



A bio-chemical application of N-GQDs and g-C₃N₄ QDs sensitized TiO₂ nanopillars for the quantitative detection of pcDNA3-HBV

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ABSTRACT

Herein, TiO₂ nanopillars (NPs)/N-doped graphene quantum dots (N-GQDs)/g-C₃N₄ QDs heterojunction efficiently suppressed the photogenerated charges recombination and improved photo-to-current conversion efficiency. The introduced N-GQDs and g-C₃N₄ QDs could result in more effective separation of the photogenerated charges, and thus produce a further increase of the photocurrent. TiO₂ NPs/N-GQDs/g-C₃N₄ QDs were firstly applied as the photoactive materials for the fabrication of the biosensors, and the primers of pcDNA3-HBV were then adsorbed on the TiO₂ NPs/N-GQDs/g-C₃N₄ QDs modified electrode under the activation of EDC/NHS. With increase of the pcDNA3-HBV concentration, the photocurrent reduced once the double helix between the primers and pcDNA3-HBV formed. The developed photoelectrochemical (PEC) biosensor showed a sensitive response to pcDNA3-HBV in a linear range of 0.01 fmol/L to 20 nmol/L with a detection limit of 0.005 fmol/L under the optimal conditions. The biosensor exhibited high sensitivity, good selectivity, good stability and reproducibility.

1. Introduction

According to the GLOBOCAN 2012, primary liver cancer is the fifth leading cancer in men and is the ninth in women with 70–90% being hepatocellular carcinoma (HCC) (Jemal et al., 2011). Being one of

the most common infections worldwide, hepatitis B virus (HBV) is one of the major public health problems and the leading cause of HCC, and resulted in 686,000 deaths per year (Mortality, 2015). HBV is a double-stranded DNA virus with an incomplete circular double-stranded DNA genome. According to more than 8% genetic variability in the full-length nucleotide sequence or 4–8% divergence in the S gene, at least 10 HBV genotypes (A to J) and their subtypes have been defined (Kurbanov et al., 2010; Tatematsu et al., 2009; Tran et al., 2008). The patients with active chronic hepatitis B have HBV DNA levels >2000 IU/mL with fluctuations that reach >20,000 IU/mL in some patients. The peripheral blood of the patient is infectious, and HBV has the intact HBV genomic sequence and has been applied for the research on the influence on hepatic cell and other cells. HBV can effectively transcribe, translate and express the related protein in the cell, which totally reflect the real status of HBV *in vivo*. It can reflect the monitor effect to HBV itself from another aspect by detecting the

HBV DNA (Baker et al., 2015). Therefore, it is very significant to detect HBV for the early warning of HCC. Here, a visible light induced PEC platform based on TiO₂ NPs/N-GQDs/g-C₃N₄ QDs for the detection of HBV was proposed.

Since g-C₃N₄ was reported because of its visible-light photocatalytic performance in 2008 (Carlsson, 2008), it is regarded as a new metal-free and robust organic semiconductor, which has been widely used benefiting from its thermal, electrical and optical characteristics (Cao et al., 2015; Hong et al., 2016; Wang et al., 2014a). However, the application of bulk g-C₃N₄ was greatly limited due to the relatively small specific surface area, low photoresponsivity and poor solubility, etc. (Ma et al., 2014). To solve these problems, many attempts have been focused on preparing different morphologies of nanostructured g-C₃N₄, such as quantum dots (QDs) (An et al., 2016; Li et al., 2016; Zhang et al., 2014b; Zou et al., 2016), nanosheets (Duan et al., 2015; Han et al., 2016) or nanospheres (Jun et al., 2013; Zhang et al., 2014a), etc. g-C₃N₄ QDs have a wider band gap and present superior catalytic activity as the conduction and valence bands (CB and VB) shift in opposite directions owing to the quantum effect (Zhou et al., 2015b). g-C₃N₄ QDs have also looked as a potential candidate of carbon-based QDs with more unique photoluminescence (PL) properties than other

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carbon-based QDs. These indicate that g-C₃N₄ QDs could act as a good co-catalyst for the semiconductor catalysts. Nevertheless, to date, there are few reports about their application in the biosensing fields (Ding et al., 2016; Feng et al., 2016; Li et al., 2014a, 2014b; Liu et al., 2016; Wang et al., 2013a, 2013b; Wang et al., 2016b, 2016c). Thus, in this work, g-C₃N₄ QDs were used to sensitize the PEC excitons transmission between TiO₂ and g-C₃N₄ QDs.

Graphene quantum dots (GQDs) are a hot newcomer of graphene family (Pan et al., 2010; Yan et al., 2010b). GQDs are carbon nanosheets of < 20 nm in lateral dimension and a quasi-zero-dimensional graphene material with a tuned band gap. It attracted great research attention recently because of its quantum confinement, edge effects (Li et al., 2011; Ponomarenko et al., 2008), excellent photoluminescence property (Yoon et al., 2016) in electronics (Bae et al., 2010; Lalwani et al., 2014), and biofields (Li et al., 2013; Peng et al., 2012; Wang et al., 2016a; Zhu et al., 2012), and so on. Due to its photon down conversion property, which could absorb photons in shorter wavelength regions and emit photons in longer wavelength regions, they have been implemented in bandgap applications recently (Liu et al., 2013a, 2013b; Tetsuka et al., 2016; Tsai et al., 2015). Owing to the high surface area, GQDs have the potential to be an ideal loading platform for various molecules (Abdullah-Al-Nahain et al., 2013; Wang, 2013) through covalent or noncovalent interaction in versatile biomedical applications (Zhang et al., 2013). The abundant functional groups, such as carboxyl, hydroxyl and epoxy groups on GQDs, endow them with good dispersion in water. In addition, compared with most of II–VI materials which contain some toxic elements, GQDs are nontoxic, environmentally friendly and reasonably biocompatible, which are favorable in practical applications. Considered the merits above, GQDs are a perfect photosensitizer for the specific activation of TiO₂.

As we know, TiO₂ is a most widely used semiconductor since Fujishima and Honda reported TiO₂ as the semiconductor photoanode illuminated by ultraviolet light (Fujishima and Honda, 1972). Nevertheless, the intrinsic wide bandgap of TiO₂ (~3.2 eV) means the poor visible light absorption and poor photo-to-current ability under the visible light illumination. In this work, g-C₃N₄ QDs and N-GQDs were utilized to sensitize TiO₂ to achieve stronger visible light absorbency by forming the offsets, which would be advantageous to control the electrical transport of carriers. Furthermore, the primers were used as the capture probes for HBV with pcDNA3 vector. Thus, a biosensor based on PEC method was developed for the pcDNA3-HBV detection as shown in Scheme 1. This PEC biosensor might offer a candidate method for pcDNA3-HBV.

2. Experimental

2.1. Preparation of TiO₂ NPs

TiO₂ NPs were prepared as reported before with slight modification (Zhou et al., 2015a). 3 g of degussa P25 TiO₂ was dispersed in 160 mL of NaOH solution (5 mol/L) with stirring for 15 min. Then the mixture was sealed in a 200 mL Teflon-lined stainless steel autoclave for 24 h at 200 °C. Then the precipitate was transferred into 250 mL of HCl solution (10%, v/v) with magnetic stirring for 10 h. After filtering and washing with the ultrapure water for 5 times, the precipitate was dried and calcined at 700 °C in air for 3 h.

2.2. Preparation of N-GQDs

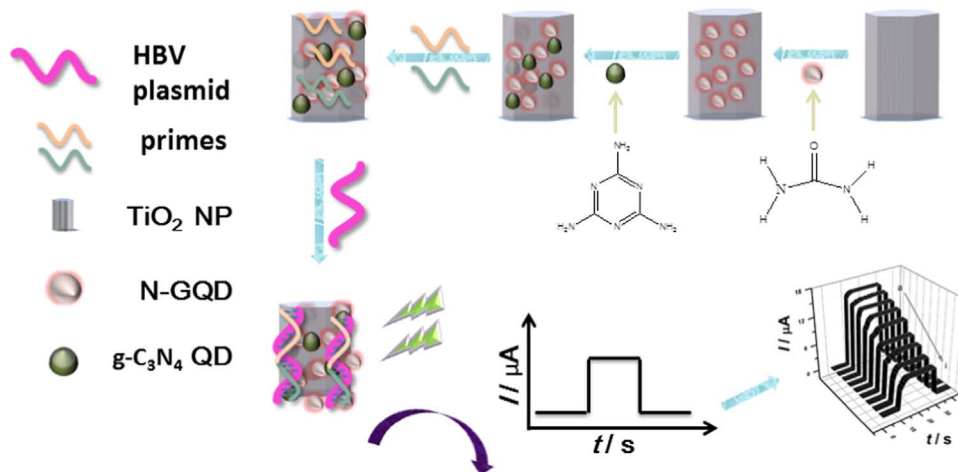
N-GQDs were prepared as described before (Zou et al., 2016). Typically, 0.3003g carbamide and 0.2627g citric acid were mixed in 6 mL ultrapure water, and then stirred to obtain a clear solution. The solution was put into the Teflon autoclave (50 mL), and then was heated at 160 °C for 8 h. The solution was centrifuged at 5000 rpm for 5 min and the final product was obtained.

2.3. Preparation of g-C₃N₄ QDs

Bulk g-C₃N₄ was obtained as previously (Pang et al., 2015). 10 g of melamine powder was put into a covered ceramic crucible and heated in a muffle furnace for 4 h at 550 °C. Bulk g-C₃N₄ was obtained after naturally cooling to room temperature. g-C₃N₄ QDs were prepared according to the literature (Wang et al., 2014b). Bulk g-C₃N₄ powders were heated at 500 °C in an open ceramic container with a ramp rate of 2 °C/min for 2 h. The obtained powder was mixed with 40 mL of concentrated H₂SO₄ and 120 mL of HNO₃ with drastic ultrasonication for 18 h. Then, the obtained solution was diluted with 800 mL of ultrapure water to form a colloidal suspension to obtain g-C₃N₄ nanosheets through a microfilter (0.45 μm) (Fig. 1G). g-C₃N₄ nanosheets were redispersed under ultrasonication in 20 mL ultrapure water. The suspension was placed into a Teflon autoclave (100 mL) and heated for 10 h at 200 °C. After cooling to room temperature, g-C₃N₄ QDs was obtained through filtering with a 0.22 μm microfilter.

2.4. Preparation of pcDNA3-HBV and PCR

pcDNA3-HBV DNA was obtained from Central Laboratory of Shandong Provincial Hospital Affiliated to Shandong University. HBV DNA with a pcDNA3 vector was transfected into the JM109. And the JM109 was cultured at 37 °C for 16 h to vastly amplify this plasmid.



Scheme 1. Schematic illustration of the PEC biosensor fabrication procedure.

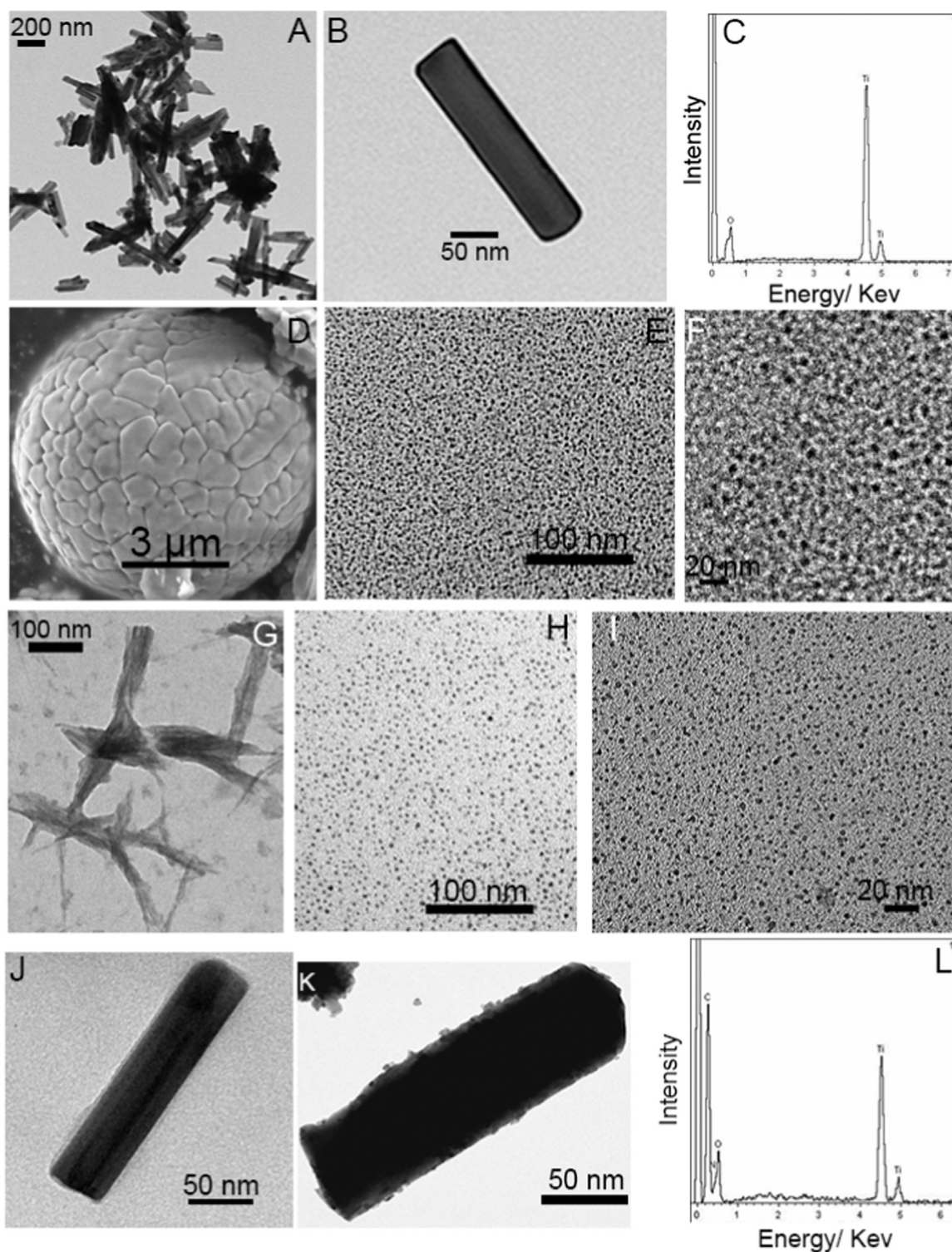


Fig. 1. TEM images of TiO₂ NPs (A, B), g-C₃N₄ nanosheets (G), TiO₂ NPs/N-GQDs (J) and TiO₂ NPs/N-GQDs/g-C₃N₄ QDs (K); SEM image of N-GQDs (D); HRTEM images of N-GQDs (E, F) and g-C₃N₄ QDs (H, I); EDS of TiO₂ NPs (C) and TiO₂ NPs/N-GQDs/g-C₃N₄ QDs (L).

Next, the pcDNA3-HBV was extracted with nucleic acid purification kit (Catalog #: AP-MM-P-50, Axygen scientific, Central Avenue, Union City, USA) according to the manufacturer's instructions. The pcDNA3-HBV plasmid was used as a template for PCR using Ex Taq (Catalog #: RR001A, TaKaRa, Development Zone, Dalian, China), according to the manufacturer's protocol. Specific primers used for PCR assays were as follows: 5'-ATGGA CATTG ACCCG TAT-3' (sense), 5'-CTAAC ATTGA GATTG CCGAG-3' (antisense) for Primer Pairs-1, and 5'-TGAAT CCTGC GGACG ACC-3' (sense), 5'-CAGCT TGGAG GCTTG AACAG-

3' (antisense) for primer Pairs-2. PCR was performed according to the following steps: 95 °C for 5 min, followed by 40 cycles of 95 °C for 5 s, 56 °C for 5 s, 72 °C for 5 s and 72 °C for 5 min, using the ABI Veriti[®]-96 PCR System.

2.5. Fabrication of the PEC biosensor

Scheme 1 displayed the fabrication process of the developed PEC biosensor. Firstly, 5 μL of nanohybridization of TiO₂ NPs (15 mg/mL)

were deposited on the bare ITO electrode. Secondly, 5 μL of N-GQDs (18 mg/mL) were deposited on TiO_2 NPs/ITO electrode with EDC/NHS. Thirdly, 5 μL of $\text{g-C}_3\text{N}_4$ QDs (20 mg/mL) were deposited on TiO_2 NPs/ $\text{g-C}_3\text{N}_4$ QDs/ITO electrode with EDC/NHS. Then, 8 μL of primers (15 g/mL) were deposited on TiO_2 NPs/ $\text{g-C}_3\text{N}_4$ QDs/N-GQDs/ITO electrode with EDC/NHS and incubated at 4 $^\circ\text{C}$ for 5 h. Finally, 1 mL of the different concentrations of pcDNA3-HBV were deposited on 3' and 5' primers/ TiO_2 NPs/ $\text{g-C}_3\text{N}_4$ QDs/N-GQDs/ITO electrode surface respectively and incubated at 4 $^\circ\text{C}$ for 5 h.

2.6. Measurement procedure

PEC and EIS tests were performed immediately on an electrochemical work station (Zahner Zennium PP211, Germany) after finishing the incubation of pcDNA3-HBV with an irradiation wavelength of 430 nm at 25 $^\circ\text{C}$. Phosphate buffered solution containing 0.1 mol/L of ascorbic acid (AA) was the electrolyte solution. 0.1 V was used the bias voltage. Light duration was 20 s and no light duration was 10 s. A three-electrode system was used (the working electrode was the modified ITO electrode, the reference electrode was a saturated calomel electrode (SCE) and the auxiliary electrode consisted of a platinum wire).

3. Results and discussion

3.1. Characterization of TiO_2 NPs, $\text{g-C}_3\text{N}_4$ QDs and N-GQDs

Fig. 1A and B showed the transmission electron microscope (TEM) morphologies of as-prepared dispersed TiO_2 NPs with almost average diameter. TEM was usually used to reveal the small size of the prepared material from JEM-1400 (JEOL, Japan). It can be seen that TiO_2 NPs were independent and Fig. 1B showed a single TiO_2 NP with a perfect pillar shape. EDS result in Fig. 1C displayed only two kinds of elements of Ti and O, which further proved the successful preparation of TiO_2 . Fig. 1D showed the scanning electron microscope (SEM) morphology of macro-collective N-GQDs. SEM image was obtained from JSM-6700F microscope (JEOL, Japan). It can be seen N-GQDs gathered as a microsphere with some micro-ravine. Fig. 1E and F described the TEM and HRTEM morphologies of the dispersive QDs, which showed the abundance of N-GQDs and their size was about 100 nm and 20 nm. HRTEM images were collected from a JEM-2100F microscope (JEOL, Japan). Fig. 1G showed the TEM morphology of the prepared $\text{g-C}_3\text{N}_4$ nanosheets during the preparation step of $\text{g-C}_3\text{N}_4$ QDs. Fig. 1H and I showed the TEM and HRTEM morphologies of the prepared $\text{g-C}_3\text{N}_4$ QDs. The output was plentiful with a uniform diameter, which verified

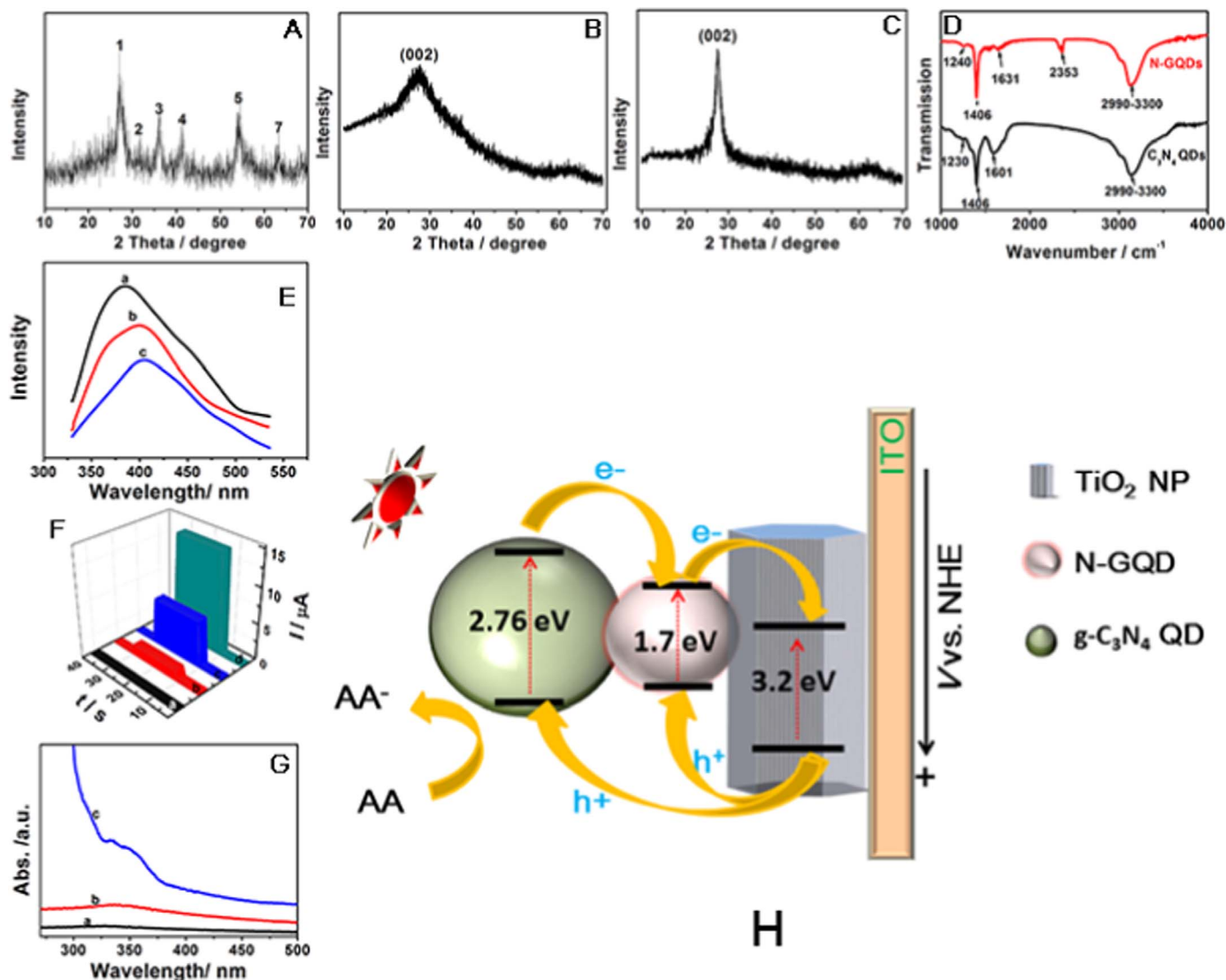


Fig. 2. XRD patterns of TiO_2 NPs (A) N-GQDs (B) and $\text{g-C}_3\text{N}_4$ QDs (C); FT-IR spectra (D) of N-GQDs (above) and $\text{g-C}_3\text{N}_4$ QDs (bottom); PL emission spectra (E) of (a) TiO_2 NPs, (b) TiO_2 NPs/N-GQDs and (c) TiO_2 NPs/N-GQDs/ $\text{g-C}_3\text{N}_4$ QDs, time-based photocurrent responses (F) of (a) ITO, (b) TiO_2 NPs, (c) TiO_2 NPs/N-GQDs and (d) TiO_2 NPs/N-GQDs/ $\text{g-C}_3\text{N}_4$ QDs and UV-vis spectra (G) of (a) TiO_2 NPs, (b) TiO_2 NPs/N-GQDs and (c) TiO_2 NPs/N-GQDs/ $\text{g-C}_3\text{N}_4$ QDs; (H) Schematic illustration of the energy level diagram.

that the preparation met the requirement. Fig. 1J and K exhibited the TEM morphologies of the TiO₂ NPs/N-GQDs and TiO₂ NPs/N-GQDs/g-C₃N₄ QDs nanocomposites. It can be seen that the N-GQDs covered as a semitransparent chiffon in Fig. 1J. However, after g-C₃N₄ QDs hybridized with TiO₂ NPs/N-GQDs, the coating was so thick that some QDs aggregated like some granules on the surface in Fig. 1K. EDS result in Fig. 1L showed that four kinds of elements, Ti, O, C and N, which suggested the perfect hybridization of TiO₂ NPs, N-GQDs and g-C₃N₄ QDs in the final sample.

Fig. 2A showed XRD patterns of TiO₂ NPs, g-C₃N₄ QDs and N-GQDs respectively. XRD patterns were collected by D8 focus diffractometer (Bruker AXS, Germany). Seven diffraction peaks can be distinguished, which respectively corresponded to (110), (101), (111), (210), (211), (220) and (002) plane indexed from 1 to 7. In Fig. 2B, a broad peak at about 26.5° was (002) plane, whose *d* value of 0.389 nm was nearly same with that of graphene (Pang et al., 2016). This result suggested that N-GQDs owned plentiful oxygen-containing functional groups (Liu et al., 1996; Peng et al., 2012). Fig. 2C displayed XRD pattern of g-C₃N₄ QDs. The peak at about 27.6° can be assigned to (002) plane with a distance of 0.325 nm and (100) peak almost disappeared due to the carboxyl measurement by H₂SO₄ and HNO₃ (Pang et al., 2015). These results showed that the carboxyl process did not influence the lattice structure of g-C₃N₄ QDs and benefited for the following fabrication of the biosensor.

Fig. 2D showed Fourier Transform Infrared (FT-IR) spectra of N-GQDs and g-C₃N₄ QDs from 1000 to 4000 cm⁻¹. FT-IR spectra were obtained from a Perkin-Elmer 580B spectrophotometer (Perkin-Elmer, USA). The above and bottom lines referred to FT-IR spectra of N-GQDs and g-C₃N₄ QDs respectively. For N-GQDs, the peak at 1240 cm⁻¹ might be the stretching vibrations of C-N bond, and the stretching vibrations of C-N bond may associate to 1251 cm⁻¹ for g-C₃N₄ QDs. C=O bending vibrations of both of the two nanomaterials located at the sharp peak at about 1406 cm⁻¹. C-O band stretching vibrations may be corresponded to the peaks centering at about 1631 (above) and 1601 (bottom) cm⁻¹ in both lines. The peak locating at 2353 cm⁻¹ might be associated to the stretching vibrations of cumulated double bonds. The broad peaks in both lines from about 2990–3300 cm⁻¹ were assigned to C-H and N-H bonds stretching vibrations. FT-IR data indicated that N-GQDs and g-C₃N₄ QDs loaded lots of functional groups of carboxyl groups and amino groups, which would be in favor of the biocompatibility and hydrophilicity in aqueous system for the detection process.

3.2. The proposed mechanism of PEC performance enhancement

Fig. 2H schematically illustrated the PEC promotion mechanism of the photo-to-electron generator of TiO₂ NPs/N-GQDs/g-C₃N₄ QDs. From Fig. 2H, it can be seen that the relative Fermi level alignment formed among TiO₂ NPs, N-GQDs and g-C₃N₄ QDs. As we know, the band gap of TiO₂, N-GQDs and g-C₃N₄ QDs is about 3.2 eV, 1.7 eV and 2.76 eV, respectively. The CB edge potential of TiO₂ is about -0.44 V (*vs.* normal hydrogen electrode, NHE) (Kanan and Nocera, 2008; Yin et al., 2009). Those of N-GQDs and g-C₃N₄ QDs are about -0.94 V (Gupta et al., 2011; Zhang et al., 2011) and -1.12 V (*vs.* NHE) (Ge et al., 2011; Yan et al., 2010a), respectively. Then, it can be calculated that VB of TiO₂, N-GQDs and g-C₃N₄ QDs are 2.76 V, 0.76 V and 1.64 V (*vs.* NHE) respectively according to $E_g = V_{VB} - V_{CB}$. In addition, it can be deduced that VB and CB energy level of TiO₂ NPs located below those of N-GQDs and g-C₃N₄ QDs. And also the CB energy level of g-C₃N₄ was negative than that of N-GQDs. While exposed under the visible light, the photogenerated electrons of g-C₃N₄ QDs and N-GQDs were excited, and then transferred quickly from the VB to the CB. Moreover, the photogenerated electrons excited from g-C₃N₄ can be easily injected into N-GQDs because of different CB energy levels. And after that, these electrons excited from g-C₃N₄ QDs and N-GQDs could be easily injected into the CB of TiO₂ NPs. Still further, the separated holes on VB of TiO₂ can enter into g-C₃N₄ and N-GQDs, which

transmission benefited for the PEC performance. And the holes would be transmitted to the interface between the electrode and the electrolyte, and be reduced by AA in the electrolyte to improve the charge transfer and separation efficiency as described in Fig. 2H (Gao et al., 2015). The above illustration explained that g-C₃N₄ QDs and N-GQDs can improve the visible light absorption property of TiO₂ NPs and this phenomenon was also proved by the results of PL, PEC and UV-vis as follows.

As we know, stronger photoluminescence (PL) value currently meant weaker photocatalytic intensity for the semiconductors (Cheng et al., 2014). Thus, in this work, PL tests were carried out to inspect the PEC behavior of TiO₂ NPs, TiO₂ NPs/N-GQDs and TiO₂ NPs/g-C₃N₄ QDs/N-GQDs nanocomposites. LS-45/55 PL spectrometer (Perkin Elmer, USA) was used to collect the PL spectra. In Fig. 2E, curve (a) showed an apparent higher PL emission intensity of TiO₂ NPs. And PL intensity in curve (b) turned lower when N-GQDs were deposited on the electrode. After modified with g-C₃N₄ QDs, PL intensity turned much lower than those of TiO₂ NPs and TiO₂ NPs/N-GQDs. As the PL tests results shown, the combination of TiO₂ NPs, N-GQDs and g-C₃N₄ QDs made the lower recombination efficiency of the photogenerated e⁻/h⁺ pairs, which indicated higher electron injection efficiency. These results proved that the viewpoint illustrated in Fig. 2H, the sensitization of N-GQDs and g-C₃N₄ QDs to TiO₂ NPs can improve the photoelectron transfer and be valued for the photoelectrons transformation, was probable.

Fig. 2F showed the time-based photocurrent responses of TiO₂ NPs, TiO₂ NPs/N-GQDs and TiO₂ NPs/N-GQDs/g-C₃N₄ QDs. Curve (a) showed there was no response on ITO electrode and curve (b) showed TiO₂ NPs owned the poor absorption property in the visible light because of its wide band gap. The combination and sensitization of N-GQDs made the photocurrent apparently increased (curve c) and the photocurrent further enhanced after modified g-C₃N₄ QDs (curve d). This phenomenon maybe prove that g-C₃N₄ QDs and N-GQDs can benefit efficiently for the electron injection between VB and CB and effectively suppressed h⁺/e⁻ pairs recombination.

UV-vis was also applied to inspect PEC properties and the absorption spectra of TiO₂ NPs, TiO₂ NPs/N-GQDs and TiO₂ NPs/N-GQDs/g-C₃N₄ QDs. The spectra were all obtained from Lambda 35 UV-vis spectrometer (Perkin-Elmer, USA). As shown in Fig. 2G (curve a), TiO₂ NPs exhibited a very weak light absorption in the visible light due to its wide band gap. Curve (b) displayed a higher light absorption between 320–350 nm, suggesting that N-GQDs increased the light absorption ability of the nanocomposite (TiO₂ NPs/N-GQDs). Moreover, a slight apparently absorption hill from 330 to 370 nm appeared in curve (c) (TiO₂ NPs/N-GQDs/g-C₃N₄ QDs), which was ascribed to the sensitization of N-GQDs and g-C₃N₄ QDs. These results could prove that the addition of N-GQDs and g-C₃N₄ QDs improved the absorbency of TiO₂ in the visible light, which improved the practical applications in the future.

3.3. Characterization of the proposed biosensor

As an effective tool to characterize the interface property of the electrodes, EIS was applied to test the fabrication procedure of the PEC biosensor. Fig. 3A displayed the Nyquist plots with different modification processes with [Fe(CN)₆]^{3-/4-} as the redox probe. As we know, the electron-transfer resistance (*R*_{et}), which equals the semicircle diameter at higher frequencies, reflects the restricted diffusion of the redox probe through the multilayer system related directly to film permeability. The linear portion at lower frequencies corresponds to the diffusion-limited process. After modification by TiO₂ NPs, *R*_{et} increased, as shown in curve (a), which was due that the deposition of TiO₂ NPs increased the impedance. Then, the immobilization of g-C₃N₄ QDs and N-GQDs led to further *R*_{et} enhancement in curve (b) and c, which resulted from the hindered interfacial electron transfer. Finally, after being incubated with the primers, *R*_{et} got higher as the forming of

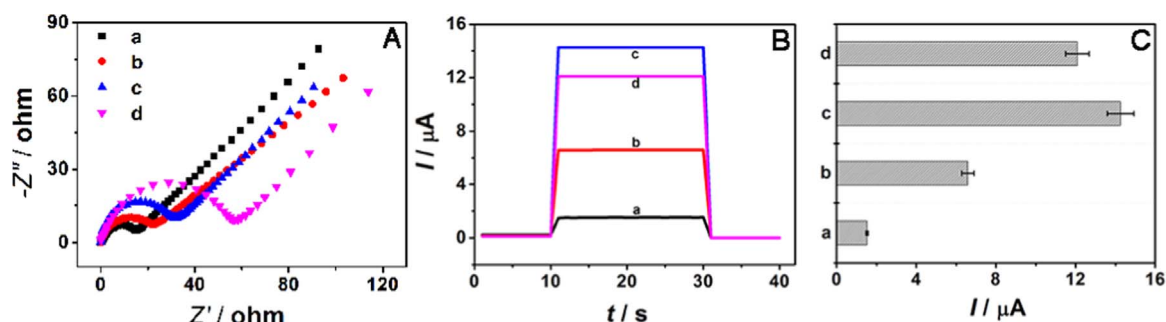


Fig. 3. (A) EIS spectra and (B) time-based photocurrent responses of (a) TiO_2 NPs/ITO, (b) TiO_2 NPs/N-GQDs/ITO and (c) TiO_2 NPs/N-GQDs/g- C_3N_4 QDs/ITO (d) 3' and 5' primers/ TiO_2 NPs/N-GQDs/g- C_3N_4 QDs/ITO.

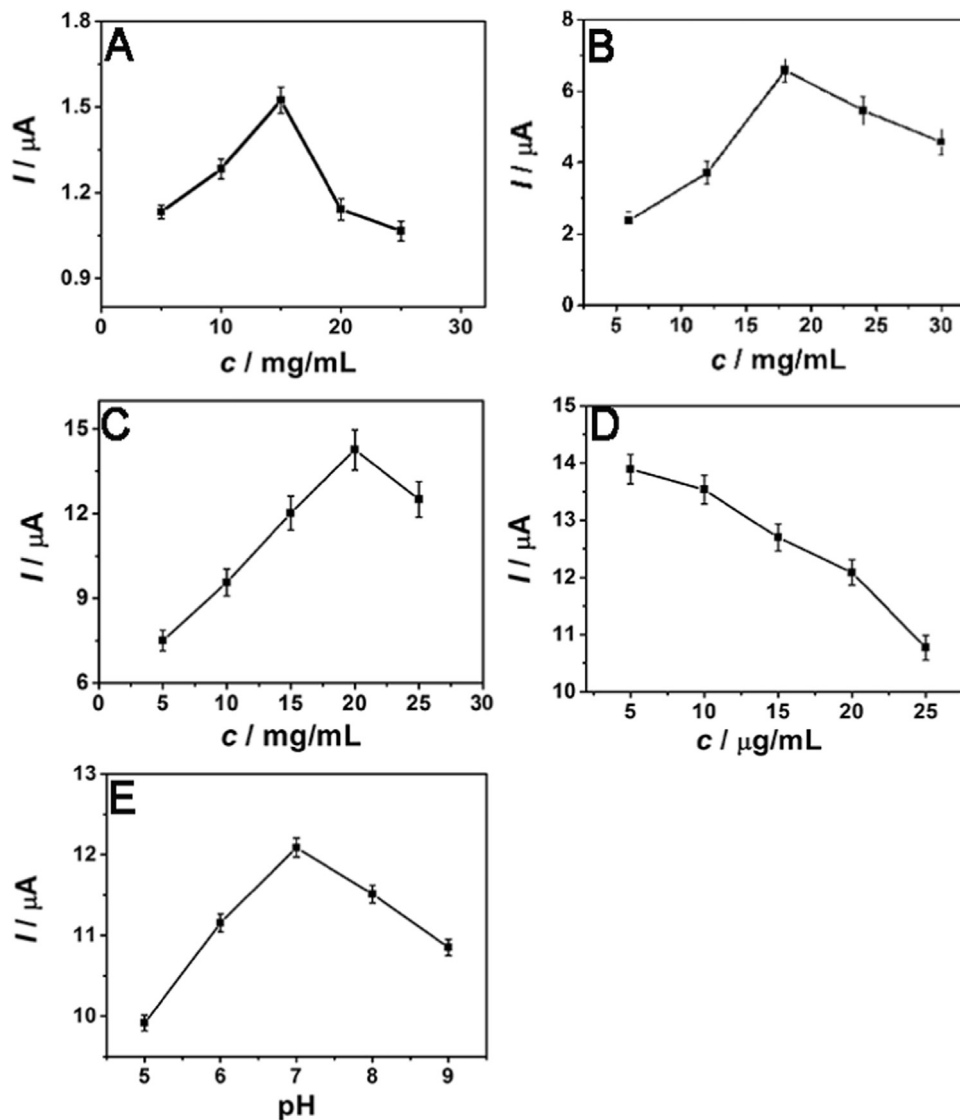


Fig. 4. The optimization of experimental conditions by time-based photocurrent responses of the concentrations of TiO_2 NPs (A), N-GQDs (B), g- C_3N_4 QDs (C), the primers (D); pH after modifying TiO_2 NPs/N-GQDs/g- C_3N_4 QDs/ITO (E).

bioaffinity complexes which further hindered the electron transfer. The above results displayed every successful fabrication step.

The fabrication process was also monitored as shown in Fig. 3B and C by PEC method. As for curve a-c, the photocurrent obviously increased benefiting from the good visible-light response after modified TiO_2 NPs, g- C_3N_4 QDs and N-GQDs layer by layer on the electrode. This could be attributed to the fact that the nanocomposite of TiO_2 NPs/N-GQDs/g- C_3N_4 QDs suppressed the interfacial electron transfer

and partly obstructed electron donor to the surface reaction with the photogenerated hole. After that, the photocurrent gradually decreased with the immobilization of the bio-anchorer, namely “the primers” (curve d). This phenomenon was ascribed to the electrostatic repulsion of negative charged backbone of DNA fragments. The above results indicated that the fabrication of the sensing interface was successful.

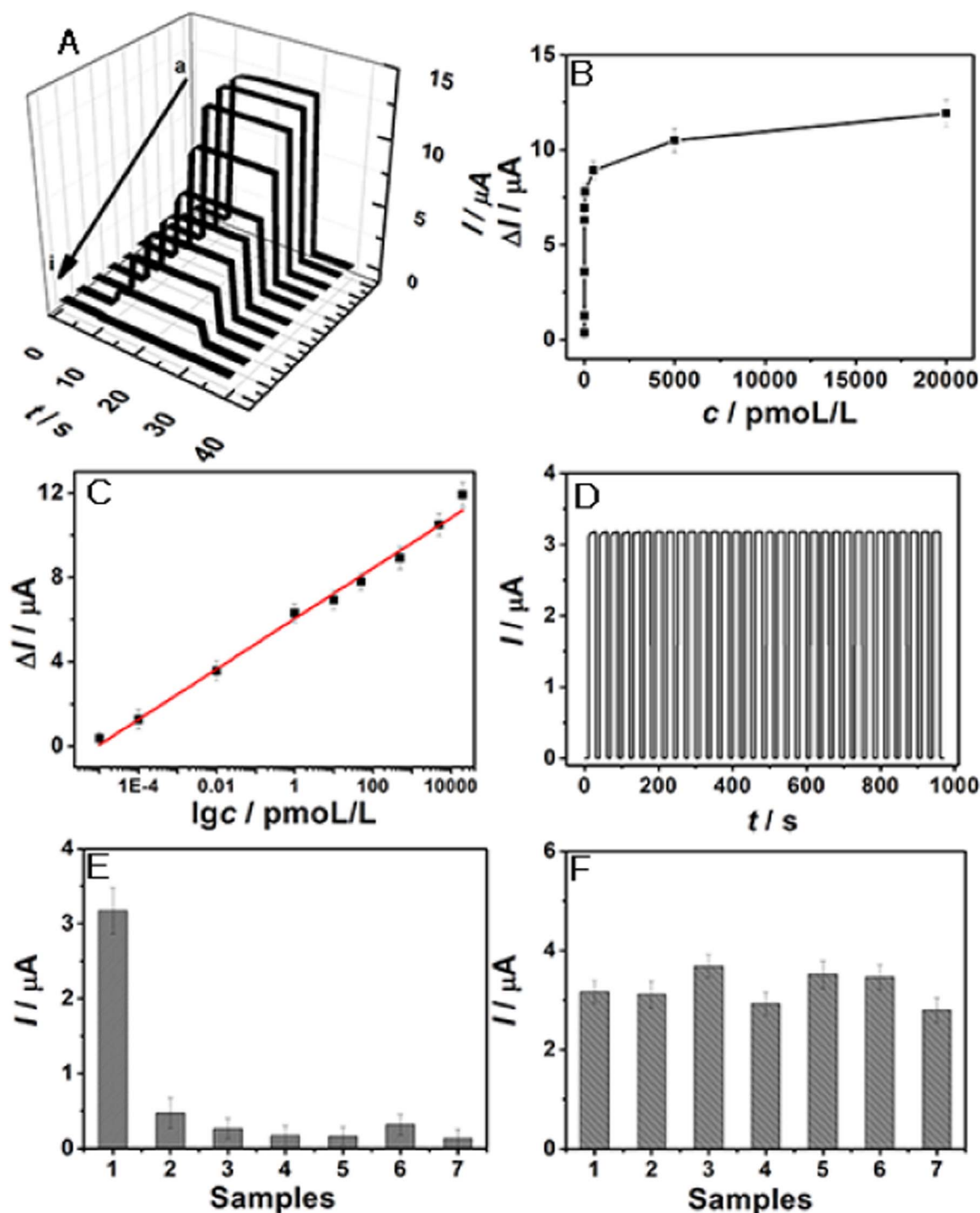


Fig. 5. (A) Time-based photocurrent responses of being incubated with different concentrations of pcDNA3-HBV; (B) Relation curve between photocurrent change (ΔI) and different pcDNA3-HBV concentrations; (C) Logarithmic calibration curve between ΔI and concentration of pcDNA3-HBV; (D) Stability of photocurrent response under the optimal condition; (E) Selectivity of the biosensor (1) 500 pmol/L pcDNA3-HBV, (2) 500 pmol/L pcDNA3, (3) 500 pmol/L pcDNA3-His, (4) 500 pmol/L pCMV5, (5) 500 pmol/L pCMV-N-HA, (6) 500 pmol/L pCMV-C-HA and (7) five kinds of gene fragments of 1000 pmol/L respectively; (F) Selectivity of the biosensor (1) 500 pmol/L pcDNA3-HBV, (2) 500 pmol/L pcDNA3-HBV +1000 pmol/L pcDNA3, (3) 500 pmol/L pcDNA3-HBV +1000 pmol/L pcDNA3-His, (4) 500 pmol/L pcDNA3-HBV +1000 pmol/L pCMV5, (5) 500 pmol/L pcDNA3-HBV +1000 pmol/L pCMV-N-HA, (6) 500 pmol/L pcDNA3-HBV +1000 pmol/L pCMV-C-HA and (7) 500 pmol/L pcDNA3-HBV + five kinds of gene fragments of 1000 pmol/L respectively.

3.4. PEC detection of pcDNA3-HBV

In order to gain the good detecting effect, the experiment condition optimization was carried out. The optimal concentrations of TiO_2 NPs (Fig. 4A), N-GQDs (Fig. 4B), $\text{g-C}_3\text{N}_4$ QDs (Fig. 4C) and the primers (Fig. 4D) were 15 mg/mL, 18 mg/mL, 20 mg/mL and 15 $\mu\text{g/mL}$, respectively. 7.0 was the optimal pH (Fig. 4E).

In this work, the different concentrations of pcDNA3-HBV could be

detected by monitoring the photocurrent signal of the PEC biosensor under the optimum experimental conditions. The photocurrent decreased with the increasing concentration of pcDNA3-HBV as shown in Fig. 5A, B and C. The decrement of the photocurrent was proportional to the logarithm of the pcDNA3-HBV concentration ranging from 0.01 fmol/L to 20 nmol/L with a correlation coefficient of 0.9949. The regression equation was $\Delta I (\mu A) = 6.036 + 1.194 \lg c_{\text{pcDNA3-HBV}} (\text{pmol/L})$. The limit of detection (LOD) was 0.005 fmol/L. The outstanding

sensitivity maybe resulted from the novel signal amplification strategy of the proposed PEC biosensor. And also the sensitization of N-GQDs and g-C₃N₄ QDs to TiO₂ NPs was attributed to good separated excitation energy and exciton conversion efficiency.

3.5. Selectivity, stability and reproducibility

The stability of the biosensor was also inspected in this work. As shown in Fig. 5D, the photocurrent response was recorded under more than 30 on/off irradiation cycles and no noticeable variation occurred, indicating the stable readout for signal collection. For the storage stability of the proposed biosensor after stored at 4 °C for 14 days, 95% of the initial response was gained for the detection of 500 pmol/L pcDNA3-HBV, indicating the satisfactory storage stability of the developed biosensor.

To investigate the specificity of the proposed biosensor, some representative interfering substances involving pcDNA3, pcDNA3-His, pCMV5, pCMV-N-HA and pCMV-C-HA were selected for the interference tests. In the tests, a constructed biosensor incubated only with 500 pmol/L pcDNA3-HBV was tested as the contrast (sample 1, Fig. 5E). Sample 7 was incubated with more than five kinds of gene fragments, which displayed the possible cross-reaction could be ignored. And also the photocurrent response was consistent with the calibration curve in Fig. 5C (500 pmol/L pcDNA3-HBV). The specificity of the biosensor was also evaluated by measuring the photocurrent responses of 1000 pmol/L pcDNA3, pcDNA3-His, pCMV5, pCMV-N-HA and pCMV-C-HA along with 500 pmol/L pcDNA3-HBV under the same experimental conditions, respectively. As shown in Fig. 5F, there was no remarkable signal increase when incubated higher concentration of non-target DNA fragments. The above results indicated that the specificity of the biosensor was suitable.

The reproducibility of the biosensor was also studied by both intra-assay and inter-assay relative standard deviation (RSD). Analyzed from the experimental results, the intra-assay RSDs were 2.3%, 3.1% and 2.6% towards 10, 30 and 50 nmol/L of pcDNA3-HBV. The inter-assay RSDs of 4.4%, 3.7% and 4.5% were obtained by measuring the same samples with five electrodes prepared independently at the same experiment conditions. The results indicated the acceptable reproducibility and precision of this biosensor.

The adding standard recovery was also carried out as shown in Table S1, the recoveries of the biosensor were 103% and 102% and the relative standard deviation (RSD) was 2.04% and 1.79%. Therefore, the fabricated biosensor could be satisfactorily applied to determination of pcDNA3-HBV.

4. Conclusions

In this study, TiO₂ NPs/N-GQDs/g-C₃N₄ QDs nanocomposite was utilized as the novel photoactive materials to develop the PEC biosensor to detect pcDNA3-HBV. The formation of TiO₂ NPs/N-GQDs/g-C₃N₄ QDs heterojunction could efficiently avoid the photo-generated electrons recombination, which promoted photo-to-current conversion. The introduced N-GQDs and g-C₃N₄ QDs could result in more effective separation of photogenerated electrons and further enhanced conversion efficiency. This proposed biosensor displayed a low detection limit of 0.005 fmol/L as well as a linear range from 0.01 fmol/L to 20 nmol/L. This novel strategy could afford a promising application in clinical diagnostics with good selectivity, high sensitivity, acceptable reproducibility and stability.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2016.12.059.

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