SUPPLEMENTARY MATERIAL

Electrochemical Sensor Based on CTAB-Nafion Modified Nano-Graphite Carbon Paste Electrode and Its Application in the Determination of Aflatoxin B1 in Food

Juan Ma[1,†], Hong Li[2,†], Siyu Huang[2,*], Xiaojing Hu[3], Tingjuan Xia[3], Dongyun Zheng[1,*]

[1] School of Biomedical Engineering, South-Central Minzu University, Wuhan 430074, China

[2] Key Laboratory of Condiment Supervision Technology, State Administration for Market Regulation, Wuhan 400713, China

[3]Wuhan Institute for Food and Cosmetic Control, Wuhan 430040, China

* Correspondence: huangsiyu@cqifdc.org.cn (S.H.); wintercloud@mail.scuec.edu.cn (D.Z.); Tel.: +86-27-6784-1231 (D.Z.)

† These authors contributed equally to this work.

1. Optimization of experimental conditions

In order to obtain the highest sensitivity for AFB1 detection, we optimized the experimental conditions using the SWV method, including accumulation potential, accumulation time, the number of CTAB–Nafion polymerization cycles and pH value of the electrolyte. The controlled variable method was used in the experiments, and the AFB1 concentrations in the substrate were all 5 nM. The experimental results are shown in Figure S1.

The CTAB–Nafion composite film was deposited onto the electrode surface via cyclic voltammetry (CV). Since the film thickness affects electrode performance, the composite film thickness was adjusted by optimizing the number of polymerization cycles, with results shown in Figure S1(A). When the CTAB–Nafion polymerization cycles ranged from 5 to 25 cycles, the peak current of AFB1 first increased and then decreased, reaching its maximum value at 10 cycles. Therefore, 10 cycles were selected as the optimal number of cycles in this system.

As shown in Figure S1(B) showed that the peak current of AFB1 increased rapidly when the enrichment time was increased from 0 to 125 s, and then gradually decreased, obtaining a maximum at 50 s. Figure S1(C), the peak current of AFB1 gradually increased from −0.2 V to 0.3 V, then decreased after peaking at the 0.1 V, indicating that the most sensitive response was found at 0.1 V. Therefore, 0.1 V and 50 s were selected as the optimal enrichment potential and time.

As the electrochemical response of an analyte can be significantly affected by the pH value of the supporting electrolyte, the detection pH value was optimized. A series of 0.1 M phosphate buffer solutions with different pH values was prepared. The influence of the pH value of the supporting electrolyte on the responses current of AFB1 is shown in Figure S1(D). Clearly, the optimal response is achieved at pH 5.0, likely due to a favorable balance between proton availability, analyte ionization, electrode surface interactions, and the stability of redox species.

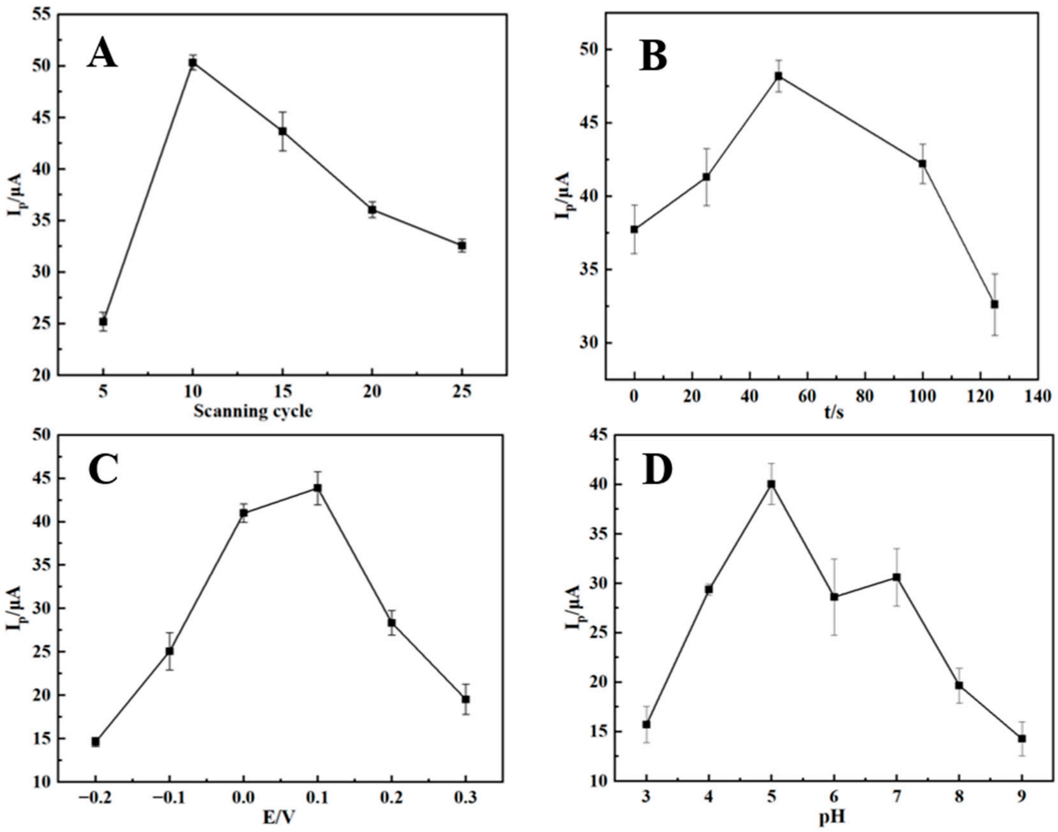

Figure S1. Effects of the CTAB–Nafion scanning cycles (A), accumulation time (B), accumulation potential (C) and pH value of electrolyte (D).

2. Calculation of the effective area of the electrode

In order to analyze the effective area of the NGCPE and CATB–Nafion/NGCPE surfaces, the effect of different scanning rates on the CV reaction with 5 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 1 M KCl buffer was investigated. As can be seen from Figure S2(A) and Figure S2(C) reversible redox peaks appeared at different scan rates, and the redox peak currents increased with the increase of scan rate. the I_{pa} of NGCPE and CATB–Nafion/NGCPE were linearly related to the scan rate ($v^{1/2}$). The linear equations were I_{pNGCPE} (μA) = 13.8 $v^{1/2}$ + 0.301 (R^2 = 0.99) and $I_{pCATB\text{–}Nafion/NGCPE}$ (μA) = 30.28 $v^{1/2}$ +

0.525 (R^2 = 0.99), respectively (Figure S2(B) and Figure S2(D)). According to the Randles-Sevcik equation [39].

$$\text{When } 25\ ^\circ\text{C},\ i_p = (2.69 \times 10^5) \times n^{3/2} v^{1/2} D^{1/2} AC \qquad (S1)$$

A: electrode area (cm^2), D: diffusion coefficient (5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ + 1 M KCl, $D_{ox}=7.63\times10^{-6}\ cm^2\cdot s^{-1}$), C: solution concentration ($mol\cdot cm^{-3}$), v: scanning rate ($V\cdot s^{-1}$), i_p: peak current (A). From Eq. (S1), the effective areas of bare NGCPE and CATB–Nafion/NGCPE were $3.71\times10^{-3} cm^2$ and $8.15\times10^{-3} cm^2$, respectively. The results indicate that the CTAB–Nafion composite material can increase the effective surface area of the electrode.

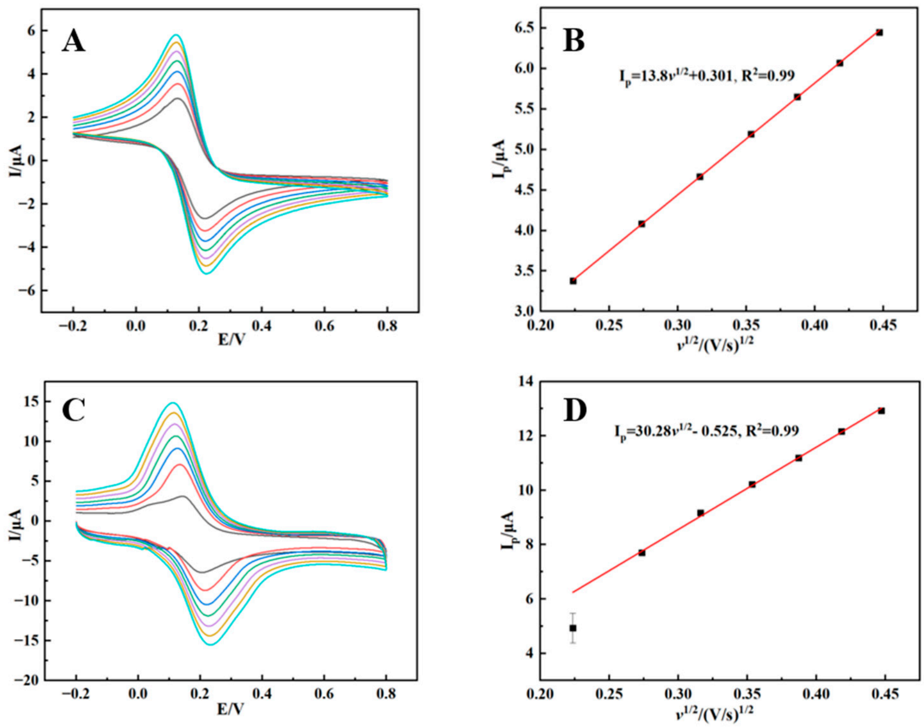

Figure S2. CV plots of I_p versus $v^{1/2}$ for bare NGCPE (A), CTAB–Nafion/NGCPE (C) in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 1 M KCl (0.05, 0.075, 0.1, 0.125, 0.15, 0.175, 0.2 $V\cdot s^{-1}$), and linear plots of I_p versus $v^{1/2}$ on bare NGCPE (B) and CTAB–NafionNGCPE (D).

Table S1. Performance comparison between CTAB–Nafion/NGCPE and other reported sensors.

Electrode	Method	LOD (nM)	LDR (nM)	Reference
MB-Apt/Au	SWV	2	8 - 4000	[40]
PANI/MIP/CNC-CNT/hypodermic needle	CV	3	0 - 25	[41]
AuNP-GQD/SPE	LSV	0.47	1 - 50	[42]
RGO-Fe_3O_4 nanorods-IL/GCE	DPV	0.096	0.064 -1 .057	[20]
CTAB-Nafion/NGCPE	SWV	0.02	0.1 - 100	This work

MB: methylene blue; Apt: aptamer; SWV: square wave voltammetry; DPV: differential pulse voltammetry; CV: cyclic voltammetry; LSV: linear sweep voltammetry; PANI: polyaniline; MIP: molecularly imprinted polymer; CNC: cellulose nanocrystals; CNT: carbon nanotubes; RGO: reduced graphene oxide; IL: Ionic liquid; GCE: glassy carbon electrode; AuNPs: gold nanoparticles; GQD: graphene quantum dots; SPE: screen-printed electrode.

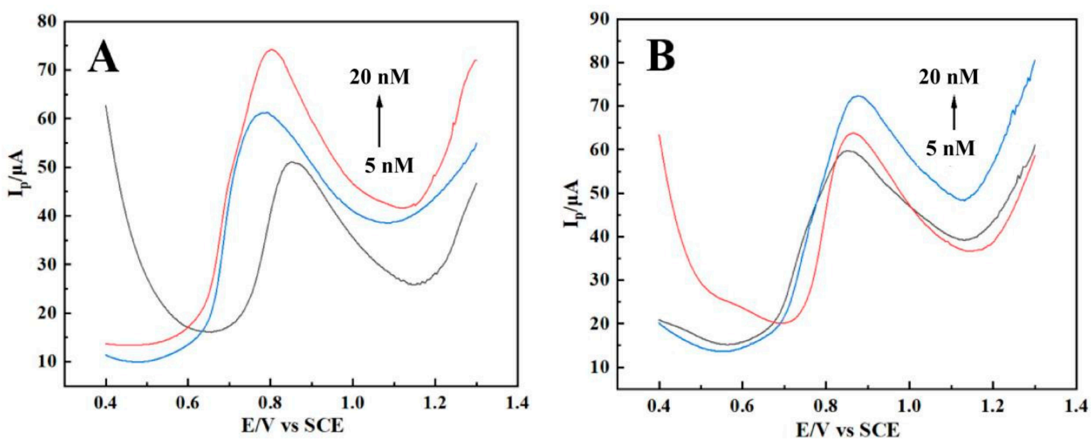

Figure S3. SWV diagrams detected in actual milk (A) and soy sauce (B) samples.

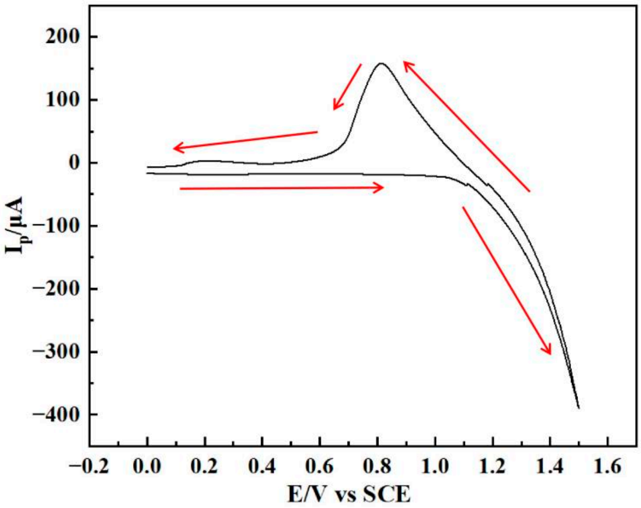

Figure S4. CV diagram of CTAB-Nafion/NGCPE in 0.1 M PBS solution containing 10 nM AFB1.

Table S2. **T-test of the sample analysis results**

Samples	**Add (nM)**	**T-value**	**Degrees of freedom**	**P-value**	**Significant (*α*=0.05)**
Milk	**5**	**-0.406**	**2**	**0.724**	**No**
	10	**-0.152**	**2**	**0.893**	**No**
	20	**0.143**	**2**	**0.899**	**No**
Soy sauce	**5**	**-0.016**	**2**	**0.988**	**No**
	10	**0.317**	**2**	**0.781**	**No**
	20	**0.276**	**2**	**0.808**	**No**