

ORIGINAL RESEARCH ARTICLE

Development of logic gates and half adders for the concurrent detection of two DNA molecules

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ABSTRACT

In this study, Graphene oxide/gold nanoparticles composite membrane electrodes (GCE/GO/AuNPs) were fabricated using drop coating and electrodeposition techniques. By analyzing changes in the probe's surface configuration before and after binding with target DNA, the electrochemical signal varies, enabling the intelligent detection of *E. coli* and Salmonella DNA. Take the target as the input signal, and $\Sigma|\Delta I|$ and $|\Delta I_{MB}/\Delta I_{FC}|$ constructs “and” and “XOR” DNA molecular logic gates for output, and proposes a new semi adder model that can be used for logic operations. Square wave voltammetry (SWV) was used to detect the current change value $\Sigma|\Delta I|$ of the two labeled probes, which was consistent with E. The logarithmic values of the concentrations of coli DNA and Sal DNA showed a good linear relationship in the range of 1.0×10^{-13} to 1.0×10^{-8} mol·L⁻¹, and the detection limits (S/N = 3) were respectively 3.2×10^{-14} mol·L⁻¹ and 1.7×10^{-14} mol·L⁻¹.

Keywords: DNA molecular logic gate; electrochemical detection; half adder; biosensor

In 1959, the American physicist Feynman first proposed the idea of molecular computing, indicating that information processing will develop from the chip age to the molecular age^[1]. In 1994, Adleman first proved through experiments that the calculation process can be carried out at the DNA level^[2]. In the following decades, many research works on molecular logic gates have been reported^[3,4]. The input object of molecular logic gate is two or more complex operations implemented at the molecular or supramolecular level, and the output signal is the logic signal obtained by the corresponding operation, so that this signal is suitable for binary Boolean logic operations of “1” and “0”, so as to realize digital operations^[5]. The construction of computer is based on logical operation. To establish and develop DNA computer, DNA molecular logic gate technology is an insurmountable only way^[6].

Graphene oxide (go) has hydroxyl groups on its surface. Carboxyl. Graphene with epoxy functional groups^[7,8] is widely used in sensors because of its hydrophilicity and good dispersion. Willner research group^[9] used go as an effective base material to realize the amplification detection of dnas and apply it to logic gate operation. This experiment introduces the application of electrochemical detection technology to the simultaneous intelligent detection of *E.coli* DNA and Salmonella DNA. The principle is shown in **Figure 1**.

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Go was modified on the surface of glassy carbon electrode, and then gold nanoparticles (aunps) were electrodeposited. Based on the principle of complementary base pairing and the strong adsorption of go on DNA single strand, the simultaneous detection of two targets was achieved. The experimental results are applied to Boolean logic operations, with two DNA sequences as inputs, which are defined as “1” when they exist, and “0” on the contrary. The electrochemical signals in various states are detected, the appropriate threshold is set, and the “and” and “XOR” DNA molecular logic gates are constructed. On this basis, a new halfadder model with logic operation function is proposed.

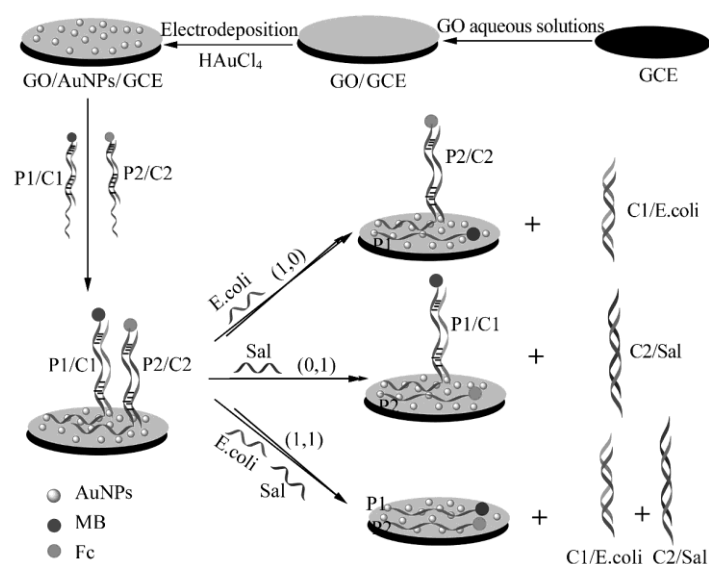


Figure 1. Schematic illustration of the dual–signaling DNA biosensor for the “half adder” construction.

1. Experimental part

1.1. Instruments and reagents

CHI760E electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd.); excellent–e–UV ultra pure water instrument (Chengdu Tangshi Corning Technology Co., Ltd.); JSM7500F field emission scanning electron microscope (Japan, electronics company).

$\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ($\text{Au} \geq 48\%$). Tris–HCl (ultra pure, Aladdin Biochemical Technology Co., Ltd.); $\text{K}_3[\text{Fe}(\text{CN})_6]$, $\text{K}_4[\text{Fe}(\text{CN})_6]$ (Tianjin Damao Reagent Factory), all reagents are analytically pure unless otherwise specified. The oligonucleotide sequence was purchased from Sangong Bioengineering (Shanghai) Co., Ltd. See **Table 1** for the specific sequence. Dissolve in $10 \text{ mmol} \cdot \text{L}^{-1}$ phosphate buffer solution (PBS) ($\text{pH} = 7.4$, containing $100 \text{ mmol} \cdot \text{L}^{-1}$ NaCl) to prepare $10 \mu\text{mol} \cdot \text{L}^{-1}$ of DNA solution, shake well and store at $4 \text{ }^\circ\text{C}$ for standby. The experimental water was ultra pure water ($18.2 \text{ M}\Omega \cdot \text{cm}$).

Table 1. Sequence of oligonucleotides used in this study.

Name	Sequence (5'–3')	Name	Sequence (5'–3')
P1	AAAAAAAAAACGCCGTATCCA–MB	<i>E.coli</i>	AACGCCGATACCA
P2	AAAAAAACTGAAATCCGGGCAT–Fc	OM–Sal	CTGTTTACAGGGCAT
C1	TGGTATCGGCGTT	NCM–Sal	GAGAAATGGCCCGTA
C2	ATGCCCGGTAAACAG	OM– <i>E.coli</i>	AACGCTGATACCA
Sal	CTGTTTACCGGGCAT	NCM– <i>E.coli</i>	TTGCGGCTATGGT

1.2. Electrode treatment and modification

Glass carbon electrode (GCE, diameter 2.0 mm) with 0.3 μm and 0.05 μm of Al_2O_3 powder is continuously polished on suede to a smooth mirror surface, washed with ultra pure water, and polished in HNO_3 (1+1). Ultrasonic drying in absolute ethanol and ultra pure water for 3 min respectively. Spare.

GO was prepared according to the literature method. Before use, ultrasonically disperse 0.10 mg of GO in 1.00 mL of ultrapure water for 20 min to obtain a light black GO suspension of $0.10 \text{ mg}\cdot\text{mL}^{-1}$. Pipette 5.0 μL of GO suspension droplets on the cleaned GCE surface. Lamp-drying to obtain GO-modified GCE, denoted as GO/GCE. The GO/GCE was put into a $6.0 \text{ mmol}\cdot\text{L}^{-1}$ HAuCl_4 solution (containing $0.10 \text{ mol}\cdot\text{L}^{-1}$ KCl), electrodeposited by potentiostatic method at a potential of -0.2 V for 2 min, taken out, rinsed with ultrapure water, and dried to obtain GO/AuNPs/GCE. Aspirate $1 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$ of P1/C1. P2/C2 DNA mixed solution, after annealing treatment, was dropped on the surface of GO/AuNPs/GCE, and stored in the dark for 2 h. The electrode surface was rinsed with $20 \text{ mmol}\cdot\text{L}^{-1}$ $\text{pH} = 7.4$ Tris-HCl buffer to remove unadsorbed DNA molecules. DNA biosensor.

1.3. Electrochemical detection

A three-electrode system was used in all electrochemical experiments: a modified GCE was used as the working electrode, a platinum electrode (2 mm in diameter) was used as the counter electrode, and a saturated calomel electrode (SCE) was used as the reference electrode. Cyclic voltammetry (CV) was used. Square wave voltammetry (SWV) was performed in $10 \text{ mmol}\cdot\text{L}^{-1}$ Tris-HCl buffer ($\text{pH} = 7.4$, containing $140 \text{ mmol}\cdot\text{L}^{-1}$ NaCl. $5 \text{ mmol}\cdot\text{L}^{-1}$ MgCl_2). CV scanning was performed under the potential window of -0.6 – 0.4 V , and the scanning speed was $0.1 \text{ V}\cdot\text{s}^{-1}$. The SWV scan was performed under the potential window of -0.6 – 0.4 V , the pulse potential was 0.04 V , the pulse amplitude was 0.025 V , and the frequency was 10 Hz . Electrochemical impedance (EIS) measurement at $\text{pH} = 7.4$ in $10 \text{ mmol}\cdot\text{L}^{-1}$ PBS (containing $0.10 \text{ mol}\cdot\text{L}^{-1}$ KCl solution. $5.0 \text{ mmol}\cdot\text{L}^{-1}$ $\text{K}_3[\text{Fe}(\text{CN})_6]$ solution. $5.0 \text{ mmol}\cdot\text{L}^{-1}$ $\text{K}_4[\text{Fe}(\text{CN})_6]$ solution), the potential is 0.187 V , the amplitude is 0.05 V , and the frequency range is 1 Hz – 100 kHz .

2. Results and analysis

2.1. Characterization of different modified electrodes

Figure 2 is a graph of the AC impedance of the sensor at different stages of assembly and at different logic input states. Curve a is bare GCE, with low impedance and high electron transfer efficiency; curve b is GO/GCE, with increased impedance, because GO modification reduces electron transfer efficiency; curve c is GCE/GO/AuNPs, with significantly lower impedance than GO/GCE. The impedance of GCE, due to the introduction of AuNPs, greatly improves the electron transfer efficiency, the above results indicate the successful assembly of the electrodes; the curve d is (0, 0) input, P1/C1, P2/C2 form a partially complementary double-stranded “standing” on the electrode surface, the increase in the negative charge density on the electrode surface reduces the electron transfer efficiency between $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and the electrode surface, resulting in an increase in impedance; curves e and f are (1, 0), respectively. In the input and (0, 1) input states, *E. coli* DNA and C1, Sal DNA and C2 are fully complementary hybridized respectively to form double strands that dissociate from the electrode surface, making free P1. P2 single-strands were adsorbed on the electrode surface respectively. Since the DNA single-strand was free to stretch and had a negative charge, it was electrostatically repulsed with the negatively charged $[\text{Fe}(\text{CN})_6]^{3-/4-}$, resulting in hindered electron transfer, so the impedance compared to (0, 0) input has increased; curve g is (1, 1) input, when *E. coli* DNA and Sal DNA are added at the same time, the free P1 and P2 single strands are adsorbed on the electrode surface due to the occurrence of hybridization reaction, further hindering the electron transfer on the electrode surface, the

impedance increases.

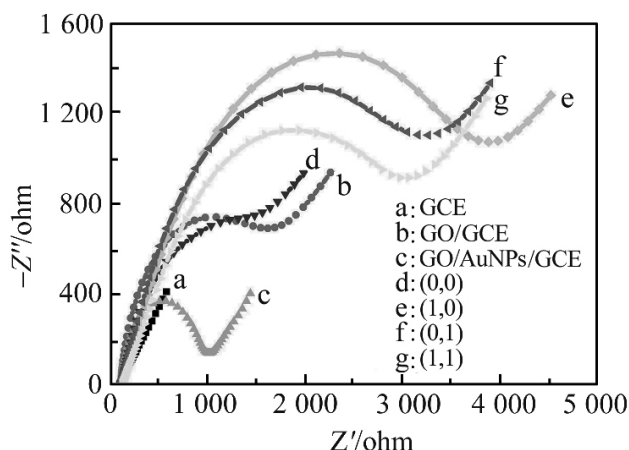


Figure 2. Nyquist diagrams of the different electrodes in 10 mmol·L⁻¹ PBS (containing 0.10 mol·L⁻¹ KCl, 5.0 mmol·L⁻¹ [Fe(CN)₆]^{3-/4-}) (a) Bare GCE; (b) GO/GCE; (c) GO/AuNPs/GCE; (d) (0, 0) input; (e) (1, 0) input; (f) (0, 1) input; (g) (1, 1) input.

Figure 3 shows the scanning electron microscope (SEM) images of different modified electrodes: (A) bare GCE. From the figure, it can be seen that the surface of GCE is smooth, indicating that the electrode is cleaned; (B) For go/gce, the typical fold structure of go can be observed; (C) For go/aunps/gce, nano gold with a particle size of about 100nm can be observed on the surface of go folds; (D) In order to fix the electrode after the labeled probe, it can be observed that the presence of DNA molecules makes aunps cluster.

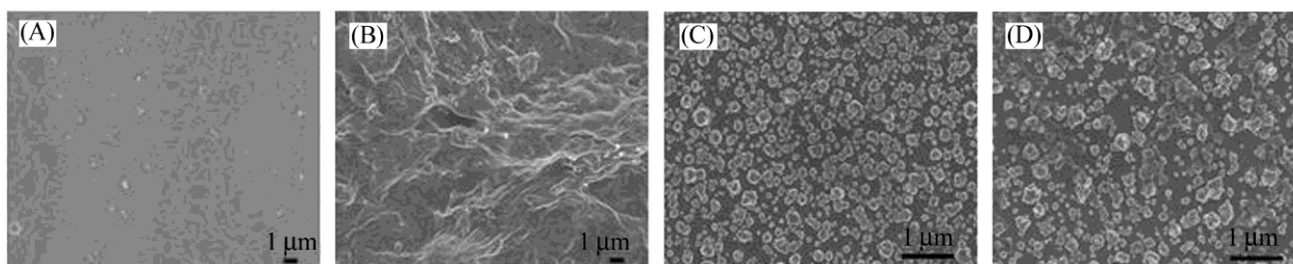


Figure 3. SEM images of different electrodes (A) Bare GCE; (B) GO/GCE; (C) GO/aunps/GCE; (D) GO/aunps/GCE with probes immobilized.

2.2. Optimization of experimental conditions

The effects of the modified electrode and the hybridization time of the target on the electrochemical signal were investigated. **Figure 4A** shows the electrochemical response of different modified electrodes, and curve a shows go/gce; curve B is go/aunps/gce, and the probe is modified for 2 h. (1, 1) under the input condition, the hybridization time of the target is the same, and the signal of curve B is significantly stronger than that of curve a, indicating that the existence of aunps increases the specific surface area of the electrode, which increases the amount of bound probes, and its good electron transfer function helps to enhance the electrochemical signals of MB and FC in the system. Therefore, go/aunps/gce modified electrode was selected. **Figure 4B** is the optimization of the hybridization time of the target. 10 μL of 1.0 μmol·L⁻¹ *E.coli* DNA and SalDNA mixture was added dropwise to the biosensor, and the measurement was performed every 1 h. The signal of Fc increased slowly at first, and then increased for 3 h. It almost reached a plateau at 4 h, but then dropped sharply after 4 h; the signal of MB also increased first, almost reached a plateau at 4 h, and then had no significant change (inset), so the hybridization time was selected as 4 h.

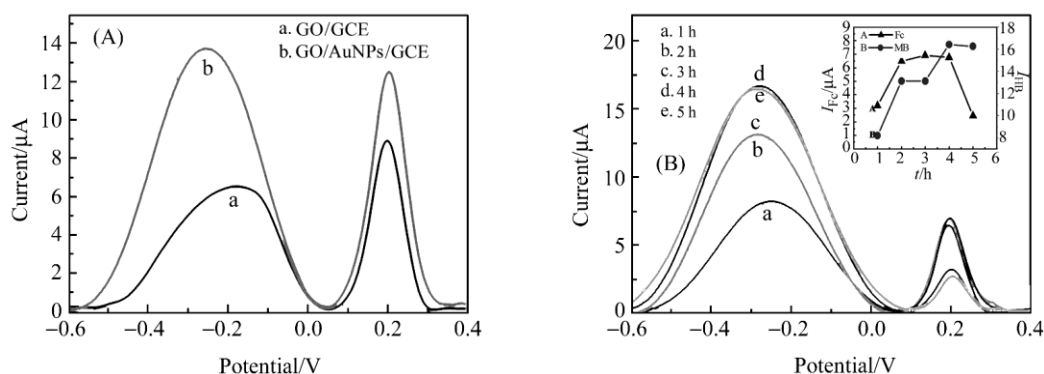


Figure 4. Optimization of detection conditions. (A) Choice of different modified electrodes, (a) GO/GCE and (b) GO/AuNPs/GCE; (B) Effect of target hybridization time on the response of biosensor.

2.3. Detection of cyclic voltammetry and square wave voltammetry in different input states

Under the optimal conditions, the CV and SWV of different input states are characterized, and the results are shown in **Figure 5**. At (0, 0) input, P1/C1 and P2/C2 form a partially hybridized double chain “standing” on the electrode surface respectively. FC and MB are far away from the electrode surface, and the electron transfer is slow. Very weak signals of FC and MB are detected (curve a); (1, 0) when inputting, *e.coli dna* and C1 form a DNA double strand and dissociate from the electrode surface, the replaced P1 single strand is adsorbed on the electrode surface, the distance between MB and the electrode surface decreases, the signal of MB increases, and the signal of FC basically remains unchanged; (0, 1) when input, C2 and *saldna* form a double strand and dissociate from the electrode surface, the replaced P2 single strand is completely adsorbed on the electrode surface, the distance between FC and the electrode surface decreases, the signal of FC increases, and the MB signal remains basically unchanged; (1, 1) when inputting, *e.coli dna* and C1, *saldna* and C2 are completely complementary, forming double strands to dissociate from the go surface, and the displaced P1 and P2 single strands are adsorbed on the electrode surface. The distance between MB and FC and the electrode surface is close, so the signals of MB and FC will increase (curve d).

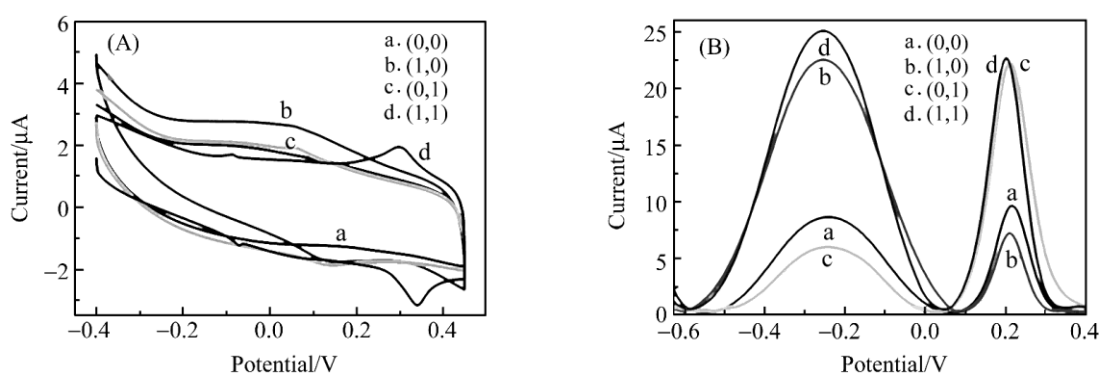


Figure 5. CV curves (A) and SWV curves (B) of the logic gates under different inputs state (a) (0, 0) input; (b) (1, 0) input; (c) (0, 1) input; (d) (1, 1) input.

2.4. Construction of “and” and “XOR” molecular logic gates

Take *e.coli dna* and *saldna* as inputs, and $\Sigma|\Delta I|$ ($\Sigma|\Delta I| = |\Delta I_{FC}| + |\Delta I_{MB}|$, $\Delta I = I - I_0$, I is the current signal after adding the target, I_0 is the current signal before adding the target) as the output, set $15 \mu\text{A}$ is the threshold, when $x > 15 \mu\text{A}$, the output is 1; when $x < 15 \mu\text{A}$, the output is 0, and the “and” type DNA molecular logic gate is constructed. The truth table is shown in **Figure 6B**.

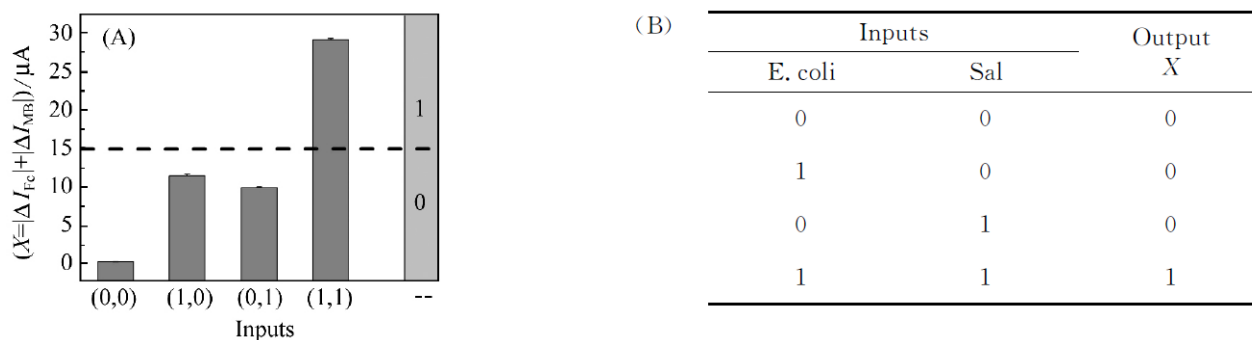


Figure 6. The result of “AND” logic gate. (A) The bar diagrams $\Sigma|\Delta I|$ detection by SWV, the dotted line indicates the threshold (15 MA); (B) The truth table of “AND” logic gat.

Take *e.coli* dna and saldna as inputs and “Y” = $|\Delta IMB/\Delta IFC|$ “as the output, set the threshold interval as 0.5–1.5, and when $y > 1.5$ or $y < 0.5$, the output is 1; when $y \in (0.5, 1.5)$, the output is 0, and the “XOR” DNA molecular logic gate is constructed. The truth table is shown in **Figure 7B**.

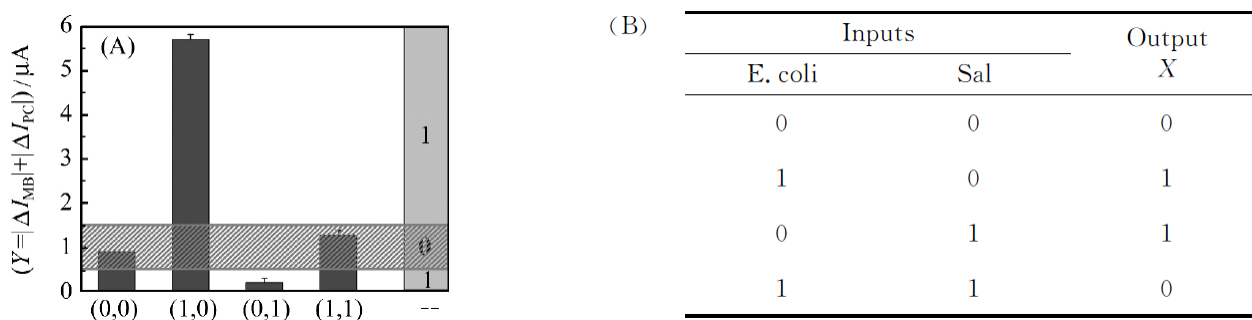


Figure 7. The result of “XOR” logic gate. (A) The bar diagrams $|\Delta IMB/\Delta IFC|$ detection by SWV, the dotted line indicates the threshold range (0.5–1.5); (B) The truth table of “XOR” logic gate.

2.5. Construction of half adder

The constructed “and” logic gate and “XOR” logic gate are integrated to form a half adder with logic function. The semi adder analyzes two kinds of molecular signals and outputs two different molecular signals according to the following rules: (1) when there is no input signal, the system does not change and there is no output; (2) When there is an input molecular signal, only sumbitoutput is activated; (3) When there are two input signals, only carrybitoutput is activated. The function of sum bit output is performed by “XOR” logic gate, while carry output is performed by “and” logic gate. The half adder only considers the addition of two one bit binary numbers, and does not consider the operation circuit from the low carry digit. See **Table 2** for the truth table.

Table 2. The truth table for half adder.

Input		Output		
A	B	Carry	Result	Sum
0	0	0	0	00
0	1	0	1	01
1	0	0	1	01
1	1	1	0	10

2.6. Detection range and detection limit of DNA biosensor

Under the optimal experimental conditions, the two target DNAs were measured respectively, and the results are shown in **Figure 8**. The concentrations of *E.coli* DNA and SalDNA are both in the range of 1.0×10^{-13} – 1.0×10^{-8} mol·L⁻¹, the logarithm of the target concentration and $\Sigma|\Delta I|$ (the sum of Fc and MB signal changes) have a good linear relationship, and the linear equations are: $\Sigma|\Delta I| = 32.03 + 2.17\log c_{E.coli}(c:\text{mol}\cdot\text{L}^{-1}, R = 0.9931)$, $\Sigma|\Delta I| = 34.14 + 2.49\log c_{Sal}(c:\text{mol}\cdot\text{L}^{-1}, R = 0.9967)$, the detection limits ($S/N = 3$) were 3.2×10^{-14} mol·L⁻¹ and 1.7×10^{-14} mol·L⁻¹.

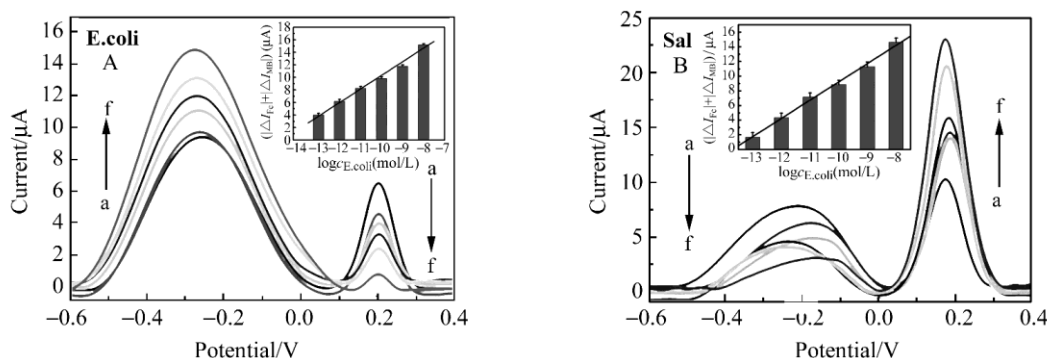


Figure 8. (A) SWV responses with different concentration of *E.coli* DNA; (B) SWV responses with different Concentration of Sal DNA.

From a to f: the concentration was 1.0×10^{-13} , 1.0×10^{-12} , 1.0×10^{-11} , 1.0×10^{-10} , 1.0×10^{-9} and 1.0×10^{-8} Mol·L⁻¹.

2.7. Sensor selectivity. Investigation of stability and reproducibility

In order to investigate the selectivity of the biosensor, target sequences were detected. One base mismatch (OM). Complete mismatch (NCM)DNA sequence response. When the mismatched base sequence is added, the signal is significantly weaker than that when the target sequence is added. This is because the mismatched DNA cannot specifically hybridize with the corresponding complementary sequence, which not only fails to open p_1/c_1 and p_2/c_2 chains, but also occupies more sites on the surface of go. The results show that the sensor has good selectivity.

The biosensor was prepared as described in 1.2. The two targets were detected at the same time, and the electrode was stored in a refrigerator at 4 °C for a week, and then detected by SWV method. The response signal did not change greatly, indicating that the DNA sensor was stable. Three identical biosensors were prepared by the same modification method. Under the same experimental conditions, the electrochemical response of (1, 1) input state was detected by SWV method, and the calculated relative standard deviation (RSD) was 3.8%, indicating that the DNA sensor had good reproducibility.

3. Conclusion

In this study, a graphene oxide/gold nanoparticle composite film–modified glassy carbon electrode was prepared by drop coating and electrodeposition as working electrode, Fc and MB as electrochemical indicators, and *E.coli* DNA and SalDNA as target molecules. The labeled DNA biosensor can realize the simultaneous intelligent analysis of *E.coli* DNA and SalDNA, the detection range is 1.0×10^{-13} – 1.0×10^{-8} mol·L⁻¹, and the detection limits ($S/N = 3$) are respectively 3.2×10^{-14} mol L⁻¹ and 1.7×10^{-14} mol L⁻¹. The research results show that the electrochemical sensor has good stability. Easy to prepare. With the advantages of low cost and good reproducibility, the established analytical method has high sensitivity and wide linear range. According to the experimental results, with *E.coli* DNA and SalDNA as input, and the sum of probe signal changes $\Sigma|\Delta I|$ as output, an “AND” type DNA molecular logic gate was constructed, and the ratio of probe signal changes

$|\Delta I_{MB}/\Delta I_{Fc}|$ was used as output The “XOR” type DNA molecular logic gate is constructed, and a new half-adder model is proposed, which can realize the addition of two binary numbers.

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Conflict of interest

The authors declare no conflict of interest.

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