Rapid Detection of antibiotics Using Self-Developed Electrochemical Analyzer and Sensor Chip

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1. Supporting Information

1.1 Reagents and materials

The aptamer single-stranded DNA (ssDNA) modified with thiol for kanamycin (HS-KAN-APT, thiol-5′-TGGGGGTTGAGGCTAAGCCGA-3′) and tetracycline (HS-TET-APT, thiol-5′-TTTTTTCTCTCGACGACATTCCGTTGATCTCTCCCTTTTGGGTTGGTGTCGT-3′), along with their respective partially complementary ssDNA (CS) modified with MB (KAN-CS-MB, 5′-TCGGCTCCCCCA-3′-methylene blue, and TET-CS-MB, 5′-TGTCGTCGAGAG-3′-methylene blue), were procured from Sangon Biotech (Shanghai, China) and stored in 1 × TE buffer (pH 7.5) at −20°C. All DNA was purified using high-pressure liquid chromatography (HPLC) and quantified with a DeNovix DS-11 FX + spectrophotometer (DeNovix Inc., Wilmington, DE, USA). The buffer solution was prepared with 20 mM Tris-HCl (pH 7.5), 140 mM NaCl, 5 mM KCl, and ultra-pure water (18.25 MΩ·cm) obtained from an ultra-pure water system (Wateer Water Treatment Equipment Co., Ltd., Sichuan, China), which we designated as 1×TNaK buffer. Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from BBI, and all other chemicals were of analytical grade. HS-KAN-APT and KAN-CS-MB, as well as HS-TET-APT and TET-CS-MB, were co-dispersed in a specific concentration of TCEP solution at 0.5 μmol/L, annealed at 95°C for 5 minutes, and subsequently cooled at a rate of 0.1°C/s until reaching 4°C before use. Kanamycin sulfate (KAN) was sourced from Shanghai Titan Scientific Co., Ltd. (Shanghai, China), tetracycline hydrochloride (TET) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and ampicillin sodium salt (AMP) and chloramphenicol (CHL) were acquired from Yuanye Bio-Technology Co., Ltd. (Shanghai, China), with their molecular formulas presented in Fig. S1. Unless otherwise specified, all antibiotics were dispersed in 1×TNaK buffer to prepare the corresponding sample solutions.

Ethylene diamine was procured from Xilong Chemical Co. (Guangdong, China). N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) and KSCN were obtained from J&K Chemicals (Beijing, China). Gold plating buffer consists of 125 mM Na_2SO_3 (Beijing Chemical Works, Beijing, China), 500 mM methanol (Beijing Jinming Biotechnology Co., Ltd., Beijing, China), and 4 mM $Na_3Au(SO_3)_2$ (Changzhou Chemical Research Institute Co., Ltd., Changzhou, China). The polystyrene (PS) substrate was sourced from Shenzhen Shunfa Plexiglass Products Co., Ltd. (Guangdong, China). The epoxy resin channel layer was acquired from Beijing Hengyue Intelligent Manufacturing Technology Co., Ltd. (Beijing, China). UV adhesive was purchased from Dongguan Jule Electronic Material Co., Ctd. (Dongguan, China).

1.2 Instrumentation

Most electrochemical experiments, including cyclic voltammetry (CV) and square wave voltammetry (SWV), were conducted using a self-developed electrochemical analyzer (abbreviated as SDEA) equipped with a lab-fabricated multi-channel PGE chip. The reference and counter electrodes were constructed from a gold fan ring, while a gold round (2.0 mm in diameter) served as the working electrode. For comparative purposes, a commercial electrochemical analyzer (CHI) produced by Shanghai Chenhua Instrument Co., Ltd. (Shanghai, China) and controlled by CHI832D, was also utilized. All measurements with the electrochemical analyzer were performed at approximately 25°C. The following parameters were employed: CV was conducted from -0.5 V to 1.2 V at a scan rate of 0.5 V/s, and SWV was executed from -0.5 V to -0.1 V at a frequency of 50 Hz.

1.3 Fabrication of the gold patterned layer (chip gold electrode, CGE) on polystyrene substrate

A piece of PS sheet measuring 75×40×1.0 mm was initially exposed to UV light (15 W, 254 nm, Philips, China) emitted by a low-pressure mercury lamp for 5 hours. Following this exposure, the PS sheet was immersed in a 100 mM phosphate buffer (pH 7.0) containing 360 mmol/L ethylene diamine and 50 mmol/L EDC at room temperature for 3 hours. After rinsing with deionized water, the selectively aminated PS substrate was treated sequentially with a 1 mmol/L $HAuCl_4$ aqueous solution for 167 minutes, followed by a 0.1 M $NaBH_4$ aqueous solution for 10 minutes. This process resulted in the formation of gold nanoparticles on the UV-exposed region of the PS substrate. Subsequently, the substrate was sonicated in a 0.5 mol/L KSCN solution for 20 minutes to remove non-specifically adsorbed gold nanoparticles from the PS surface, thereby preventing non-specific over-plating in the subsequent plating process. Finally, the activated PS was immersed in gold plating for approximately 6-7 hours. After rinsing with deionized water, the PS gold electrode substrate was baked at 60°C for 1 hour and was then ready for use. The schematic diagram of the entire process and the physical product are shown in Fig. S2.

1.4 Water treatment

Environmental water samples, including lake water from Nanhu Park in Changchun, China, and tap water from the Changchun Institute of Applied Chemistry, were filtered using a pure cotton fiber qualifier filter to eliminate precipitates and suspensions. These samples underwent a two-step (filter-back flush) processing method. Initially, the water samples were placed in a 10 mL syringe and manually filtered through a 0.22 μm pore size mixed cellulose esters (MCE) needle filter (Sangon Biotech, Shanghai, China). The needle filter, which contained retentates including microbial contaminants, was rinsed with 1 mL of sterile water to remove any residual corrosive liquids. Subsequently, the retentates were recovered into

a 1 mL syringe by back flushing the filter with 100 µL of sterile water. The treated water samples were stored at 4℃ and utilized within 6 hours.

2. Supporting Figures

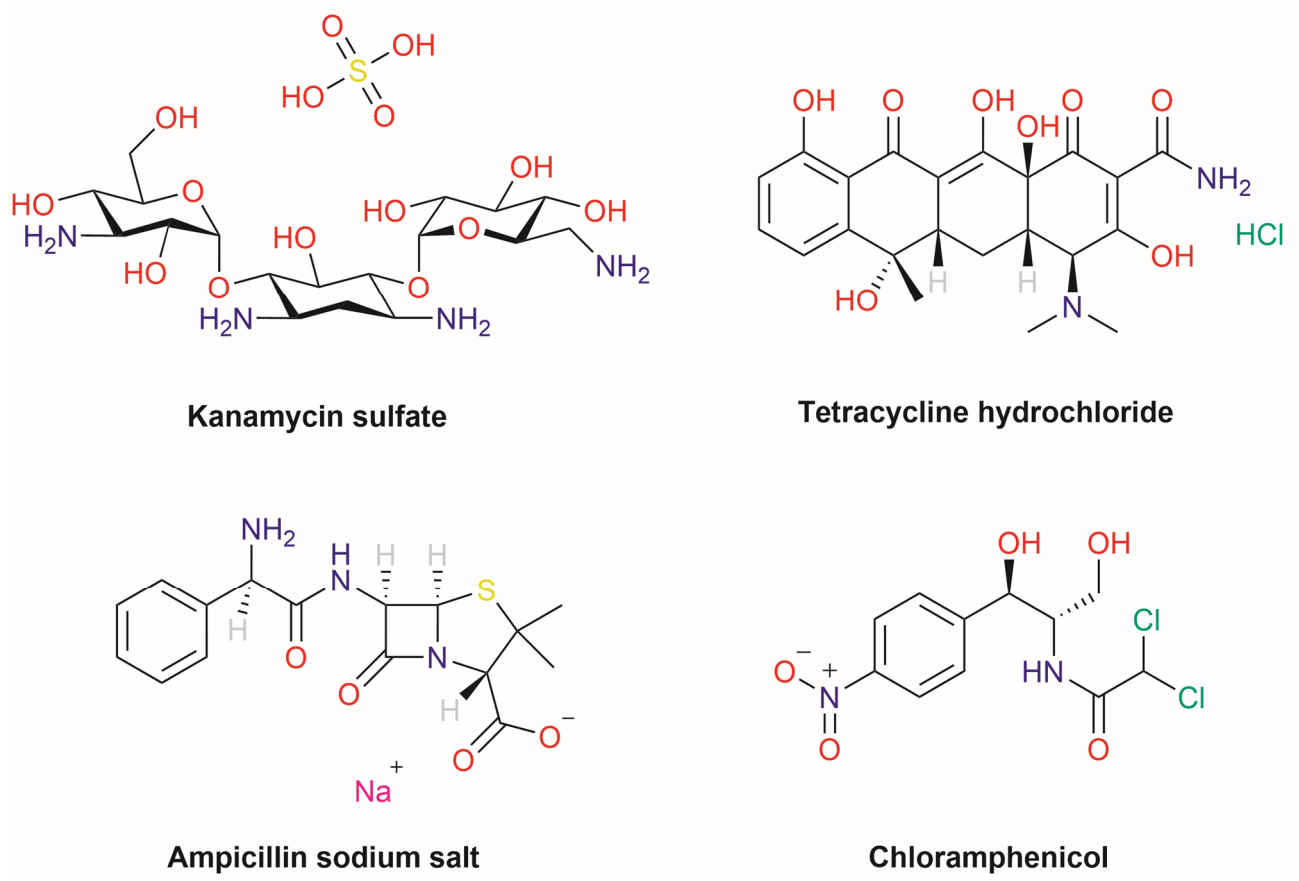


Fig. S1 The molecular formulas of various antibiotics utilized in the study.

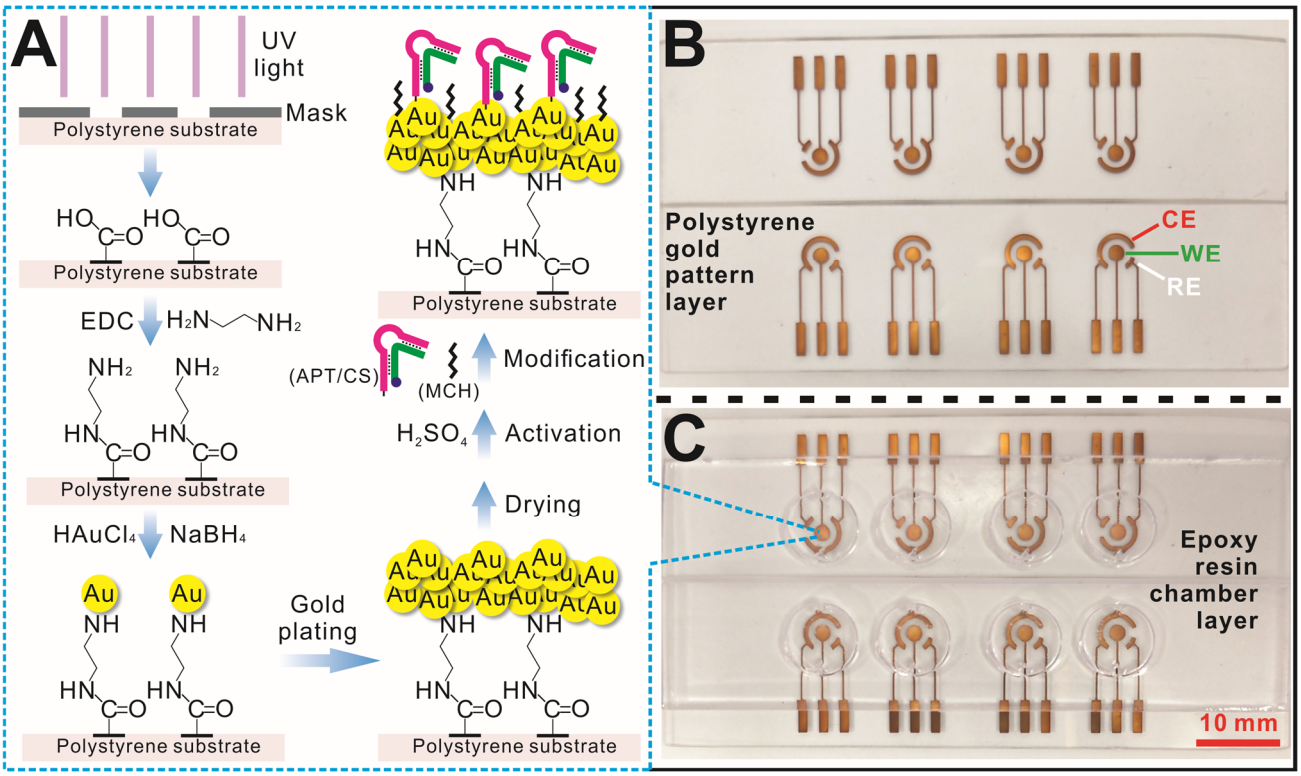


Fig. S2 (A) Schematic diagram of CGE and sensor manufacturing process. (B) Diagram of the eight-channel CGE. (C) An eight-channel CGE chip.

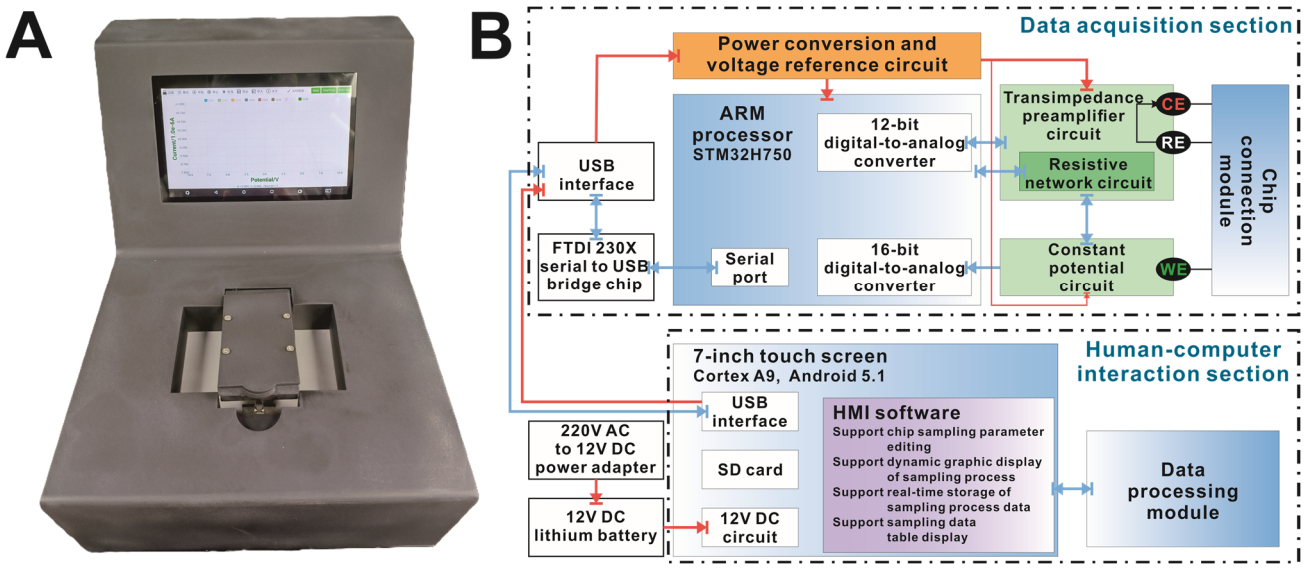


Fig. S3 (A) The appearance of the self-developed electrochemical analyzer (SDEA). (B) A thumbnail representation of the system architecture of SDEA.

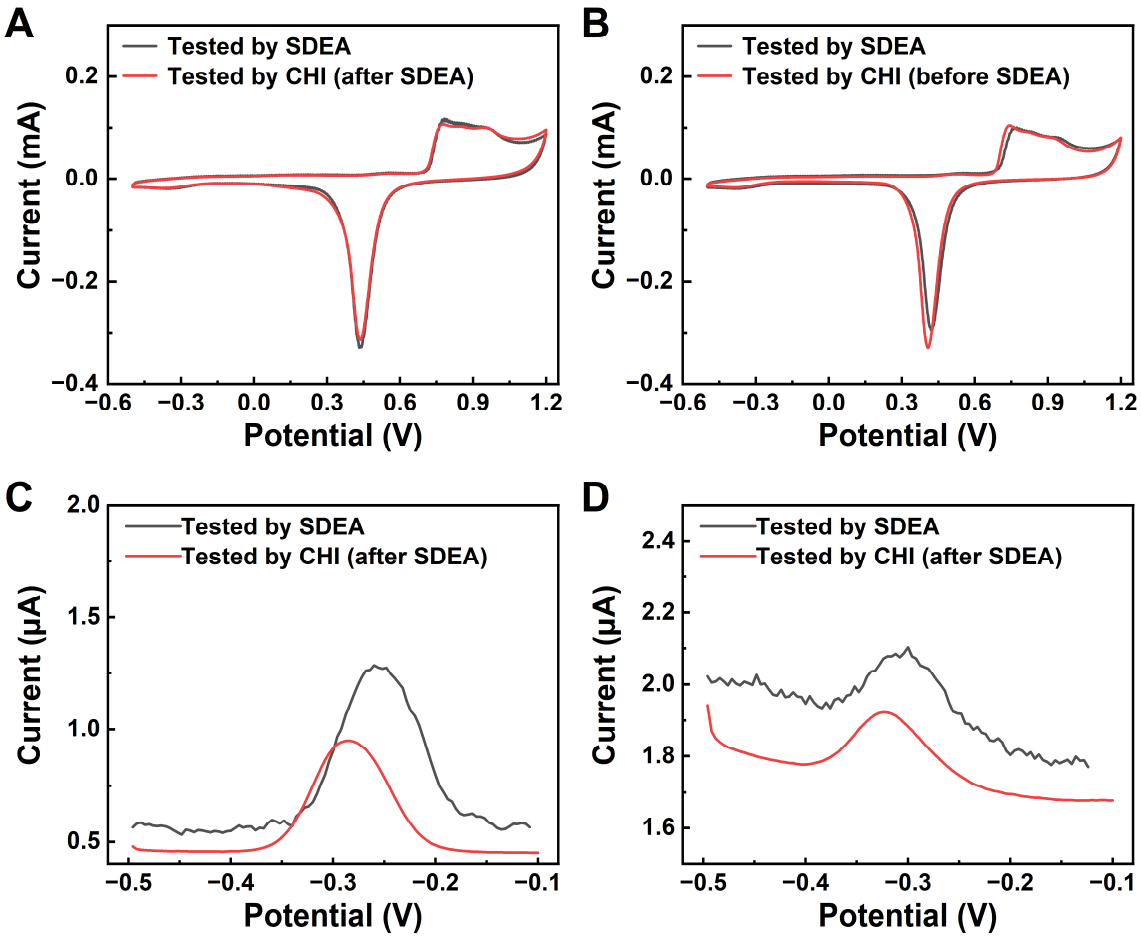


Fig. S4 (A) For the same bare CGE, the SDEA scans first, and the CHI scans later. (B) For the same bare CGE, the CHI scans first, and the SDEA scans subsequently. (C) For the same modified CGE, the SDEA scans first, followed by the CHI. (D) For the same modified CGE, the CHI scans first, and the SDEA scans later.

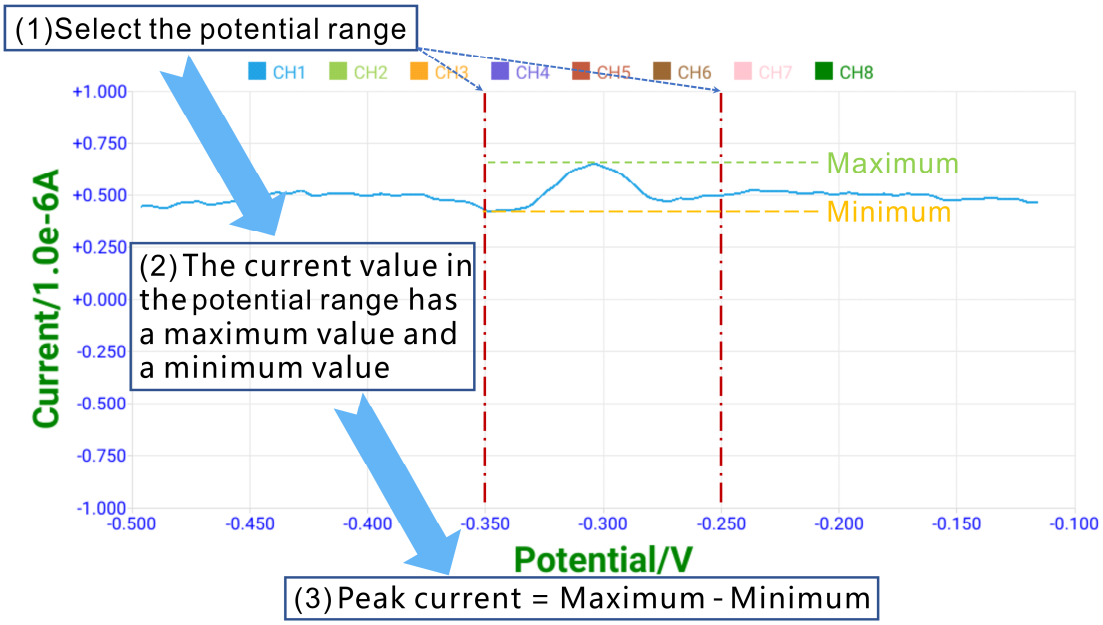


Fig. S5 Schematic diagram of the current peak algorithm built into the SDEA.

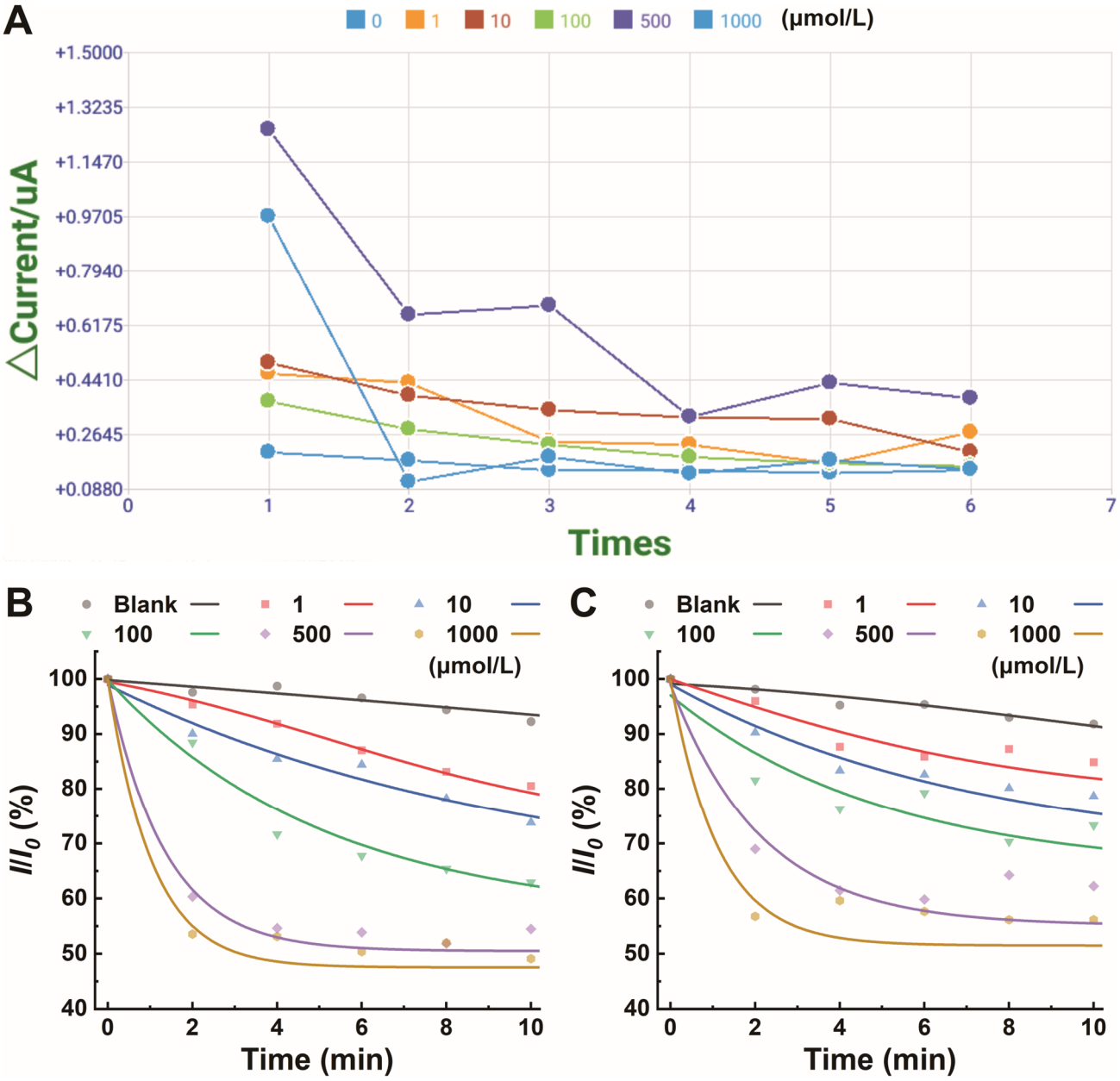


Fig. S6 (A) The screenshot of the SDEA illustrates the real-time data processed by its built-in peak current algorithm, with the horizontal axis representing the number of scans and the vertical axis indicating the peak current value. (B) Results of manual processing for parallel experiment group 2. (C) Results of manual processing for parallel experiment group 3.

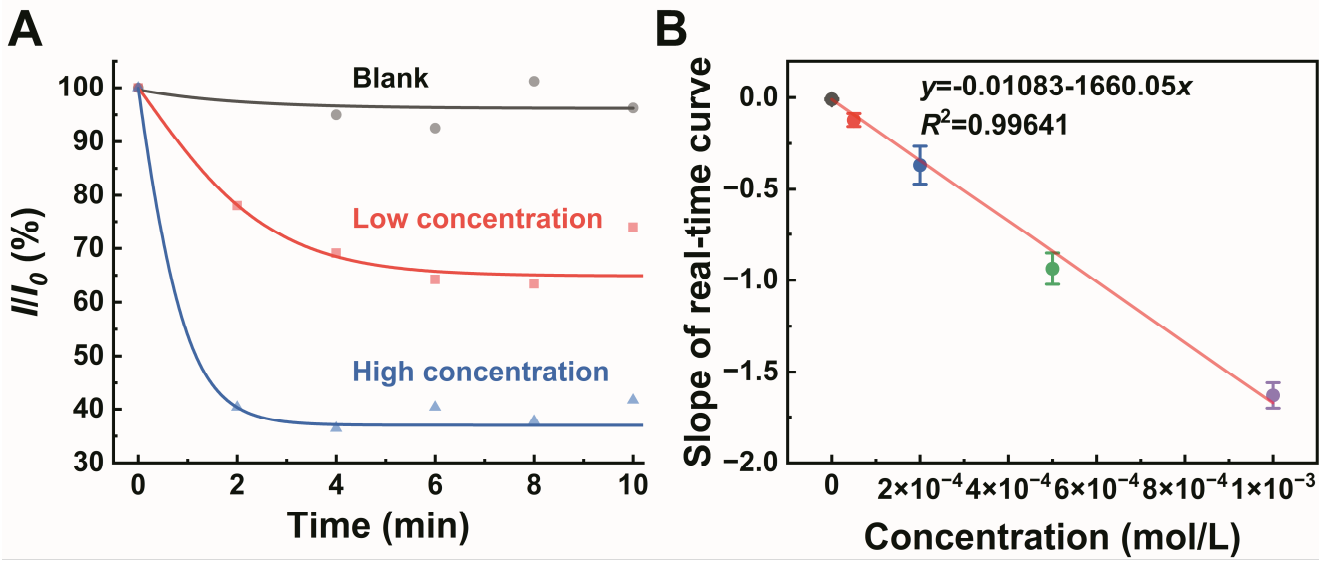


Fig. S7 (A) Real-time results of tetracycline detection. (B) The variation trend of initial point slope in real-time data fitting curves with concentration and their corresponding fitting curve, error bar: SD, n=2-3.

3. Supporting Tables

Table S1. A list of some electrochemical workstation parameters.

Model	Voltage range	Current range	Input bias current	Current measurement resolution	Instrument dimensions	Function	Number of channels
SDEA	±10 V	±400 mA	<10 pA	1 pA	354×252×258 mm	CV, SWV, Data processing	8
Shanghai ChenhuaCHI 832D	±10 V	±250 mA	<1 pA	0.03 fA	320×280×120 mm	CV, LSV, i-t, DPV, SWV, EIS, etc.	1
PINE WaveNow	±4 V	±100 mA	<2 pA	313 pA	165×100×29 mm	LSV, CV, SWV, DPV, etc.	1
Metrohm 910 PSTAT mini	±2.048V	±200 μA	/	1 pA	80×54×23 mm	LSV, CV, SWV, DPV, AP, PAD	1
Shenzhen Refresh Biosensing BioSYS-P15E Max	±300 mV	±0.1 nA	/	10 pA	45×21×12 mm	CV, LSV, DPV, SWV, i-t, RES, etc.	1

Table S2. The performances of kanamycin sensing in real water samples (n=3).

Sample	Spiked KAN concentration (μmol/L)	Detected KAN concentration (μmol/L)	RSD (%)	Recovery (%)
Nanhu Lake water	100	108.0	4.544	108.0
	500	525.7	3.887	105.1
Tap water	500	507.8	4.672	101.6

Table S3. The performances of tetracycline sensing in real water samples (n=3-4).

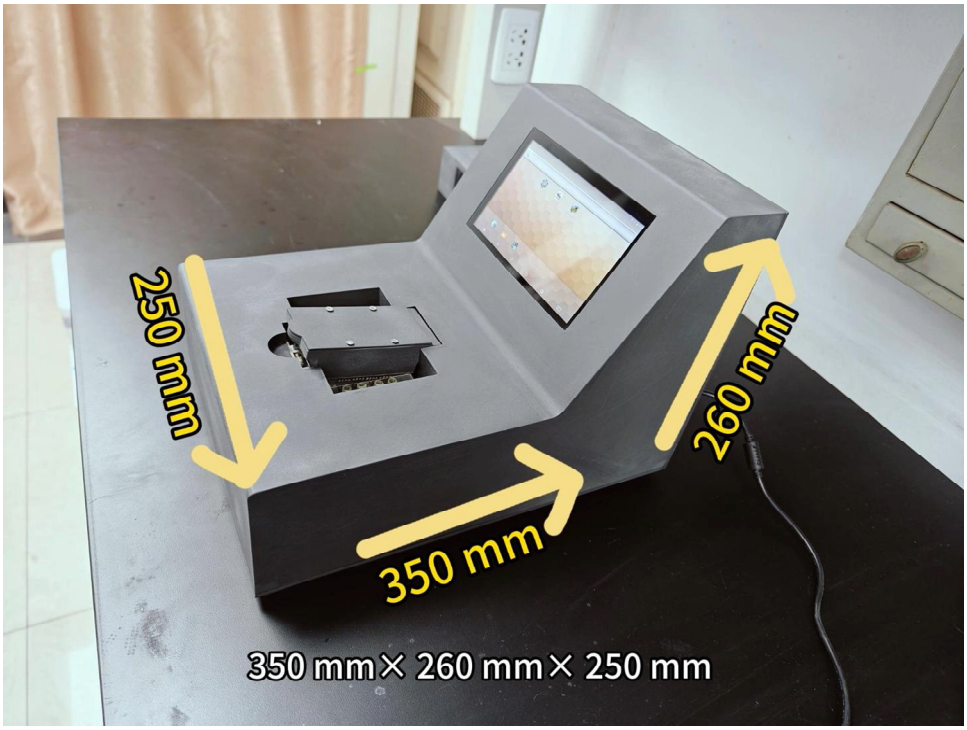
Sample	Spiked TET concentration (μmol/L)	Detected TET concentration (μmol/L)	RSD (%)	Recovery (%)
Nanhu Lake water	500	529.8	2.295	106.0
Tap water	500	516.3	3.003	103.3

Table S4. Performance comparison of state-of-the-art antibiotic detection technologies.

Method	Target	LOD (mol/L)	Time	Portability	Real-time	Multiplex	Ref.
HPLC-ESI-MS/MS	Multi-class	10^{-9}-10^{-7}	>60 min	No	No	Limited	[1]
AuNPs/DNA+HCR	Kanamycin	6.8×10^{-7}	>60 min	No	No	No	[2]
Graphene FET aptasensor	Kanamycin	1.06×10^{-9}	20 s	No	No	No	[3]
Electrochemilumines cence sensor	Kanamycin	1.5×10^{-12}	>60 min	No	No	No	[4]
ELISA+ Paper-based colorimetric sensor	Tetracycline Chloramphenicol	1.12×10^{-9} 1.55×10^{-10}	~8 min	Yes	No	No	[5]
Fluorescent sensor+ smartphone-POCT	Tetracycline	4.17×10^{-7}	1 min	Yes	Yes	No	[6]
Molecularly imprinted photoelectrochemical sensor	Tetracycline	7×10^{-12}	~7 min	No	Yes	No	[7]
Electrochemical aptasensor+SDEA	**Kanamycin** **Tetracycline**	**4.9×10^{-7} or 9.6×10^{-7}** **4.51×10^{-5} or 8.8×10^{-6}**	**20 or 5-10 min**	**Yes**	**Yes**	**Yes (8 ch)**	**This work**

4. Supporting Video

Video S1. Function introduction and operation demonstration of self-developed electrochemical analyzer.



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