences detection sensitivity. In this study, under the optimized buffer condition ($\text{pH} = 7.0$), Anti-FLA was applied to the electrode surface using a drop-casting method at concentrations of 10, 30, 50, 70, 90, and 110 $\mu\text{g}/\text{mL}$. Differential pulse voltammetry (DPV) was employed to monitor the peak current (I_p), and the results are presented in Figure 3B.

In the concentration range below 70 $\mu\text{g}/\text{mL}$, the antibody coverage on the electrode surface was insufficient, resulting in a limited number of active capture sites per unit area. Consequently, the amount of FLA bound increased proportionally with the antibody concentration. Simultaneously, the deposition of immune complexes at the interface led to the gradual formation of a resistive biolayer, which hindered the diffusion of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple and manifested as a decrease in I_p . When the Anti-FLA concentration reached 70 $\mu\text{g}/\text{mL}$, I_p dropped to a minimum value of 84.32 μA . At this point, a uniform monolayer of antibody molecules formed a dense and well-organized immunorecognition layer on the electrode surface, maximizing interfacial charge-transfer resistance and specific recognition efficiency. However, when the antibody concentration exceeded 70 $\mu\text{g}/\text{mL}$, increased electrostatic repulsion and steric hindrance among antibody molecules disrupted the ordered monolayer, leading to the formation of a disordered, multilayered structure. This non-ideal adsorption increased nonspecific binding sites and physically blocked some effective binding regions, ultimately reducing the actual binding efficiency between antigen and antibody. Electrochemically, the porous structure of the multilayer provided more diffusion pathways for the redox probe [34], decreasing the electron-transfer resistance and causing I_p to increase again. Therefore, antibody concentra-

tion affects sensor performance via a dual mechanism of “binding site saturation–steric hindrance,” and both under- and over-saturation can impair detection efficiency. A concentration of 70 $\mu\text{g/mL}$ was determined to be optimal, providing a well-defined interfacial modification parameter for constructing the immunosensor and laying a solid foundation for accurate quantification of FLA in practical samples. Based on both the I_p response curve and surface adsorption behavior, 70 $\mu\text{g/mL}$ was established as the optimal Anti-FLA concentration.

4.2.4. Optimization of Redox Probe Concentration

The concentration of the redox probe is another key factor that influences the electrochemical signal intensity of immunosensors. It affects both electron transfer efficiency and the diffusion behavior of electroactive species at the electrode interface. Under the optimized conditions (buffer pH = 7.0 and Anti-FLA concentration of 70 $\mu\text{g/mL}$), LSV was employed to systematically investigate the effect of different $\text{K}_3\text{Fe}(\text{CN})_6$ concentrations (0.1, 1, 5, and 10 mM) on the sensor's response, with particular focus on the change in I_p before and after immunoreaction. The results are shown in Figure 3C.

The data indicate that the maximum I_p difference ($\Delta I_p = 39.02 \mu\text{A}$) was achieved at a $\text{K}_3\text{Fe}(\text{CN})_6$ concentration of 5 mM, significantly higher than those observed at 0.1 mM (2.18 μA), 1 mM (11.81 μA), and 10 mM (10.02 μA). This result is attributed to the following: at low concentrations ($\leq 1 \text{ mM}$), the limited diffusion flux of probe molecules restricts the number of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ species involved in electron transfer, resulting in reduced signal amplitude. Conversely, at high concentrations ($\geq 10 \text{ mM}$), increased electrostatic repulsion among probe molecules and nonspecific interactions with immune complexes hinder efficient electron transfer. At the optimal concentration of 5 mM, the negatively charged $[\text{Fe}(\text{CN})_6]^{3-/4-}$ species effectively couple electrostatically with the positively charged groups of the PANI/Nano- Fe_3O_4 composite film at the electrode interface, significantly amplifying the change in electron-transfer resistance induced by the immunoreaction. Thus, 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ was identified as the optimal redox probe concentration, providing the most pronounced signal response and supporting stable electrochemical output in subsequent FLA detection.

4.3. Performance Evaluation of the Immunosensor

4.3.1. Analytical Performance

Under the optimized conditions (buffer pH = 7.0, Anti-FLA concentration 70 $\mu\text{g/mL}$, and probe concentration 5 mM), DPV was conducted to detect a series of FLA concentrations (0.5, 10, 20, 30, 40, 50, 60, 70, and 80 ng/mL), and the results are presented in Figure 4A. A linear regression analysis was performed to establish the quantitative relationship between FLA concentration and I_p , and the limit of detection (LOD) was calculated using the 3σ method [35]. The experimental results demonstrated a good linear response between FLA concentration and I_p in the range of 0.5–80 ng/mL, with a fitted regression equation of $I = -1.551C + 174.602$ ($R^2 = 0.996$), where C is the FLA concentration (ng/mL), and the negative slope indicates a resistive response as current decreases with increasing concentration. Based on the calibration curve slope ($k = -1.551$) and a signal-to-noise ratio (S/N) of 3, the calculated LOD was 0.354 ng/mL. These performance results highlight the advantages of the PANI/Nano- Fe_3O_4 /Anti-FLA/BSA/GCE immunosensor, including simple fabrication, rapid detection, high versatility, and excellent sensitivity with a low detection limit.

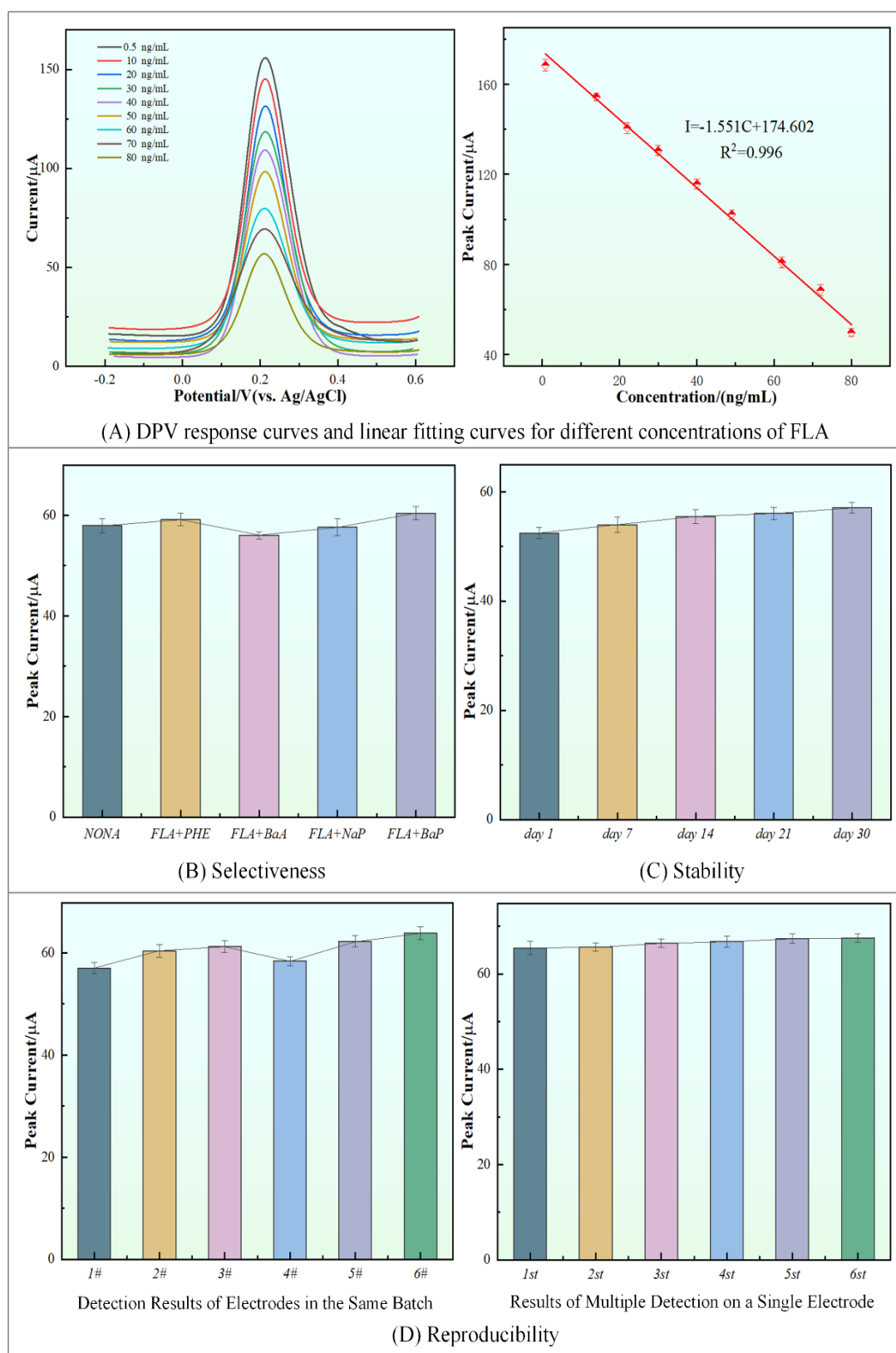


Figure 4. Comprehensive performance evaluation of the electrochemical immunosensor.

4.3.2. Selectivity, Stability, and Reproducibility

The selectivity of the immunosensor was evaluated by examining its response to structurally similar compounds. In a system containing 60 ng/mL FLA, potential interfering substances—phenanthrene (PHE), benzo[a]anthracene (BaA), naphthalene (NaP), and

benzo[a]pyrene (BaP)—were introduced at 100-fold excess concentrations. As shown in Figure 4B, the relative changes in current response were all less than 5% compared with the baseline value without interferences, indicating excellent anti-interference performance and high molecular recognition specificity of the sensor toward FLA.

The sensor's stability was assessed via long-term storage experiments. The fabricated PANI/Nano-Fe₃O₄/Anti-FLA/BSA/GCE sensors were stored at 4 °C and tested for their response to 60 ng/mL FLA on days 1, 7, 14, 21, and 30. As depicted in Figure 4C, the peak current retained over 95% of its initial value after 30 days, confirming the sensor's robust storage stability and suitability for long-term detection applications.

Reproducibility was assessed by evaluating both inter-electrode variation ($n = 6$ sensors from the same batch) and intra-electrode repeatability ($n = 6$ repeated measurements on the same sensor). As shown in Figure 4D, the RSD for both tests was well below 5%, demonstrating the high consistency of the fabrication process and measurement results.

Overall, the PANI/Nano-Fe₃O₄/Anti-FLA/BSA/GCE immunosensor exhibits outstanding selectivity, stability, and reproducibility, providing a reliable technical foundation for long-term and large-scale trace FLA monitoring in marine environments.

4.3.3. Real Seawater Sample Analysis

To validate the applicability of the immunosensor in complex matrices, real seawater samples were collected from three representative sites (S_1 , S_2 , S_3) in Maowei Sea, Qinzhou, Guangxi. After filtration through a 0.45 μm membrane and pH adjustment to 7.0 with NaOH, the optimized sensor was employed to detect FLA at five spiked concentration levels (10–50 ng/mL). Differential pulse voltammetry (DPV) was used to record I_p , which was substituted into the calibration equation ($I = -1.551C + 174.602$) to quantify FLA concentrations. The accuracy was assessed by standard addition recovery experiments, with the results summarized in Table 4. The data revealed spatial heterogeneity in FLA concentrations across the sampling sites, likely due to localized industrial discharge, shipping activities, and other environmental factors. The recoveries at the five spiking levels ranged from 95.44% to 97.51%, with RSDs below 3%, demonstrating excellent detection accuracy. These results indicate that the sensor enables precise FLA detection in real seawater matrices with minimal sample pretreatment—only simple filtration and pH adjustment are required—effectively addressing the challenge of trace-level FLA monitoring in complex marine environments. This provides a highly efficient solution for in situ detection of PAHs.

Table 4. Recovery results of FLA spiking in real seawater samples.

Experiment	Added ng/mL	Theory I_p μA	Site —	Detection I_p μA	$\bar{I}_p$ μA	Found ng/mL	Recovery %
Experiment 1	10	159.09	S_1	161.24	159.80	9.54	95.44
			S_2	159.41			
			S_3	158.75			
Experiment 2	20	143.58	S_1	145.91	144.35	19.50	97.51
			S_2	144.22			
			S_3	142.93			
Experiment 3	30	128.07	S_1	131.25	129.29	29.21	97.38
			S_2	128.81			
			S_3	127.82			
Experiment 4	40	112.56	S_1	114.63	114.54	38.72	96.81
			S_2	115.51			
			S_3	113.49			

Table 4. Cont.

Experiment	Added ng/mL	Theory I_p μA	Site —	Detection I_p μA	$\bar{I}_p$ μA	Found ng/mL	Recovery %
Experiment 5	50	97.05	S ₁	99.26	96.97	50.04	97.10
			S ₂	96.31			
			S ₃	102.34			

5. Conclusions

To address the challenge of trace FLA detection in marine environments, we developed a PANI/Nano-Fe₃O₄/Anti-FLA/BSA/GCE electrochemical immunosensor. Anti-FLA antibodies were covalently immobilized onto a PANI/Nano-Fe₃O₄ composite-modified GCE surface via EDC/NHS to promote physical adsorption, and BSA was used to block nonspecific binding sites. Specific antigen–antibody recognition enabled selective and quantitative detection of FLA in complex matrices. Optimization experiments demonstrated a good linear response over the 0.5–80 ng/mL concentration range, with a calibration equation of $I = -1.551C + 174.602$ ($R^2 = 0.996$). The LOD, calculated using the 3σ criterion, was as low as 0.354 ng/mL. Recovery tests in real seawater samples yielded recoveries between 95.44% and 97.51%, with RSDs below 3%, indicating excellent anti-matrix interference capability and analytical accuracy. Leveraging the synergistic properties of the nanocomposite material and the high specificity of the antibody recognition mechanism, this immunosensor offers a sensitive and selective approach for rapid on-site detection of PAHs. Its superior performance demonstrates strong potential for practical environmental monitoring applications.

Author Contributions: Conceptualization, X.H. and H.H.; methodology, X.H. and Q.L.; software, X.H., R.X. and H.H.; validation, X.H., X.W., J.Y., R.X. and H.H.; formal analysis, X.H., J.Y. and H.H.; investigation, X.H., X.W., R.L., Q.L. and H.H.; resources, X.H.; data curation, X.W., R.X. and H.H.; writing—original draft, X.H.; writing—review and editing, H.H.; visualization, X.H. and Z.A.; supervision, X.W., R.L. and Z.A.; project administration, X.H., X.W., R.L., J.Y., Z.A. and Q.L.; funding acquisition, X.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by 2025 “Guangxi Glory” Study and Research Program (Special Project for Science and Technology Talents) and 2026 Guangxi Special Fund for Talent Cultivation Supporting Marine Economy—Talent Training Project for Marine Energy Utilization Research Team.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mrdaković, M.; Ilijin, L.; Vlahović, M.; Filipović, A.; Grčić, A.; Todorović, D.; Perić-Mataruga, V. Effects of dietary fluoranthene on nymphs of *Blaptica dubia* S. (Blattodea: Blaberidae). *Environ. Sci. Pollut. Res.* **2019**, *26*, 6216–6222. [CrossRef] [PubMed]
2. Takam, P.; Schaeffer, A.; Laovitthayangoon, S.; Chareerntantakul, W.; Sillapawattana, P. Toxic effect of polycyclic aromatic hydrocarbons (PAHs) on co-culture model of human alveolar epithelial cells (A549) and macrophages (THP-1). *Environ. Sci. Eur.* **2024**, *36*, 176. [CrossRef]
3. Cao, X.; Wang, S.; Tian, S.; Lou, H.; Kong, Y.; Yang, Z.; Liu, J. Spectroscopic and molecular modeling studies on the interactions of fluoranthene with bovine hemoglobin. *Spectrosc. Acta Pt. A-Molec. Biomolec. Spectr.* **2018**, *203*, 301–307. [CrossRef] [PubMed]

4. Chi, T.; Liu, Z.; Zhang, B.; Zhu, L.; Dong, C.; Li, H.; Jin, Y.; Zhu, L.; Hu, B. Fluoranthene slow down sulfamethazine migration in soil via π - π interaction to increase the abundance of antibiotic resistance genes. *Environ. Pollut.* **2024**, *358*, 124532. [CrossRef] [PubMed]
5. Sun, K.; Song, Y.; Liu, Z.; Jing, M.; Wan, J.; Tang, J.; Liu, R. Toxicity assessment of Fluoranthene, Benz(a)anthracene and its mixed pollution in soil: Studies at the molecular and animal levels. *Ecotoxicol. Environ. Saf.* **2020**, *202*, 110864. [CrossRef] [PubMed]
6. Wang, M.; Tian, L.; Sun, J.; Zhang, Y.; Zhai, H.; Li, D.; Wan, L. Distribution, sources and toxicity of polycyclic aromatic hydrocarbons in Liuxi River, China. *Chem. Ecol.* **2022**, *38*, 312–326. [CrossRef]
7. Huang, Y.; Wei, J.; Song, J.; Chen, M.; Luo, Y. Determination of low levels of polycyclic aromatic hydrocarbons in soil by high performance liquid chromatography with tandem fluorescence and diode-array detectors. *Chemosphere* **2013**, *92*, 1010–1016. [CrossRef] [PubMed]
8. Li, C.F.; Wong, J.W.C.; Huie, C.W.; Choi, M.M.F. On-line flow injection-cloud point preconcentration of polycyclic aromatic hydrocarbons coupled with high-performance liquid chromatography. *J. Chromatogr. A* **2008**, *1214*, 11–16. [CrossRef] [PubMed]
9. Ma, L.L.; Chu, S.G.; Wang, X.T.; Cheng, H.X.; Liu, X.F.; Xu, X.B. Polycyclic aromatic hydrocarbons in the surface soils from outskirts of Beijing, China. *Chemosphere* **2005**, *58*, 1355–1363. [CrossRef] [PubMed]
10. Wang, S.; Zhang, X.; Zhou, Q.; Liu, Z.; Xu, Z. Magnetic hypercrosslinked polymer microspheres for the detection, spatial distribution, source identification and potential risks assessment of five polycyclic aromatic hydrocarbons in city river of plateau lake, Southwest China. *J. Hazard. Mater.* **2024**, *476*, 135026. [CrossRef] [PubMed]
11. Hu, X.; Pang, L.; Wu, M.; Wang, C.; Li, J. Nanoleaf-derived carbon materials as a sensitivity coating for solid-phase microextraction of polycyclic aromatic hydrocarbons. *Anal. Bioanal. Chem.* **2024**, *416*, 277–285. Available online: <https://link.springer.com/article/10.1007/s00216-023-05016-8> (accessed on 14 May 2025). [PubMed]
12. Jing, L.; Shi, Y.; Cui, J.; Zhang, X.; Zhan, J. Hydrophobic gold nanostructures via electrochemical deposition for sensitive SERS detection of persistent toxic substances. *RSC Adv.* **2015**, *5*, 13443–13450. [CrossRef]
13. Sun, F.; Littlejohn, D.; Gibson, M.D. Ultrasonication extraction and solid phase extraction clean-up for determination of US EPA 16 priority pollutant polycyclic aromatic hydrocarbons in soils by reversed-phase liquid chromatography with ultraviolet absorption detection. *Anal. Chim. Acta* **1998**, *364*, 1–11. [CrossRef]
14. Coelho, N.d.S.; Menezes, H.C.; Cardeal, Z.d.L. Development of new PDMS in tube extraction microdevice for enhanced monitoring of polycyclic aromatic hydrocarbons and their derivatives in water. *Talanta* **2025**, *281*, 126882. [CrossRef] [PubMed]
15. Tijunelyte, I.; Betelu, S.; Moreau, J.; Ignatiadis, I.; Berho, C.; Lidgi-Guigui, N.; Guénin, E.; David, C.; Vergnole, S.; Rinnert, E.; et al. Diazonium Salt-Based Surface-Enhanced Raman Spectroscopy Nanosensor: Detection and Quantitation of Aromatic Hydrocarbons in Water Samples. *Sensors* **2017**, *17*, 1198. [CrossRef] [PubMed]
16. Yu, X.; Kong, L.; Lan, T.; Chen, J.; Fu, Y.; Sun, Q.; Huang, M.; Wang, J.; Huang, Z.; Liu, T. An integrated enrichment-enhancement membrane SERS substrate for rapid detection of polycyclic aromatic hydrocarbons in aquatic environments. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2025**, *328*, 125429. [CrossRef] [PubMed]
17. Zheng, X.; Tian, D.; Duan, S.; Wei, M.; Liu, S.; Zhou, C.; Li, Q.; Wu, G. Polypyrrole Composite Film for Highly Sensitive and Selective Electrochemical Determination Sensors. *Electrochim. Acta* **2014**, *130*, 187–193. [CrossRef]
18. Kazemi, F.; Naghib, S.M.; Zare, Y.; Rhee, K.Y. Biosensing Applications of Polyaniline (PANI)-Based Nanocomposites: A Review. *Polym. Rev.* **2021**, *61*, 553–597. [CrossRef]
19. Ab Mutalib, T.N.A.B.T.; Tan, S.J.; Foo, K.L.; Liew, Y.M.; Heah, C.Y.; Abdullah, M.M.A.B. Properties of polyaniline/graphene oxide (PANI/GO) composites: Effect of GO loading. *Polym. Bull.* **2021**, *78*, 4835–4847. [CrossRef]
20. Ramana, G.V.; Kumar, P.S.; Srikanth, V.V.S.S.; Padya, B.; Jain, P.K. Electrochemically Active Polyaniline (PANI) Coated Carbon Nanopipes and PANi Nanofibers Containing Composite. *J. Nanosci. Nanotechnol.* **2015**, *15*, 1338–1343. [CrossRef] [PubMed]
21. Cai, Y.; Yan, Z.H.; Wang, N.Y.; Cai, Q.Y.; Yao, S.Z. Preparation of naphthyl functionalized magnetic nanoparticles for extraction of polycyclic aromatic hydrocarbons from river waters. *RSC Adv.* **2015**, *5*, 56189–56197. [CrossRef]
22. Shi, X.; Zhang, X.; Gao, W.; Zhang, Y.; He, D. Removal of microplastics from water by magnetic nano-Fe₃O₄. *Sci. Total Environ.* **2022**, *802*, 149838. [CrossRef] [PubMed]
23. Yao, X.; Kou, X.; Qiu, J. Generation mechanism of negative dielectric properties of nano-Fe₃O₄/PANI composites. *Mater. Chem. Phys.* **2018**, *208*, 177–182. [CrossRef]
24. Zolfigol, M.A.; Moosavi-Zare, A.R.; Moosavi, P.; Khakyzadeh, V.; Zare, A. Nano-ferrous ferric oxide (nano-Fe₃O₄): Powerful, reusable, and stable catalyst for N-Boc protection of amines. *Comptes Rendus Chim.* **2013**, *16*, 962–966. [CrossRef]
25. Isaev, V.A.; Grishenkova, O.V.; Zaykov, Y.P. Theory of cyclic voltammetry for electrochemical nucleation and growth. *J. Solid State Electrochem.* **2018**, *22*, 2775–2778. [CrossRef]
26. Chen, G.; Yuan, Z.; Wei, Y.; Fu, X.; Jiang, K. Electron Beam Interaction with Carbon Nanotubes in Scanning Electron Microscope: Mechanisms and Applications. *Adv. Funct. Mater.* **2024**, *34*, 2404682. [CrossRef]
27. Nhu, C.T.; Quoc, T.V.; Quang, L.D.; Dang, P.N.; Hong, S.N.; Duc, T.C.; Thanh, T.B. Novel, compact electrochemical and impedance instrumentation. *Instrum. Sci. Technol.* **2025**, *53*, 28–43. [CrossRef]

28. Choi, H.W.; Song, Y.S. Rheological Equivalent Circuit Model Using Electrochemical Impedance Analysis. *Macromol. Theory Simul.* **2025**, *34*, 2400069. [[CrossRef](#)]
29. Mohamad, A.A. Cyclic voltammetry of hybrid supercapacitors: A characterization review. *Inorg. Chem. Commun.* **2025**, *172*, 113677. [[CrossRef](#)]
30. El-Latif, E.I.A.; Kebede, M.A.; Sekar, K.; Hameed, T.A.; Yahia, I.S.; Gao, H.; Sheha, E. Modeling the Diffusion Coefficient of Charge Carriers in Metal Ion Batteries using the Randles-Sevcik Equation. *Adv. Theory Simul.* **2025**, *8*, 2500346. [[CrossRef](#)]
31. Emiliano, C.B.; Bellin, C.d.O.; Lope, M.C. Trapezoidal Cyclic Voltammetry as a Baseline for Determining Reverse Peak Current from Cyclic Voltammograms. *J. Electrochem. Sci. Technol.* **2024**, *15*, 405–413. [[CrossRef](#)]
32. Gulotta, A.; Polimeni, M.; Lenton, S.; Starr, C.G.; Stradner, A.; Zaccarelli, E.; Schurtenberger, P. Combining Scattering Experiments and Colloid Theory to Characterize Charge Effects in Concentrated Antibody Solutions. *Mol. Pharm.* **2024**, *21*, 2250–2271. [[CrossRef](#)] [[PubMed](#)]
33. van der Zon, A.A.; Höchsmann, A.; Bos, T.S.; Neusüß, C.; Somsen, G.W.; Jooß, K.; Haselberg, R.; Gargano, A.F. Characterization of monoclonal antibody charge variants under near-native separation conditions using nanoflow sheath liquid capillary electrophoresis-mass spectrometry. *Anal. Chim. Acta* **2024**, *1331*, 343287. [[CrossRef](#)] [[PubMed](#)]
34. Gupta, S.; Dubey, A.; Rathore, A.S. Role of Charge Heterogeneity on Physical Stability of Monoclonal Antibody Biotherapeutic Products. *Pharm. Res.* **2024**, *41*, 1443–1454. [[CrossRef](#)] [[PubMed](#)]
35. Hao, H.; Qiu, C.; Qu, W.; Zhuang, Y.; Han, X.; Tao, W.; Gu, Y.; Zhao, Z.; Liu, H.; Wang, W. Research on the Detection of Hg(II) in Seawater Using GR-MWCNTs/CeO₂-Modified Electrodes. *Chemosensors* **2024**, *12*, 128. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.