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# Ultrasensitive and Highly Selective Electrochemical Biosensor for HIV Gene Detection Based on Amino-Reduced Graphene Oxide and β-cyclodextrin Modified Glassy Carbon Electrode

*Jiawen Li, Xiaoyu Jin, Mengmeng Feng, Shan Huang*[*]*, Jinrong Feng*[*]

College of Chemistry and Materials Science, Guangxi Key Laboratory of Natural Polymer Chemistry and Physics, Nanning Normal University, Nanning 530001, P. R. China
[*]E-mail: huangs@whu.edu.cn, 1278281915@qq.com

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A differential pulse voltammetry (DPV) platform for the sensing of human immunodeficiency virus (HIV) gene was described through the self-assembling of amino-reduced graphene oxide ($NH_2$-rGO) and β-cyclodextrin (β-CD) on the surface of glassy carbon electrode (GCE). It is making use of the single-stranded DNA (ssDNA) with sequence hybridized with HIV gene. The ssDNA was attached on $NH_2$-rGO/β-CD modified GCE ($NH_2$-rGO/β-CD/GCE) surface, then methylene blue (MB) was interacted with ssDNA to general obvious DPV signal. The HIV gene was then initiated to hybridize with ssDNA on $NH_2$-rGO/β-CD/GCE surface, causing a dramatic decrease in DPV response of MB at potential of −0.25 V (vs. Ag/AgCl). Under optimal experimental conditions, response was linear in the 0.05 pM to 1 pM HIV gene concentration range. The electrochemical biosensor displayed an ultrahigh sensitivity with detection limit of 8.7 fM and excellent selectivity. The modified electrode displayed good reproducibility, ultra-high sensitivity, and terrific selectivity. This electrochemical biosensor was successfully used for the determination of HIV gene in human serum samples.

**Keywords:** HIV gene; Differential pulse voltammetry; Amino-reduced graphene oxide; Biosensor; β-Cyclodextrin

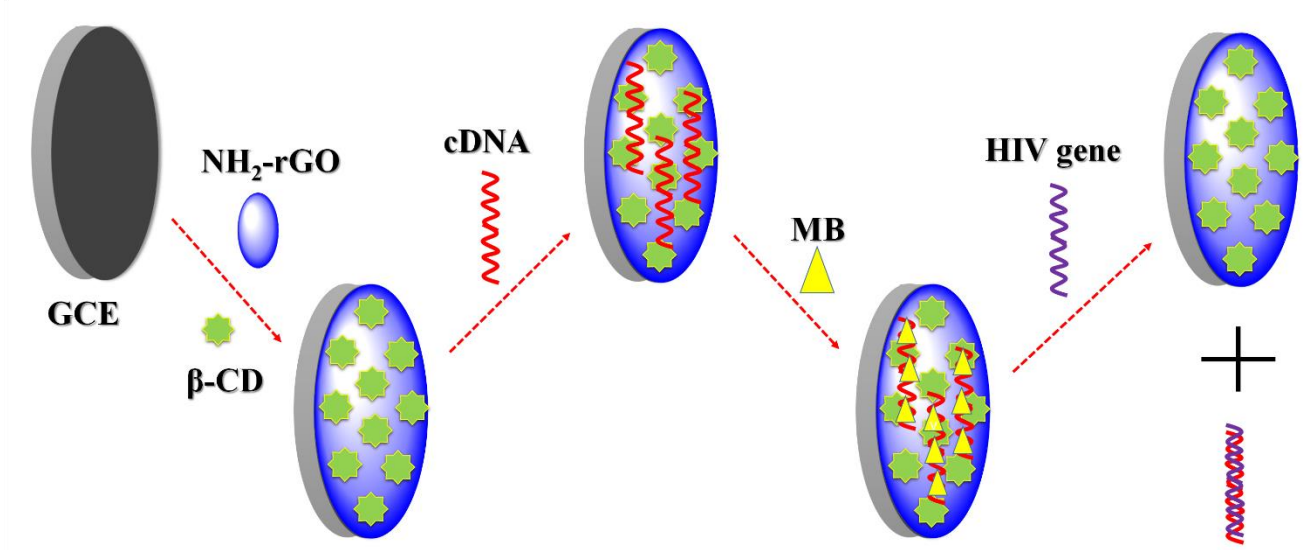
## 1. INTRODUCTION

Human immunodeficiency virus (HIV) is a kind of serious viruses in retrovirus group. HIV infection causes one of the most severe diseases−acquired immunodeficiency syndrome (AIDS) that causes numerous deaths in the world [1,2]. Since HIV genes can attack human immune system and destruct the T4 lymphocytes, the human body is unable to defend itself against several diseases, leading to the death of human. Therefore, rapid, highly specific, and ultrasensitive HIV gene detection system is required to facilitate identification of early diagnosis, prevention of virus's propagation, and clinical

therapy of AIDS. Toward this goal, many HIV gene detection strategies were proposed and used in laboratory areas, including surface enhanced raman scattering [3], colorimetric method [4], light scattering [5], and fluorescence spectrometry [6]. Nevertheless, these methods and techniques are ponderous, expensive, and complicated for clinical diagnosis. In contrast, electrochemical techniques have several advantages over the above methods for HIV gene because of its easy operation, high stability, high sensitivity, and good sensitivity [7−11]. Especially, differential pulse voltammetry (DPV) can simultaneously detect HIV gene in complex biological matrix without elimination processes. Li et al. reported the electrochemical biosensor for HIV gene sensing via DPV with detection limit of 0.21 pM [12].

Graphene oxide (GO) is one kind of oxidized derivative of graphene with single atom-thick and two-dimensional structure. The phenol hydroxyl, epoxide groups, and carboxylic groups of GO make it be widely used for developing electrochemical sensors for several targets [13−15]. In particular, due to its extraordinarily good electronic transport properties, amino-reduced graphene oxide ($NH_2$-rGO) becomes a good electrode material for constructing electrochemical-based sensing assays [16,17]. Liu et al. reported a $NH_2$-rich graphene nanostructure modified electrode for the detection of Cu(II) [18]. Our group described an electrochemical platform for the determination of Cu(II) in real water samples via $NH_2$-rGO and β-cyclodextrin (β-CD) modified glassy carbon electrode (GCE) with wider linear range and lower detection limit [19].

Herein, an ultrasensitive and highly selective electrochemical biosensor for HIV gene detection via DPV analysis based on $NH_2$-rGO/β-CD composite modified GCE ($NH_2$-rGO/β-CD/GCE) was fabricated (Scheme 1). In this electrochemical biosensor, $NH_2$-rGO is used to enhance the proton conductivity of GCE and facilitate the accumulation of the complementary HIV gene sequence. Meanwhile, β-CD is used to promote the dispersion of bulk $NH_2$-rGO [19]. The $NH_2$-rGO nanosheets can strongly bind with the complementary HIV gene sequences (capture DNA) via the hydrophobic and the $\pi - \pi$ stacking interactions between nucleobases and nanosheets, so such interactions can protect the dissociation of the capture DNA from the $NH_2$-rGO nanosheets. Methylene blue (MB), which is a widely used organic phenothiazine dye during chemical sensing processes, can bind to single-stranded DNA (ssDNA) sequences via guanine bases present at ssDNA and electrostatic interaction [20]. In the absence of HIV gene, the capture DNA adsorbs onto the surface of $NH_2$-rGO/β-CD/GCE and subsequently stimulates the assembly of MB molecules on $NH_2$-rGO/β-CD/GCE surface, leading to a strong DPV signal coming from the adsorbed MB molecules. After the addition of HIV gene, HIV gene hybridizes with the capture DNA to construct double-stranded DNA (dsDNA). The dsDNA shows weak binding ability with $NH_2$-rGO/β-CD composite, leading to the desorption of dsDNA from the $NH_2$-rGO/β-CD/GCE surface. Therefore, the amount of MB absorbed on $NH_2$-rGO/β-CD/GCE surface is much less compared to its initial amount, resulting in the decrease of the DPV signal. Consequently, the selectivity and sensitivity for HIV gene determination are all increased by using $NH_2$-rGO/β-CD composite and the specific HIV gene capture DNA. The fabricated $NH_2$-rGO/β-CD/GCE can be used to detect HIV gene in human serum samples with good recovery. This work displays a promising electrochemical sensing path for developing ultrasensitive and highly selective electrochemical biosensor to detect some serious disease-related viral genes.




**Scheme 1.** Schematic of the construction of the electrochemical biosensor and its application for HIV gene.

## 2. MATERIALS AND METHODS

### *2.1 Reagents*

$NH_2$-rGO was obtained from XFNANO Materials Tech. Co. Ltd. (http://www.xfnano.com/). All ssDNA sequences were purchased from Shanghai Sangon Biological Engineering Technological Co. Ltd. (http://www.sangon.com/) and purified by high-performance liquid chromatography. The sequences of ssDNA were list as below:

Capture DNA (cDNA): 5'-GGG TGG AAA ATC TCT AGC A-3';

Target HIV gene: 5'-ACT GCT AGA GAT TTT CCA CAT-3';

Single-base mismatch DNA (T1-DNA): 5'-AC**A** GCT AGA GAT TTT CCA CAT-3';

Four-base mismatch DNA (T2-DNA): 5'-AC**A** GC**C** A**C**A G**G**T TTT CCA CAT-3';

Non-complementary DNA (N-DNA): 5'-CAG TAG CTG TCG GGG ATA AGC-3';

Hepatitis B virus (HBV) gene (B-DNA): 5'-CTG GAT CCT GCG CGG GAC GTC CTT-3';

Hepatitis C virus (HCV) gene (C-DNA): 5'-GGC GAC GCG GGA TCC GAC GTT-3';

Human papilloma virus (HPV) gene (P-DNA): 5'-CAA GCA GAA CCG GAC AGA CCC CAT-3'.

All ssDNA sequences were dissolved in 10 mM Tris-HCl (pH 7.4) buffer with 1 mM ethylenediamine tetracetic acid (EDTA). All solution was stored at 4 °C. Human serum albumin (HSA), bovine serum albumin (BSA), and other biological reagents were purchased from Aladdin (http://www.aladdin-e.com/us_en/). MB, β-CD, and other chemical reagents were purchased from Sinopharm Chemical Reagent Factory (http://en.reagent.com.cn/). All reagents were of analytical grade and used as received without any further purification.

*2.2 Apparatus*

Electrochemical experiments were performed on Chenhua CHI-660E electrochemical workstation (http://www.chinstr.com/). Ag/AgCl electrode and platinum wire were employed as the reference and the counter electrodes. GCE and modified GCEs were used as working electrode. All pH measurements were made with Sartorius basic PB-10 pH meter (https://www.sartorius.com.cn/sartoriusCN/zh/CNY). Ultrapure water with resistivity of 18.2 MΩ cm was produced by Millipore-Q Academic purification set (http://www.merckmillipore.com/).

*2.3 Fabrication of electrochemical biosensor*

GCE was firstly polished with 1.0 μm, 0.3 μm, and 0.05 μm alumina slurry, then GCE was rinsed thoroughly with ultrapure water. Finally, GCE was sonicated in 0.5 M $H_2SO_4$, ethanol, and ultrapure water subsequently for 5 min. 10 mg $NH_2$-rGO and 20 mg β-CD were dispersed in 10 mL ultrapure water, then the mixture was sonicated for 30 min to form a homogenous dispersion. After that, 5 μL $NH_2$-rGO/β-CD dispersion was dropped onto the surface of GCE and dried under an infrared lamp. $NH_2$-rGO/β-CD/GCE was finally rinsed with ultrapure water to remove the loose adsorbed materials.

*2.4 Procedures for HIV gene determination*

Firstly, 5 μL cDNA (3 μM) was dropped onto the $NH_2$-rGO/β-CD/GCE surface. Then the electrode was incubated at 4 °C for overnight. Then, the electrode was rinsed with 10 mM phosphate buffer saline (PBS, pH 7.4) to obtain cDNA/$NH_2$-rGO/β-CD/GCE. Such cDNA/$NH_2$-rGO/β-CD/GCE was immersed in MB solution (20 mM) for stirring with 30 min to adsorb MB molecules onto the surface of cDNA/$NH_2$-rGO/β-CD/GCE. The electrode was rinsed with 10 mM PBS (pH 7.4) to obtain MB/cDNA/$NH_2$-rGO/β-CD/GCE. The DPV responses of MB/cDNA/$NH_2$-rGO/β-CD/GCE to increasing concentration of HIV gene in 20 mM Tris-HCl (pH 7.4) were carefully investigated. Electrochemical measurements were carried out with potential range of −0.5 to 0 V, pulse amplitude of 0.05 V, pulse width of 0.05 s, sampling width of 0.0167 s, pulse period of 0.5 s, and quiet time of 2 s. Cyclic voltammetry (CV) experiments were carried out in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution with 0.1 M KCl. CV experiments were carried out with potential range of −0.1 to 0.6 V and scan rate of 50 mV $s^{-1}$. Electrochemical impedance spectrometry (EIS) experiments were performed in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution with 0.1 M KCl in the frequency range of $10^5$ to 0.1 Hz.

*2.5 Samples preparation*

Human serum samples were obtained from the volunteers with healthy body and informed consent. All experiments were performed in compliance with the laws and institutional guidelines. For getting rid of blood cells, these samples were centrifuged at 10000 g for 5 min. Perchloric acid ($HClO_4$, 20% v/v) was added into the solution to precipitate proteins in human plasma samples. Then, the samples

were vigorous stirred for 5 min and centrifuged at 10000 g for 20 min. The supernatant was collected and stored at 4 °C. Then, human serum samples were diluted 100 times with 10 mM Tris-HCl (pH 7.4) with 10 mM EDTA and different concentration of target HIV gene was added into the reaction system. Final concentrations of HIV gene were 0.1 pM and 0.5 pM, respectively. The recovery of HIV gene in real human serum samples was determined by the method.

## 3. RESULTS AND DISCUSSION

### *3.1 Electrochemical characterization of electrodes*

CV responses of 5 mM $[Fe(CN)_6]^{3-/4-}$ at different electrodes surfaces in 0.1 M KCl solution were shown in Fig. 1A. It seems that the redox peak currents of $[Fe(CN)_6]^{3-/4-}$ increase apparently after the modification of $NH_2$-rGO and $NH_2$-rGO/β-CD on GCE surface, suggesting the better electron transfer ability of $NH_2$-rGO [19]. However, β-CD hinders the electron transfer efficiency of $[Fe(CN)_6]^{3-/4-}$ toward GCE surface [19]. All these phenomena are highly consisting with the results reported before [19]. After the continuous deposition of cDNA, the redox peak currents of $[Fe(CN)_6]^{3-/4-}$ toward $NH_2$-rGO/β-CD/GCE surface decrease significantly. The large steric hindrance and the electrostatic repulsive-force of cDNA observably hinder the electron transfer process of $[Fe(CN)_6]^{3-/4-}$ at $NH_2$-rGO/β-CD/GCE surface [21]. In addition, the assembly of MB on cDNA/$NH_2$-rGO/β-CD/GCE surface facilitates the electron transfer and accelerates the diffusion of $[Fe(CN)_6]^{3-/4-}$ to the electrode surface (Fig. 1A). The results obtained from EIS highly agrees well with the result obtained by CV (Fig. 1B).

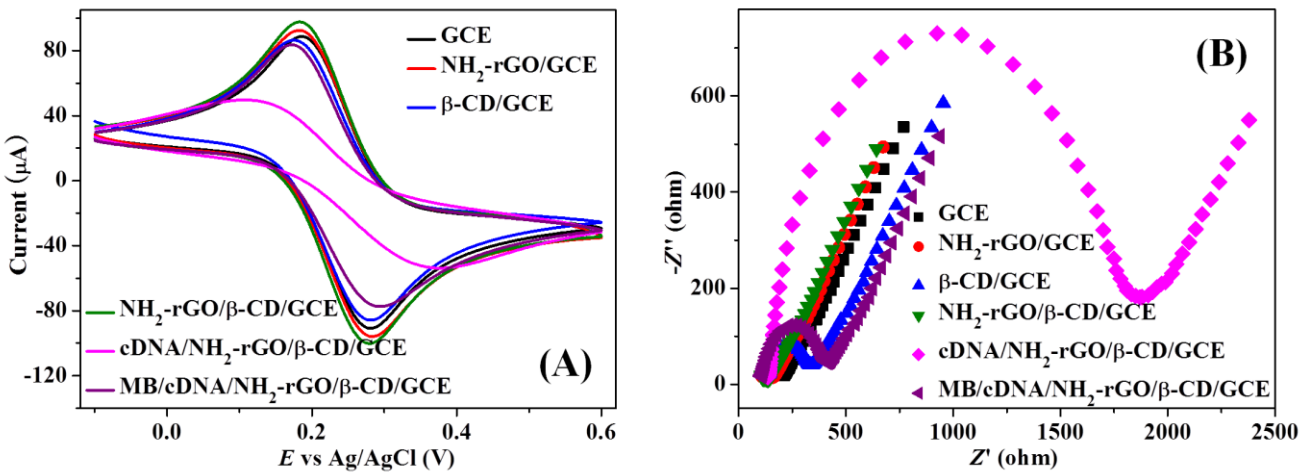



**Figure 1.** (A) CV responses of $[Fe(CN)_6]^{3-/4-}$ at different electrodes surfaces in 0.1 M KCl solution from −0.1 and 0.6 V with scan rate of 50 mV $s^{-1}$. (B) Nyquist plots of $[Fe(CN)_6]^{3-/4-}$ at different electrodes surfaces in 0.1 M KCl solution over the frequency range of $10^5$ Hz to 0.1 Hz.

Electrochemical property of electrode was compared by calculating the standard electrochemical rate constant ($k^{\Theta}$) through the Nicholson's method [22]. Fig. 2 showed the characteristics of quasi-reversibility of different modified electrodes. The $k^{\Theta}$ value is determined via the equations [22,23]:

$$\psi = k^{\Theta}(\frac{D_O}{D_R})^{\alpha/2}\sqrt{\frac{RT}{\pi nFD_O v}} \qquad (1)$$

$$\psi = \frac{-0.6288 + 0.0021n\Delta E_p}{1 - 0.017n\Delta E_p} \qquad (2)$$

In equations (1) and (2), $\psi$ is the dimensionless kinetic parameter and $\alpha$ is the charge transfer coefficient ($\alpha$ = 0.5), respectively [23]. $\Delta E_p$ is the peak potential separation. $D_O$ ($D_O$ = 7.63 × $10^{-6}$ $cm^2$ $s^{-1}$) and $D_R$ ($D_R$ = 6.32 × $10^{-6}$ $cm^2$ $s^{-1}$) are the diffusion coefficients of $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$, respectively [24]. $F$, $R$, and $T$ have their common meanings. According to the inserts in Fig. 2, the calculated $k^{\Theta}$ values of $[Fe(CN)_6]^{3-/4-}$ at the surfaces of GCE, $NH_2$-rGO/GCE, β-CD/GCE, and $NH_2$-rGO/β-CD/GCE were about 4.71 × $10^{-3}$, 4.88 × $10^{-3}$, 3.96 × $10^{-3}$, and 5.22 × $10^{-3}$ cm $s^{-1}$, respectively. The $k^{\Theta}$ value of $NH_2$-rGO/β-CD/GCE is higher than those of other electrodes, indicating much faster electron transfer kinetics of $[Fe(CN)_6]^{3-/4-}$ toward $NH_2$-rGO/β-CD/GCE.

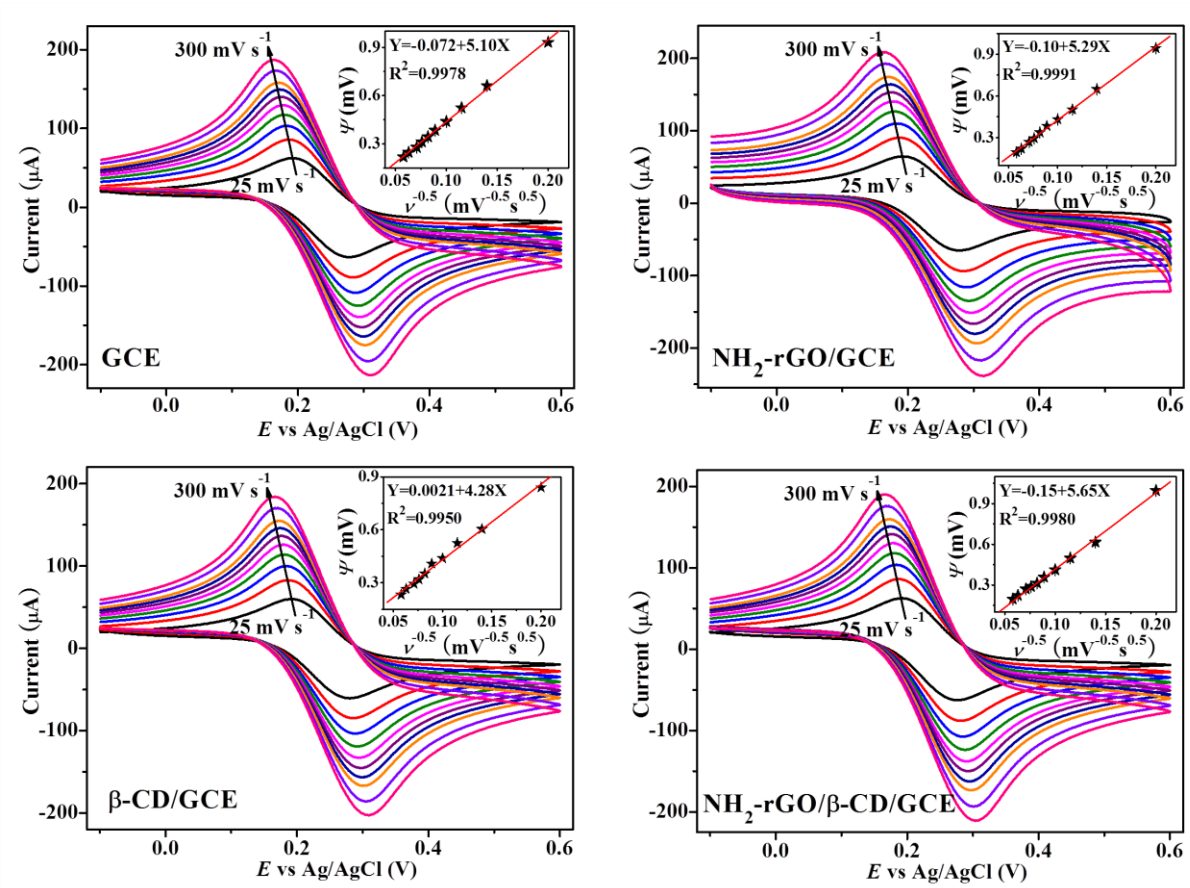



**Figure 2.** CVs and associated kinetic analyses of $[Fe(CN)_6]^{3-/4-}$ toward GCE, β-CD/GCE, $NH_2$-rGO/GCE, and $NH_2$-rGO/β-CD/GCE in 0.1 M KCl solution. Insert were the plots of $\psi - \nu^{-1/2}$ curves. Sweep rates: 25, 50, 75, 100, 125, 150, 175, 200, 250, and 300 mV $s^{-1}$.

Chronocoulumetry curves of different electrodes were recorded by using 0.1 mM $K_3Fe(CN)_6$ as the model complex [24]. The effective surface area ($A$) of different electrode is calculated according to the equation [25]:

$$Q(t) = \frac{2nFAcD_O^{1/2}t^{1/2}}{\pi^{1/2}} + Q_{dl} + Q_{ads} \qquad (3)$$

Herein, $Q(t)$ is the charge of electron transfer at specific time ($t$), $n$ is the number of electron transfer, $c$ is the concentration of substrate, $Q_{dl}$ is the double layer charge, and $Q_{ads}$ is the Faradic charge, respectively. Fig. 3A and Fig. 3B showed the plots of $Q - t$ and $Q - t^{1/2}$ curves of 0.1 mM $K_3Fe(CN)_6$ at the surfaces of GCE, $NH_2$-rGO/GCE, β-CD/GCE, and $NH_2$-rGO/β-CD/GCE in 0.1 M KCl solution with the potentials swept from 0.15 to 0.25 V. According to the slope of $Q - t^{1/2}$ curves, the calculated $A$ value of $NH_2$-rGO/β-CD/GCE is 0.216 $cm^2$, which is much higher than the effective surface areas of GCE (0.086 $cm^2$), $NH_2$-rGO/GCE (0.159 $cm^2$), and β-CD/GCE (0.067 $cm^2$). The current response signal will be enhanced at $NH_2$-rGO/β-CD/GCE surface with high effective surface area, which is good for the construction of ultrasensitive electrochemical platform for HIV gene detection [19].

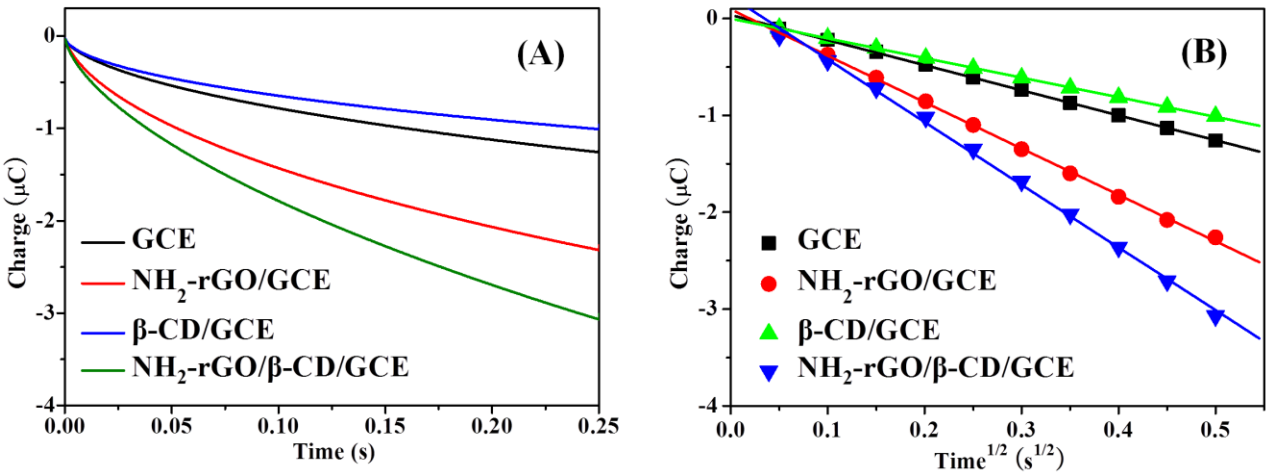



**Figure 3.** Plots of $Q - t$ (A) and $Q - t^{1/2}$ (B) curves of 0.1 mM $K_3Fe(CN)_6$ at the surfaces of GCE, β-CD/GCE, $NH_2$-rGO/GCE, and $NH_2$-rGO/β-CD/GCE in 0.1 M KCl solution with the potentials swept from 0.15 to 0.25 V.

### *3.2 Detection of HIV gene*

For sensitive detection of trace HIV gene with MB/cDNA/$NH_2$-rGO/β-CD/GCE, we optimized these detection parameters: (a) Amount of $NH_2$-rGO; (b) Concentration of cDNA; (c) Buffer pH value; (d) Incubation time of MB; (e) Reaction time of HIV gene. The DPV peak current reduction value ($\Delta I = I - I_0$) is used as the criterion to optimal the experimental conditions. $I_0$ and $I$ are the DPV peak current of the electrode without and with HIV gene, respectively. As shown in Fig. 4, these experimental conditions were found to give best results: (a) 1.0 mg $mL^{-1}$ $NH_2$-rGO; (b) 3 μM cDNA; (c) Buffer with pH 7.4; (d) Incubation time of MB of 30 min; (e) Reaction time of HIV gene of 20 min.

Under these optimum conditions, MB/cDNA/$NH_2$-rGO/β-CD/GCE was employed for the determination of HIV gene through DPV technique. Fig. 5A showed the DPV responses of MB/cDNA/$NH_2$-rGO/β-CD/GCE for increasing concentration of HIV gene from 0.05 to 2000 pM. It is noticeable that the DPV signal decreases as the concentration of HIV gene increases (Fig. 5A). Furthermore, a good relationship is obtained between the Δ*I* value and the logarithm value of HIV gene concentration (Fig. 5B).

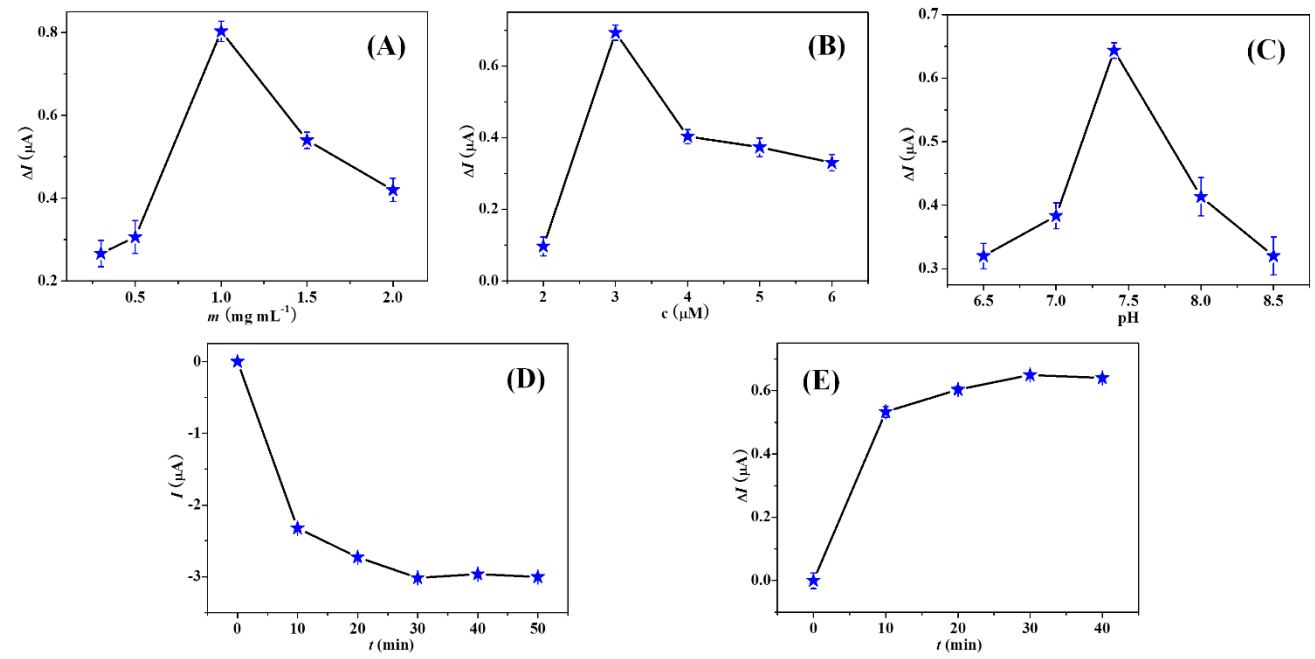



**Figure 4.** Influences of amount of $NH_2$-rGO (A), concentration of cDNA (B), buffer pH value (C), incubation time of MB (D), and reaction time of HIV gene (E) on the determination of HIV gene.

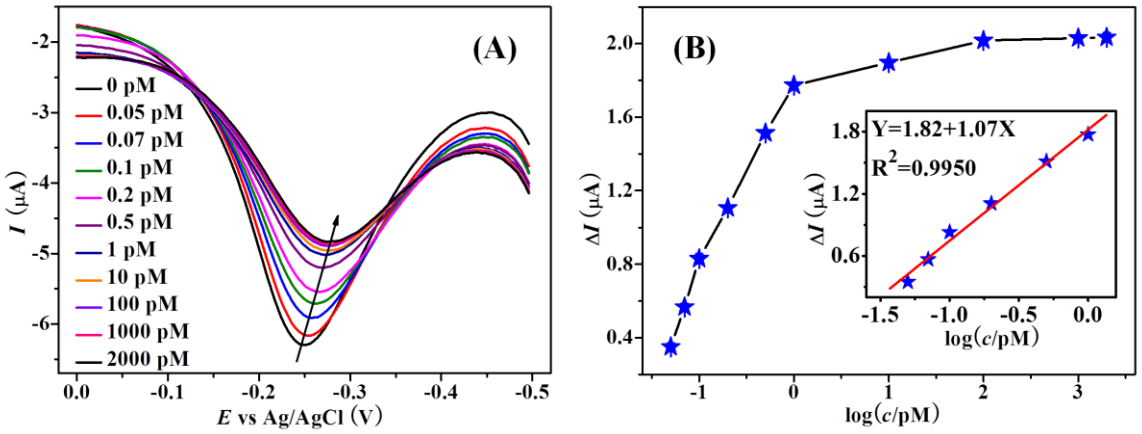



**Figure 5.** (A) DPV responses of MB/cDNA/$NH_2$-rGO/β-CD/GCE for increasing concentration of HIV gene from 0.05 to 2000 pM. (B) DPV peak current reduction value versus the logarithm value of HIV gene concentration. Inset was the corresponding calibration curve. Results were expressed as the average of three independent experiments. Error bars represent standard deviations.

When the HIV gene concentration is in the range of 0.05 to 1 pM, the Δ*I* value at negative potential of around −0.25 V is directly proportional to the logarithm value of HIV gene concentration with linear regression equation of Δ*I* (μA) = 1.07 log[*c*/(pM)] + 1.82 and correlation coefficient of 0.9950. Due to the high loading amount and the efficient electrochemical signal of MB on cDNA/$NH_2$-rGO/β-CD/GCE surface, the detection limit of HIV gene is 8.7 fM at 3*S*/*N* (*S* and *N* are the signal and noise, respectively). Compared with some electrochemical methods for different nucleic acids [26−30], such strategy possesses considerable sensitivity and relatively low detection limit for HIV gene with such simple procedure of electrode preparation (Table 1).

**Table 1.** Comparison of different electrochemical methods for different nucleic acids.

| Modifier | Technique | Linear range (nM) | Detection limit (pM) | Ref |
|---|---|---|---|---|
| DNA/GE | Amperometric | 5 − 500 | 7.05 | 11 |
| DNA/TSDR/SPE | DPV | 0.001 − 100 | 0.21 | 12 |
| CS/$Fe_3O_4$/SPE | DPV | 0.05 – 0.3 | 50 | 26 |
| Nafion–graphene/SPCE | DPV | 40 − 2560 | 5000 | 10 |
| Exo III and guanine nanowire/GE | Amperometric | 0.01 – 100 | 3.6 | 9 |
| DNA/TSDR/GE | DPV | 0.001 − 10 | 0.88 | 8 |
| Multi-wall carbon nanotubes/GCE | DPV | 10 − 400 | 141.2 | 27 |
| Enzyme and gold nanoparticles | DPV | 0.2 – 388 | 100 | 28 |
| DNA hybridization/SPE | DPV | 0.04 − 400 | 0.65 | 29 |
| Silver-dendrimer/GCE | ASV | 0.1 – 0.3 | 0.78 | 30 |
| $NH_2$-rGO/β-CD/ GCE | DPV | 0.00005 – 0.001 | 0.0087 | This work |

*3.3 Selectivity, reproducibility and stability*

Specificity of the electrochemical biosensor was investigated by detecting different biomolecules, such as T1-DNA, T2-DNA, N-DNA, B-DNA, C-DNA, P-DNA, HSA, and BSA. Fig. 6A showed the DPV responses of MB/cDNA/$NH_2$-rGO/β-CD/GCE for these different biomolecules. As exhibited in Fig. 6B, the Δ*I* value responses to 1 pM N-DNA, 2 pM HSA, 2 pM BSA, 2 pM B-DNA, 2 pM C-DNA, and 2 pM P-DNA are very small which can be omitted within experimental errors. Although the Δ*I* value responses to 1 pM T1-DNA and 1 pM T2-DNA are relative higher than other interferences, the Δ*I* value

response to 0.1 pM HIV gene is about three or seven times higher than those of T1-DNA and 1 pM T2-DNA, respectively. These results confirm the high selectivity of this electrochemical biosensor for target HIV gene, which implies the potential application for real samples detection.

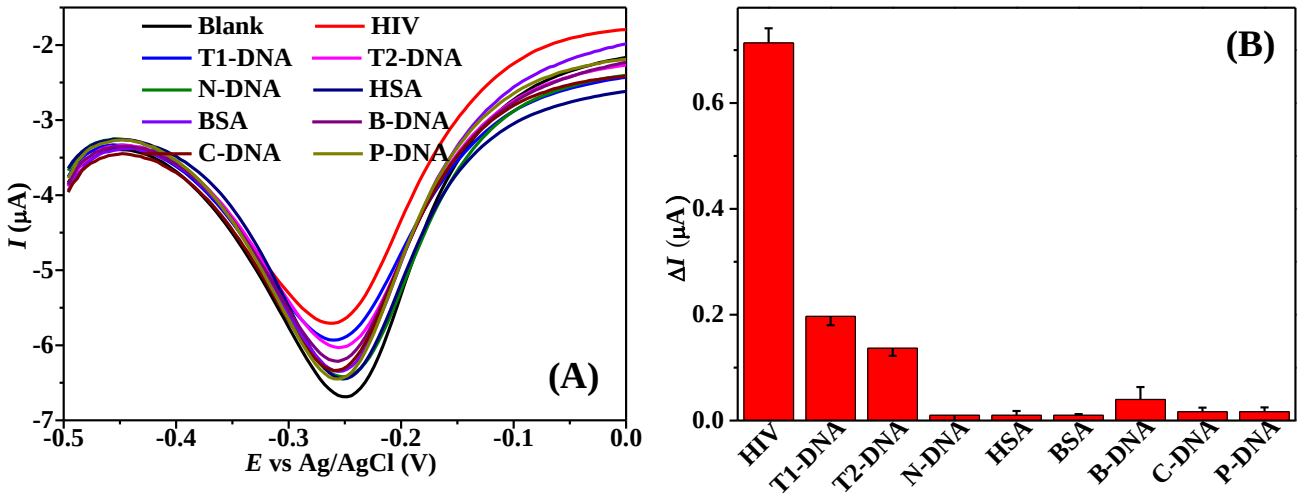



**Figure 6.** (A) DPV responses of MB/cDNA/$NH_2$-rGO/β-CD/GCE for different biomolecules. (B) DPV peak current reduction value responding to different biomolecules. The concentration of HIV gene was 0.1 pM. The concentrations of T1-DNA, T2-DNA, and N-DNA were all 1 pM, while the concentrations of HSA, BSA, B-DNA, C-DNA, and P-DNA were all 2 pM. Error bars represent the standard deviations for three times successive assays.

The relative standard deviation (RSD) of one same electrode response to 0.1 pM HIV gene was 0.82% for five times successive assays. The RSD of five different electrodes responses to 0.1 pM HIV gene was 1.3% for five times successive assays. Most importantly, when same electrode was placed in water for one week, the DPV signal of this electrode for 0.1 pM HIV gene was approximately 97.2% of its initial DPV signal. All these phenomena confirmed the excellent reproducibility and the good long-term stability of such electrochemical biosensor for target HIV gene.

### *3.4 Real samples detection*

This electrochemical biosensor was used to detect HIV gene in human serum samples from healthy volunteers. As illustrated in Table 2, inconspicuous variation of DPV signal was existed in these human serum samples, indicating that these human serum samples did not contain any concentration of HIV gene. The recovery of HIV gene in human serum samples was detected by adding two different concentrations of HIV gene (0.1 pM and 0.5 pM) into human serum samples, and the recovery values of HIV gene were in the range of 96.0% to 106.6% for three times successive assays. Since the target HIV gene is derived from the genom of HIV, such electrochemical biosensor exhibits huge potential for clinical analysis of HIV with high selectivity and good reproducibility.

**Table 2.** Detection of HIV gene in human serum samples ($n$ = 3).

| Sample | Added (pM) | Found (pM) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| Human serum sample (1) | 0 | 0 | – | – |
| | 0.1 | 0.0979 − 0.1066 | 97.9 − 106.6 | 2.6 |
| | 0.5 | 0.495 − 0.519 | 99.0 − 103.8 | 1.5 |
| Human serum sample (2) | 0 | 0 | – | – |
| | 0.1 | 0.096 − 0.104 | 96.0 − 104.0 | 1.1 |
| | 0.5 | 0.484 – 0.51 | 96.8 − 102.0 | 4.3 |
| Human serum sample (3) | 0 | 0 | – | – |
| | 0.1 | 0.0977 − 0.104 | 97.7 – 104.0 | 3.1 |
| | 0.5 | 0.497 – 0.519 | 99.4 − 103.8 | 2.5 |

## 4. CONCLUSIONS

An ultrasensitive and highly selective electrochemical biosensor was established for HIV gene via DPV based on the self-assembly of $NH_2$-rGO/β-CD on GCE surface. The better biocompatibility of β-CD and the excellent electron transfer ability of $NH_2$-rGO together with the specific hybridization interaction maintained the superior sensitivity for HIV gene detection. The prepared electrode exhibited faster DPV response, lower detection limit, excellent reproducibility, and good stability toward the detection of HIV gene. This electrochemical biosensing platform is convenient without complicated treatment process and additional contamination, offering great potential applications toward various viral nucleic acids. This electrochemical strategy was used for HIV gene in human serum samples, implying the huge practical application in monitoring HIV in clinical diagnosis.

COMPLIANCE WITH ETHICAL STANDARDS

There are no conflicts to declare. Human serum samples were obtained from the volunteers with healthy body and informed consent. All experiments were performed in compliance with the laws and institutional guidelines.

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