



A high sensitive visible light-driven photoelectrochemical aptasensor for shrimp allergen tropomyosin detection using graphitic carbon nitride-TiO₂ nanocomposite

Mahmoud Amouzadeh Tabrizi^{a,*}, Mojtaba Shamsipur^{b,*}, Reza Saber^{a,c}, Saeed Sarkar^{a,d},
Vahid Ebrahimi^c

^a Research Center for Science and Technology in Medicine, Tehran University of Medical Sciences, Tehran, Iran

^b Department of Chemistry, Razi University, Kermanshah, Iran

^c School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Iran

^d Department of Medical Physics and Biomedical Engineering Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:

Photoelectrochemical
g-C₃N₄-TiO₂
Aptasensor
Food allergy
Tropomyosin

ABSTRACT

Herein, for the first time a visible-light-driven photoelectrochemical (PEC) aptasensor for shrimp tropomyosin determination was fabricated by using graphitic carbon nitride (g-C₃N₄) and titanium dioxide (TiO₂) as photoactive nanomaterials, ascorbic acid (AA) as electron donor and ruthenium (III) hexaammine (Ru(NH₃)₆³⁺) as signal enhancer. The surface of an ITO electrode was first modified with g-C₃N₄, TiO₂, and polyethyleneimine (PEI) and then the amine terminal aptamer_{TROP} probe was attached to PEI by the use of glutaraldehyde (GA) as cross-linker. After that, Ru(NH₃)₆³⁺ was adsorbed on aptamer to enhance the photocurrent signal. The principle of proposed PEC aptasensor is based on the formation of a selective complex between tropomyosin and immobilized aptamer_{TROP} probe on the surface of ITO/g-C₃N₄-TiO₂/PEI/apptamer_{TROP}-Ru(NH₃)₆³⁺. After the incubation of tropomyosin with TROP aptamer probe, the photocurrent signal decreased due to releasing adsorbed Ru(NH₃)₆³⁺ on aptamer and preventing AA from scavenging photogenerated holes to the photoactive modified electrode. Under the optimized conditions, the fabricated PEC aptasensor was used for the determination of shrimp tropomyosin in the concentration range of 1–400 ng mL⁻¹ with a limit of detection of 0.23 ng mL⁻¹. The proposed PEC aptasensor exhibited high selectivity, sensitivity, and good stability.

1. Introduction

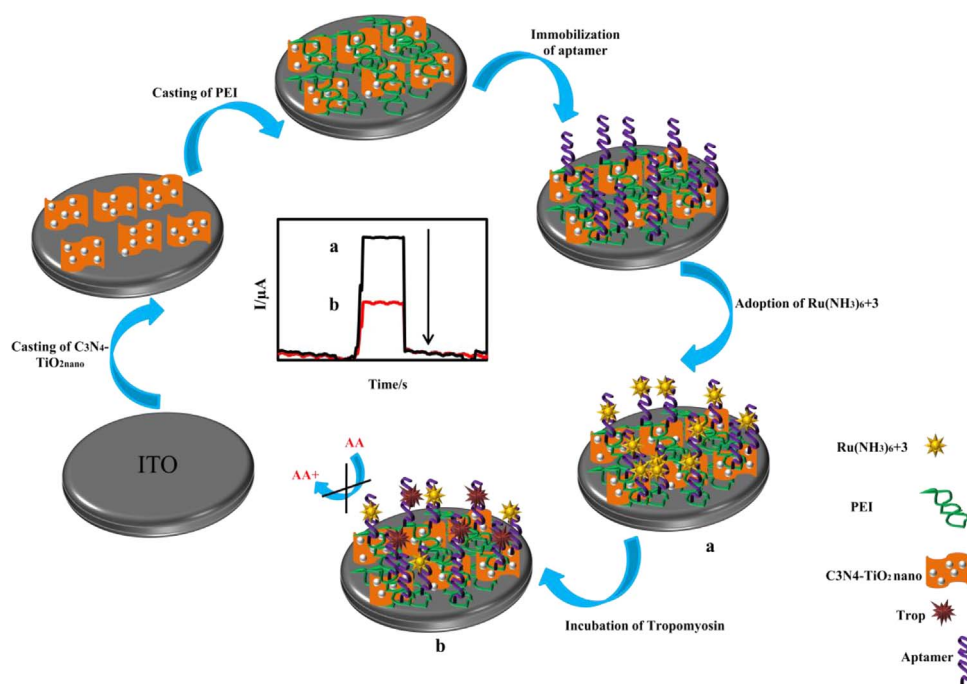
The seafood allergies affect approximately 6.5 million people in a year (Gordon, 2006). Although most of the allergenic source materials in sea foods are deactivated by cooking, but some of them are heat stable. Tropomyosin (TROP), a kind of proteins in the muscle shellfish such as shrimp, is a major heat stable allergenic source. Commonly, antibody-based enzyme-linked immunosorbent assay (ELISA) has been applied to recognize the tropomyosin poisoning (Zhang et al., 2014). But this method suffers some disadvantages such as expensive fabrication process, instability in the antibody and enzyme, needs a lab operator with a high level of experience, limited linear response range, and time-consuming determination process. However, the biggest advantage of antibody-based biosensor is the specificity and affinity of these probes to target analytes (Amouzadeh Tabrizi et al., 2016; Teresa Fernández-Abedul et al., 2015; Wen et al., 2017; H. Zhang et al., 2016).

Due to the reduce economic losses and improve the public health and food safety, the fabrication of a sensor for the determination of tropomyosin is necessary. Photoelectrochemical (PEC) sensors are a kind of electrochemical methods which includes a light source and a suitable photoactive modified electrode. The light source excites a photoactive modified electrode to generate a photocurrent signal which it is recorded by the electrochemical device (Liu et al., 2016b).

Several photoactive nanomaterials have been used for the fabrication of PEC biosensors (Liu et al., 2015, 2016c; Shu et al., 2016; Zeng et al., 2013; L. Zhang et al. 2016b; Zhang et al. 2011a; Zhao et al., 2015, 2014). Graphitic carbon nitride (g-C₃N₄) is a novel photoactive nanomaterial consisting of carbon and nitrogen, has a visible-light-driven band gap of 2.69 eV (Chen et al., 2013; Hou et al., 2016). Among the various methods have been reported for the synthesis of graphitic carbon nitride (Jiang et al., 2014; Ong et al., 2016; Zheng et al., 2012; Zhu et al., 2014), the pyrolysis of melamine is a common method for

* Corresponding authors.

E-mail addresses: mahmoud.tabrizi@gmail.com (M. Amouzadeh Tabrizi), mshamsipur@yahoo.com (M. Shamsipur).



Scheme 1. The schematic illustration for fabrication of PEC aptasensor.

the synthesis of this carbon-nitrogen rich material (Amiri et al., 2016; Das et al., 2016). The g-C₃N₄ have various potential applications in many fields of science ranging such as photodegradation of water pollution components (Yan et al., 2009; Zhu et al., 2014), water splitting (Hong et al., 2017; Kong et al., 2016), carbon dioxide reduction (Walsh et al., 2016; Yu et al., 2015), bioimaging (Zhang et al., 2013), and biosensor (Fan et al., 2016; He et al., 2015; Li et al., 2016b; Liu et al., 2016a; Sun and Qi, 2016).

To the best of our knowledge, no electrochemical PEC aptasensor for the determination of TROP has been reported yet. In this study, for the first time, a PEC aptasensor was fabricated base on immobilization of the amino terminal of the amine terminal aptamer_{TROP} probe via glutaraldehyde (GA) on the surface of ITO modified with graphitic carbon nitride/titanium dioxide/polyethyleneimine (g-C₃N₄-TiO₂/PEI). The proposed PEC aptasensor exhibited high analytical performance in terms of selectivity, stability, sensitivity, linear range (LR), and limit of detection (LOD).

2. Experimental section

2.1. Reagents and chemicals

All chemicals were of analytical reagent grade and used without further purification. Melamine, potassium chloride (KCl), potassium hexacyanoferrate (III) (K₃[Fe(CN)₆]), potassium hexacyanoferrate (II) (K₄[Fe(CN)₆]), magnesium chloride (MgCl₂), tris hydrochloride (Tris-HCl), potassium hydroxide (KOH) and phosphoric acid (H₄PO₄) were obtained from Merck (Darmstadt, Germany). Hexaammine ruthenium (III) chloride (Ru(NH₃)₆³⁺)Cl₃, polyethylene imine (PEI), titanium(IV) oxide (TiO₂), bovine serum albumin (BSA) streptavidin, and lysozyme were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tropomyosin was obtained from Medical Biology Research Center, Tehran University of Medical Sciences, Tehran, Iran. Double distilled water was used throughout. The probe aptamer was modified at the 5'-terminus with an NH₂ group and its sequence was as follow: 5'-NH₂-(CH₂)₆-5'-TACTAACGGTACAAGCTACCAGGCCGCAACGTTGACCTAGAACGACCTGCCAGACCCGAACGTTGACCTAGAACG-3' (Zhang et al., 2017).

2.2. Apparatus

The Fourier Transform infrared spectra were obtained using a Bruker vector 22 Fourier transform infrared (FTIR) spectrometer. Energy Dispersive X-ray (EDS) analysis was performed with a VEGA, Model TESCAN-LMU. Transmission electron microscopy (TEM) was performed on a HITACHI H-8100 EM with an accelerating voltage of 200 kV. Atomic force microscopy (AFM) measurement was made on DME DualScope Scanner DS95-200 (Herlev, Denmark). The photo-electrochemical (PEC) and cyclic voltammetry (CV) studies were performed using an Autolab potentiostat-galvanostat model PGSTAT30 (Autolab, Netherlands). A three-electrode system was employed with an Ag|AgCl (saturated KCl) electrode as a reference electrode, a Pt wire as a counter electrode and the ITO/g-C₃N₄-TiO₂/PEI/apptamer_{TROP} as a working electrode. A 150 W Xe lamp was used as an irradiation source. The ultrasonication process was carried out using an ultrasonic cleaner (Elma-E30H, Powerful cleaning with 37 kHz cavitation).

2.3. Fabrication of g-C₃N₄

The g-C₃N₄ was synthesized according to a procedure described in a previous literature (Amiri et al., 2016). In brief, an amount of 2.0 g of white melamine powder was transferred into an oven and the temperature of the oven was then increased to 520 °C and held for 4.0 h under argon condition with a ramp rate of about 3 °C/min. The obtained yellow powder was grounded and used for further characterizations (Fig. S1, Supplementary data). Finally, 5 mg of g-C₃N₄ was dispersed in 1 mL of isopropanol alcohol with ultrasonic agitation for 2 h to achieve a well-dispersed suspension.

2.4. Fabrication of aptasensor

The ITO electrode was ultrasonicated for 10 min in ethanol, acetone and, distilled water, respectively. An amount of 2.0 mg of g-C₃N₄ and 1.0 mg of TiO₂ was dispersed in 1 mL of isopropanol alcohol for 5 h. Then, 5.0 μL of g-C₃N₄-TiO₂ (5 mg mL⁻¹) solution was dropped onto the surface of ITO electrode and allowed to dry at ambient temperature. Next, 3.0 μL of an aqueous solution of PEI (1%) was cast on the

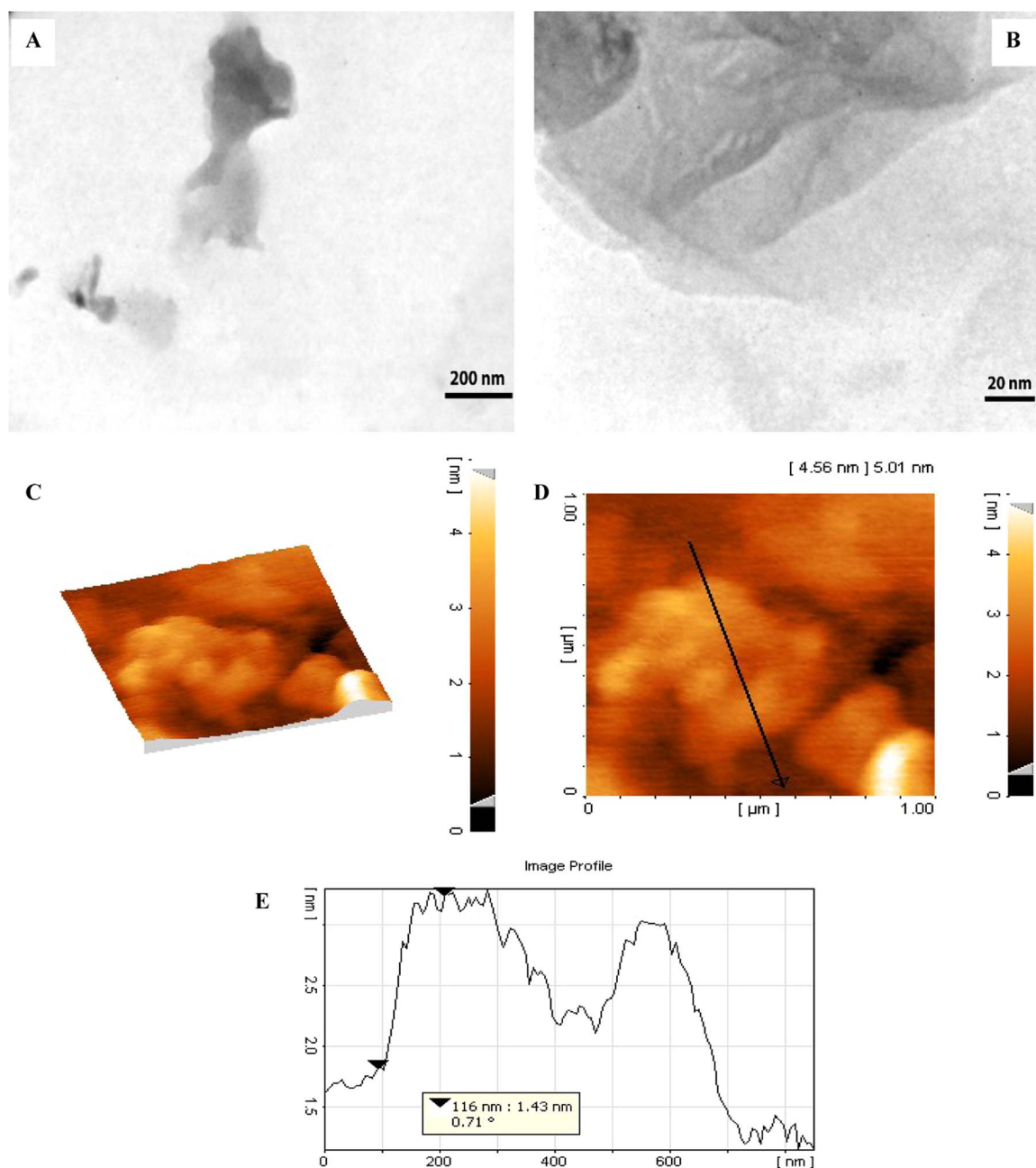


Fig. 1. (A, B) TEM, (C, D) AFM images of g-C₃N₄ and, (E) surface profile of the synthesized g-C₃N₄.

surface ITO/g-C₃N₄-TiO₂. Then, 6 μ L of 2.5% GA solution was dropped onto the surface of ITO/g-C₃N₄-TiO₂/PEI for 30 min. After rinsing with 0.1 M phosphate buffer solution (PBS, pH 7.4), the electrode was immersed in amine terminal TROP aptamer working solution (1 mg mL⁻¹, 0.1 M PBS and pH 7.4) for 4 h at 4 °C to immobilize amine terminal aptamer_{TROP} on the surface of ITO/g-C₃N₄-TiO₂/PEI electrode. Subsequently, the electrode was rinsed with distilled water to wash away un-immobilized aptamers. After that, the fabricated aptasensor was incubated with 20.0 μ L BSA (2%) for 1.0 h at room temperature to block non-specific sites. The ITO/g-C₃N₄-TiO₂/PEI/apptamer electrode was then rinsed thoroughly with water to wash away the loosely BSA. Finally, ITO/g-C₃N₄-TiO₂/PEI/apptamer electrode was immersed on 0.2 mM Ru(NH₃)₆⁺³ for 60 min to interact with guanines and adenines of aptamer (Lopes et al., 2010). In the next step, the ITO/g-C₃N₄-TiO₂/PEI/apptamer_{TROP}- Ru(NH₃)₆⁺³ was transferred into the bonding buffer solution (20.0 mM Tris-HCl, 50 mM NaCl, 5.0 mM KCl, 1.0 mM MgCl₂, pH 7.4) and different concentrations of the TROP to

incubate with aptamer_{TROP} probe for 80 min at 4 °C. Finally, the electrode was thoroughly cleaned with washing buffer to remove unbonded tropomyosin. Scheme 1 shows the schematic illustration of proposed PEC aptasensor fabrication and the sensing mechanisms employed.

3. Results and discussion

3.1. Characterization of g-C₃N₄

The morphological characterization of the g-C₃N₄ was examined by TEM and AFM (Fig. 1). The TEM (Fig. 1A, B) and AFM images (Fig. 1C, D) of g-C₃N₄ show that the g-C₃N₄ has a nanosheet structure (Das et al., 2016). Also, the cross-sectional view indicates that the average thickness of g-C₃N₄ is about 1.4 nm (Fig. 1E). The TEM (A) and AFM (B, C) images of TiO₂ nanoparticles were shown in Fig. S2. The average diameter size of TiO₂ nanoparticles was 24 $\pm$ 0.5 nm.

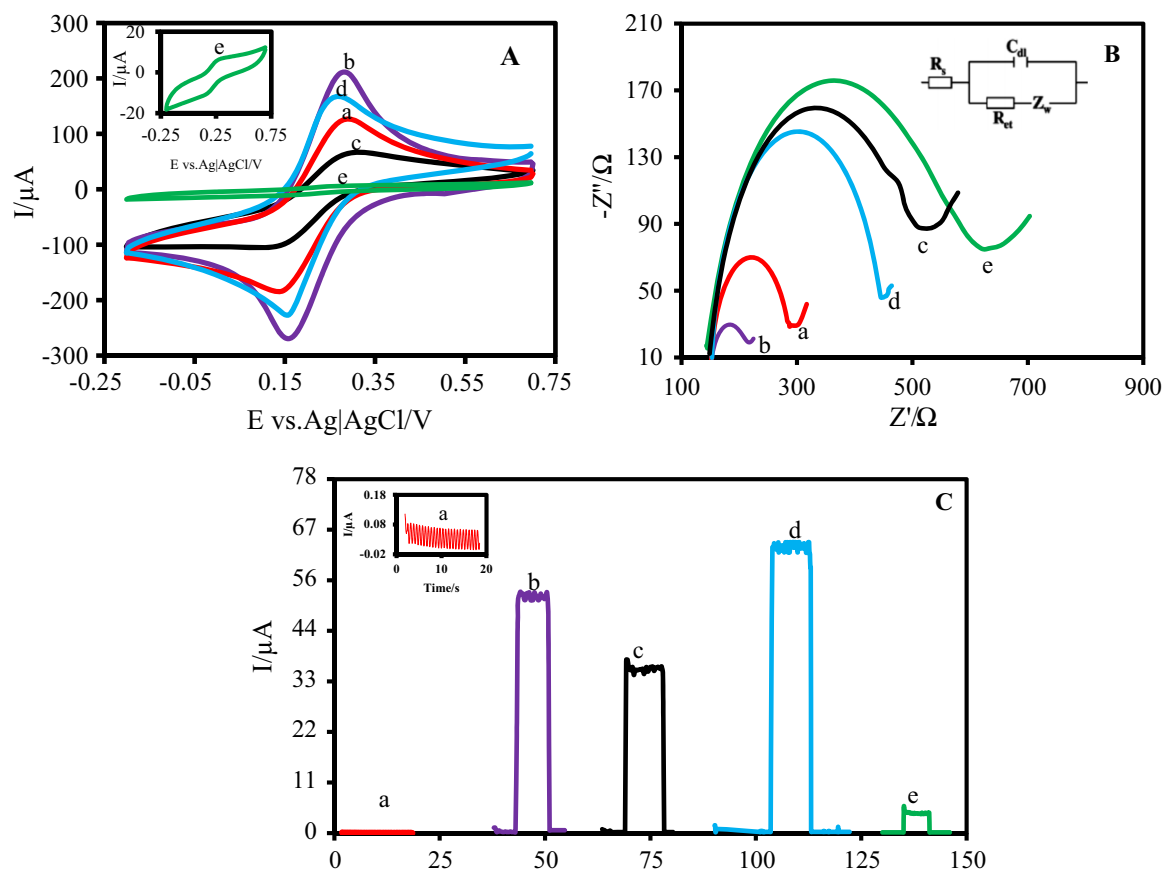


Fig. 2. (A) Cyclic voltammograms and (B) EIS for a ITO (a), ITO/g-C₃N₄-TiO₂/PEI (b) ITO/g-C₃N₄-TiO₂/PEI aptamer_{TROP} (c) and ITO/g-C₃N₄-TiO₂/PEI/apptamer_{TROP}/TROP (d) in a solution containing 5.0 mM Fe(CN)₆^{3-/4-} couple (1:1) and 0.1 M KCl, pH 7.4. (C) Time-based photocurrent response of ITO (a), ITO/g-C₃N₄-TiO₂/PEI (b), ITO/g-C₃N₄-TiO₂/PEI/apptamer_{TROP} (c) ITO/g-C₃N₄-TiO₂/PEI/apptamer_{TROP}-Ru(NH₃)₆³⁺ (d) and, ITO/g-C₃N₄-TiO₂/PEI/apptamer_{TROP}/tropomyosin (e) in solution containing 0.1 mM AA (0.1 M PBS, pH 7.4). The inset of Fig. 3B shows the equivalent electric circuit compatible with the Nyquist diagrams. R_s: solution resistance, R_{et}: electron transfer resistance, C_{dl}: double layer capacitance, Z_w: Warburg impedance. A bias potential: 0.22 V.

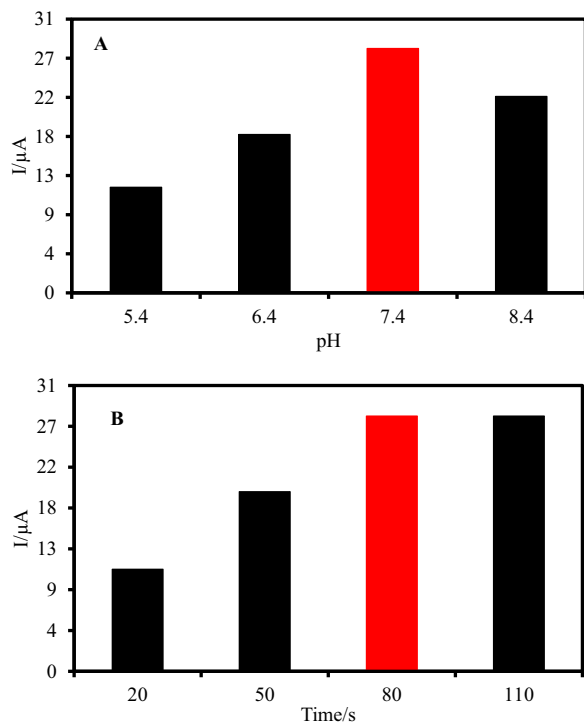


Fig. 3. (A) Effect of incubation time (B) and pH of the solution on the response of PEC aptasensor to tropomyosin in a 0.1 M phosphate solution.

Furthermore, the surface morphology of the ITO/g-C₃N₄-TiO₂ (A, B) and ITO/g-C₃N₄-TiO₂/PEI (C, D) electrodes were also characterized by SEM and AFM (Fig. S3). It can be clearly seen that after the casting of PEI on the surface of modified electrode with g-C₃N₄-TiO₂ the surface morphology of electrode has been changed.

Fig. S4A shows the UV–visible absorption spectroscopy of g-C₃N₄. The absorption peak at 315 nm attributed to the absorption range characteristic to $\pi \rightarrow \pi^*$ electronic transition in g-C₃N₄ (Das et al., 2016; Sun et al., 2016; Zhang et al., 2013). The elemental analysis of the synthesized g-C₃N₄ was carried out by EDS (Fig. S4B). As shown in this figure, the g-C₃N₄ displays the peaks corresponding to C and N elements (Chen et al., 2014; Jiang et al., 2014). The g-C₃N₄ nanocomposite was also characterized by FTIR (Fig. S4C, Supplementary data). The characteristic stretching vibration band at 3382 cm⁻¹ shows the existence of the -NH₂ group of g-C₃N₄. The absorption band in the range of 1230–1650 cm⁻¹ are also attributed to C–N and C=N stretching vibrations of g-C₃N₄ (Amiri et al., 2016; Zhou et al., 2016). The elemental analysis of TiO₂ nanoparticles was also carried using EDS (Fig. S5, Supplementary data). The EDS result clearly indicates that the prepared TiO₂ nanoparticles contain Ti and O elements.

3.2. Electrochemical characterization of the sensing interface

The Cyclic voltammetry (CV), Electrochemical impedance spectroscopy (EIS), and time-based photocurrent measurements were utilized to confirm the stepwise changes of the ITO electrode (Fig. 2). As shown in Fig. 2A, the peak current of Fe(CN)₆^{3-/4-} on ITO/g-C₃N₄-TiO₂/PEI electrode (curve_b) was higher than that observed on ITO electrode (curve_a). The reasonable explanation is that the positively charged

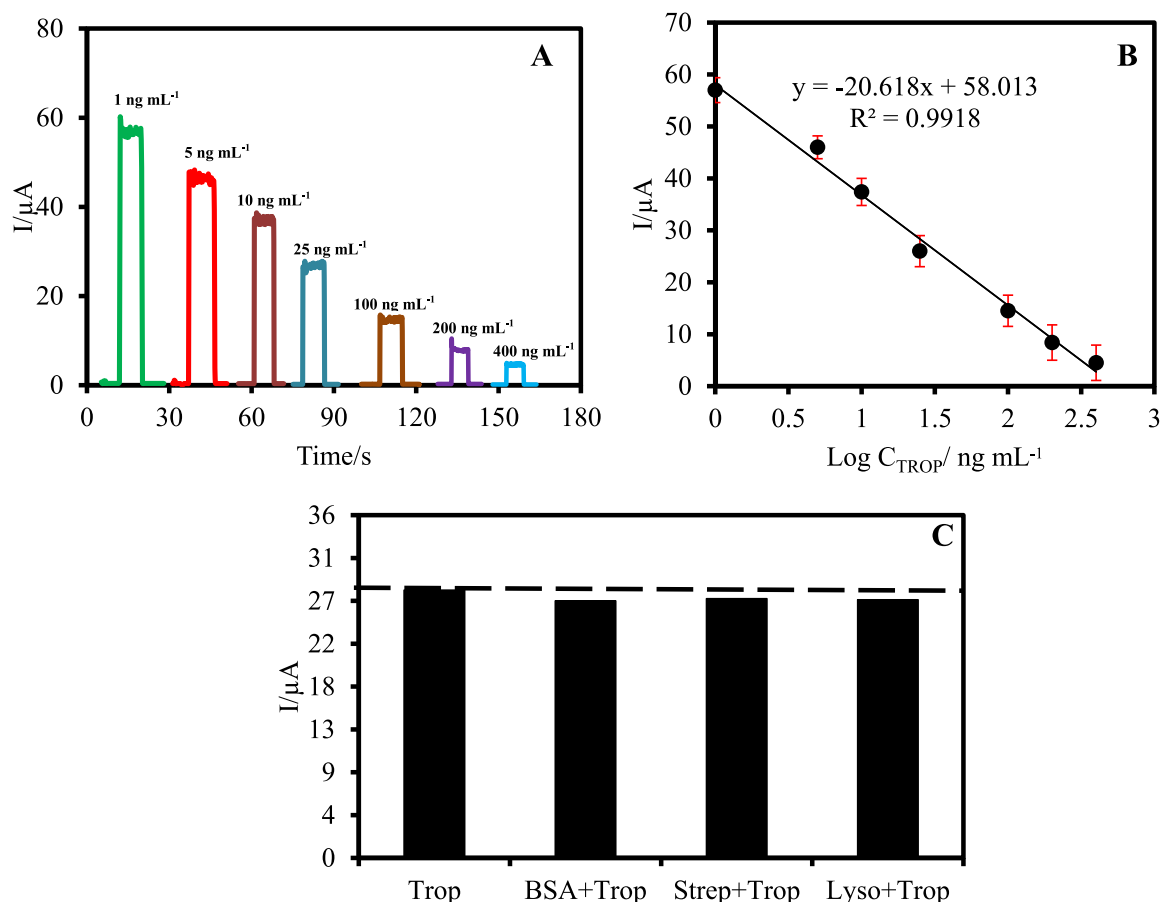


Fig. 4. (A) Time-based photocurrent response and (B) logarithmic calibration curve for PEC aptasensor for the detection of different amounts of tropomyosin (1, 5, 10, 25, 100, 200, and 400 ng mL⁻¹). Error bars were the standard deviation of four replicate determinations. Applied potential: +0.2 V. (C) Selectivity of the proposed PEC aptasensor was shown for tropomyosin in presence of 10 times excess of BSA, streptavidin, lysozyme and tropomyosin. The concentration of tropomyosin is 25.0 ng mL⁻¹ and that of the others were 250.0 ng mL⁻¹.

PEI promoted the transfer of the negatively charged $\text{Fe}(\text{CN})_6^{3-/4-}$ probe to the ITO/g-C₃N₄-TiO₂/PEI surface (Shangguan et al., 2015). But, after the immobilization of TROP aptamer on ITO/g-C₃N₄-TiO₂/PEI surface, the peak current of $\text{Fe}(\text{CN})_6^{3-/4-}$ decreased (curve_c). It is because of the strong electrostatic repulsion interaction between negatively charged aptamer_{TROP} and negatively charged $\text{Fe}(\text{CN})_6^{3-/4-}$ probe. But, after the adsorption of $\text{Ru}(\text{NH}_3)_6^{+3}$ on aptamer_{TROP}, the peak current increased. It was explained that adsorbed $\text{Ru}(\text{NH}_3)_6^{+3}$ endorsed electron-transfer on the surface of the electrode (curve_c). After the incubation of aptamer_{TROP} with TROP (25.0 ng mL⁻¹), the peak current decreased (curve_d). The reasonable explanation is that the formation of aptamer_{TROP}/TROP complex led to a mass-transfer limiting of $\text{Fe}(\text{CN})_6^{3-/4-}$ to the electrode surface.

Fig. 2B also shows the EIS behavior of $\text{Fe}(\text{CN})_6^{3-/4-}$, during stepwise changes of the electrodes. It can be seen that the Nyquist diameter ($R_{\text{et}}=195\ \Omega$) of ITO (curve_b) was higher than the ITO/g-C₃N₄-TiO₂/PEI ($R_{\text{et}}=83\ \Omega$) (curve a). But, after the immobilization of aptamer_{TROP} on the surface of ITO/g-C₃N₄-TiO₂/PEI (curve_c), the R_{et} of electrode increased to 620.0 Ω . Also, after the adsorption of $\text{Ru}(\text{NH}_3)_6^{+3}$ on ITO/g-C₃N₄-TiO₂/PEI/aptamer_{TROP}, the R_{et} of electrode decreased to 420.0 Ω (curve_d). Finally, the incubation of TROP with aptamer on ITO/g-C₃N₄-TiO₂/PEI/aptamer_{TROP} electrode led to a dramatical increase in the R_{et} of the electrode to 797.0 Ω . The inset of Fig. 2B is the equivalent circuit of the EIS.

The time-based photocurrent response of the ITO (curve_a), ITO/g-C₃N₄-TiO₂/PEI (curve_b), ITO/g-C₃N₄-TiO₂/PEI/aptamer_{TROP} (curve_c) ITO/g-C₃N₄-TiO₂/PEI/aptamer_{TROP}- $\text{Ru}(\text{NH}_3)_6^{+3}$ (curve_d) and, ITO/g-C₃N₄-TiO₂/PEI/aptamer_{TROP}/TROP (curve_e) in PBS (0.1 M, pH = 7.4) containing 0.1 mM AA was shown in Fig. 2C. No photo-

current was generated for ITO electrode under visible light illumination (curve_a), indicating that the ITO electrode is not a suitable electrode for observing the photocurrent signal. However, ITO/g-C₃N₄-TiO₂/PEI electrode showed strong photocurrent signal of 16 μA (curve_b). After the immobilizing of aptamer on the surface of the electrode, photo-generated electron transfer was blocked, therefore the photocurrent intensity decreased (curve_c). But after the adsorbing of $\text{Ru}(\text{NH}_3)_6^{+3}$ on the aptamer, the photocurrent intensity was increased (curve_d) due to enhancing electron transfer (Shangguan et al., 2015). However, after the incubation of tropomyosin (25.0 ng mL⁻¹) with the aptamer, the photocurrent intensity decreased dramatically (curve_e). It explained that the incubated TROP which is a high-molecular-weight protein (36.6 and 38.5 KD) (Zhang et al., 2017) not only hindered the diffusion of the AA to the electrode surface but also decreased the amount of adsorbed $\text{Ru}(\text{NH}_3)_6^{+3}$ on the electrode surface.

3.3. Optimization of effective parameters on aptasensor response

The influence of incubation time and pH of the solution on the response of PEC aptasensor to 25.0 ng mL⁻¹ of TROP were studied (Fig. 3). As shown in Fig. 3A, the response of PEC aptasensor increased rapidly with increasing incubation time up to 80 min, and remained unchanged at longer incubation times, suggesting that the formation of tropomyosin/aptamer_{TROP} aggregate on the electrode surface has reached a saturation level. Also, Fig. 3B shows the influence pH value on the response of aptasensor to tropomyosin. As shown in this figure, with increasing pH from 5.4 to 8.4, the responses of aptasensor increased from pH 5.4 to 8.4 and reached the maximum at pH 7.4 and then decreased with higher pH value. This was because that the

alkaline or acid solutions would break the tropomyosin/aptamer linkage and inactivate the biomolecules. Therefore, the accumulation time of 80 min and, solutions with a pH 7.4 have been chosen as the optimum condition for determination of tropomyosin in throughout this work.

3.4. Detection of tropomyosin

Under the optimized experimental conditions, the proposed PEC aptasensor was employed for the determination of TROP (Fig. 4A). It can be seen that the photocurrent intensity decreased with increasing TROP concentration. The current had a good linear relationship versus the logarithm of the concentration of tropomyosin in the dynamic linear range from 1 to 400 ng mL⁻¹ with a regression equation of I_p (μA) = -20.618 Log C_[tropomyosin] (ng mL⁻¹) + 58.013 and a correlation coefficient of 0.9918 (Fig. 4B). The limit of detection (LOD) was 0.23 ng mL⁻¹ (3σ/S), where σ is the standard deviation of the blank measurements and S is the slope. The error-bars represent the calculated standard deviation for the four measurements. The obtained LOD was lower than the previous ELISA base (Seiki et al., 2007; Shibahara et al., 2007), and aptamer base system (Zhang et al., 2017) but higher than sandwich ELISA based on the monoclonal antibody for Kuruma prawn tropomyosin (Zhang et al., 2014). The effect interfering BSA, streptavidin, and lysozyme was studied on the determination of TROP (Fig. 4C). As shown in this figure, the proposed PEC aptasensor has higher selectivity to TROP. The concentrations of the interfering substances were 10.0 times of TROP (25.0 ng mL⁻¹). The stability of the proposed PEC aptasensor was then evaluated. The relative standard deviation (RSD) was found to be 4.3% after 14 days. The reproducibility was also evaluated for determinations of 25 ng mL⁻¹ of TROP with five different sensors. The relative standard deviation (RSD) was calculated to be for TROP 4.6%. Therefore, the analytical performances of proposed PEC aptasensor such as stability, precision, and reproducibility are comparable with other PEC aptasensors in the literature (Li et al., 2016a; Shangguan et al., 2015; Zhang et al., 2011b). The proposed aptasensor was also employed for the determination of TROP in human serum to examine its applicability for real sample analysis. The human serum samples were diluted 10.0 times with PBS (0.1 M, pH 7.4). The recoveries of samples were obtained by using a standard addition method (Table S1).

4. Conclusions

Summerly, a novel PEC aptasensor base on ITO/ g-C₃N₄-TiO₂/PEI/ aptamer_{TROP} electrode has been developed for the determination of TROP. The amine terminal aptamer_{TROP} probe was attached on the ITO/g-C₃N₄/TiO₂/PEI electrode surface via GA crosslinker. To increase of photoelectrochemical intensity of the signal, the Ru(NH₃)₆³⁺ probe was absorbed on aptamer. Under the optimum condition, the proposed PEC aptasensor shows high sensitivity, selectivity, and stability to TROP. However, the LOD of proposed aptasensor is higher than sandwich ELISA based on the monoclonal antibody for kuruma prawn tropomyosin. We believe that the proposed aptasensor could provide a unique opportunity for application in the broad field of food, clinical and biological science.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the

online version at doi:10.1016/j.bios.2017.06.040.

References

- Amiri, M., Salehniya, H., Habibi-Yangjeh, A., 2016. Ind. Eng. Chem. Res. 55, 8114–8122.
- Amouzadeh Tabrizi, M., Shamsipur, M., Mostafaie, A., 2016. Mater. Sci. Eng. C 59, 965–969.
- Chen, L., Huang, D., Ren, S., Dong, T., Chi, Y., Chen, G., 2013. Nanoscale 5, 225–230.
- Chen, L., Zeng, X., Si, P., Chen, Y., Chi, Y., Kim, D.-H., Chen, G., 2014. Anal. Chem. 86, 4188–4195.
- Das, D., Shinde, S.L., Nanda, K.K., 2016. ACS Appl. Mater. Interfaces 8, 2181–2186.
- Fan, D., Guo, C., Ma, H., Zhao, D., Li, Y., Wu, D., Wei, Q., 2016. Biosens. Bioelectron. 75, 116–122.
- Gordon, S.M., 2006. Peanut Butter, Milk, and Other Deadly Threats: What You Should Know About Food Allergies. Enslow Publishers, Berkeley Heights, NJ, 112.
- He, Y., Li, J., Liu, Y., 2015. Anal. Chem. 87, 9777–9785.
- Hong, Y., Fang, Z., Yin, B., Luo, B., Zhao, Y., Shi, W., Li, C., 2017. Int. J. Hydrog. Energy, 42, 6738–6745.
- Hou, H., Gao, F., Wang, L., Shang, M., Yang, Z., Zheng, J., Yang, W., 2016. J. Mater. Chem. A 4, 6276–6281.
- Jiang, D., Zhang, Y., Chu, H., Liu, J., Wan, J., Chen, M., 2014. RSC Adv. 4, 16163–16171.
- Kong, H.J., Won, D.H., Kim, J., Woo, S.I., 2016. Chem. Mater. 28, 1318–1324.
- Li, J., Zhang, Y., Kuang, X., Wang, Z., Wei, Q., 2016a. Biosens. Bioelectron. 85, 764–770.
- Li, X., Zhou, Y., Xu, Y., Xu, H., Wang, M., Yin, H., Ai, S., 2016b. Anal. Chim. Acta 934, 36–43.
- Liu, S., Cao, H., Wang, Z., Tu, W., Dai, Z., 2015. Chem. Commun. 51, 14259–14262.
- Liu, Y., Ma, H., Zhang, Y., Pang, X., Fan, D., Wu, D., Wei, Q., 2016a. Biosens. Bioelectron. 86, 439–445.
- Liu, Y., Yan, K., Zhang, J., 2016b. ACS Appl. Mater. Interfaces 8, 28255–28264.
- Liu, Y., Zhang, Y., Wu, D., Fan, D., Pang, X., Zhang, Y., Ma, H., Sun, X., Wei, Q., 2016c. Biosens. Bioelectron. 86, 301–307.
- Lopes, L.M.F., Garcia, A.R., Brogueira, P., Ilharco, L.M., 2010. J. Phys. Chem. B 114, 3987–3998.
- Ong, W.-J., Tan, L.-L., Ng, Y.H., Yong, S.-T., Chai, S.-P., 2016. Chem. Rev. 116, 7159–7329.
- Seiki, K., Oda, H., Yoshioka, H., Sakai, S., Urisu, A., Akiyama, H., Ohno, Y., 2007. J. Agric. Food Chem. 55, 9345–9350.
- Shangguan, L., Zhu, W., Xue, Y., Liu, S., 2015. Biosens. Bioelectron. 64, 611–617.
- Shibahara, Y., Oka, M., Tominaga, K., Ii, T., Umeda, M., Uneo, N., Abe, A., Ohashi, E., Ushio, H., Shiomi, K., 2007. Determination of Crustacean allergen in food products by sandwich ELISA. J. Jpn. Soc. Food Sci. 54, 280–286.
- Shu, J., Qiu, Z., Zhou, Q., Lin, Y., Lu, M., Tang, D., 2016. Anal. Chem. 88, 2958–2966.
- Sun, A.-L., Qi, Q.-A., 2016. Analyst 141, 4366–4372.
- Sun, M., Fang, Y., Kong, Y., Sun, S., Yu, Z., Umar, A., 2016. Dalton Trans. 45, 12702–12709.
- Teresa Fernández-Abedul, M., Begoña González-García, M., Agustín, C.-G., Escarpa, A., González, M.C., López, M.Á., 2015. Agricultural and Food Electroanalysis, Chapter 9: Electrochemical Immunosensors. John Wiley & Sons, Ltd, 223–293.
- Walsh, J.J., Jiang, C., Tang, J., Cowan, A.J., 2016. Phys. Chem. Chem. Phys. 18, 24825–24829.
- Wen, W., Yan, X., Zhu, C., Du, D., Lin, Y., 2017. Anal. Chem. 89, 138–156.
- Yan, S.C., Li, Z.S., Zou, Z.G., 2009. Langmuir 25, 10397–10401.
- Yu, W., Xu, D., Peng, T., 2015. J. Mater. Chem. A 3, 19936–19947.
- Zeng, X., Ma, S., Bao, J., Tu, W., Dai, Z., 2013. Anal. Chem. 85, 11720–11724.
- Zhang, H., Lu, Y., Ushio, H., Shiomi, K., 2014. Food Chem. 150, 151–157.
- Zhang, H., Ma, L., Li, P., Zheng, J., 2016a. Biosens. Bioelectron. 85, 343–350.
- Zhang, L., Sun, Y., Liang, Y.-Y., He, J.-P., Zhao, W.-W., Xu, J.-J., Chen, H.-Y., 2016b. Biosens. Bioelectron. 85, 930–934.
- Zhang, X., Li, S., Jin, X., Li, X., 2011a. Biosens. Bioelectron. 26, 3674–3678.
- Zhang, X., Li, S., Jin, X., Zhang, S., 2011b. Chem. Commun. 47, 4929–4931.
- Zhang, X., Xie, X., Wang, H., Zhang, J., Pan, B., Xie, Y., 2013. J. Am. Chem. Soc. 135, 18–21.
- Zhang, Y., Wu, Q., Wei, X., Zhang, J., Mo, S., 2017. Microchim. Acta 184, 633–639.
- Zhao, W.-W., Xu, J.-J., Chen, H.-Y., 2014. Chem. Rev. 114, 7421–7441.
- Zhao, W.-W., Wang, J., Zhu, Y.-C., Xu, J.-J., Chen, H.-Y., 2015. Anal. Chem. 87, 9520–9531.
- Zheng, Y., Liu, J., Liang, J., Jaroniec, M., Qiao, S.Z., 2012. Energy Environ. Sci. 5, 6717–6731.
- Zhou, D., Wang, M., Dong, J., Ai, S., 2016. Electrochim. Acta 205, 95–101.
- Zhu, J., Xiao, P., Li, H., Carabineiro, S.A.C., 2014. ACS Appl. Mater. Interfaces 6, 16449–16465.

Supporting information

A high sensitive visible light-driven photoelectrochemical aptasensor for shrimp allergen tropomyosin detection using graphitic carbon nitride-TiO₂ nanocomposite

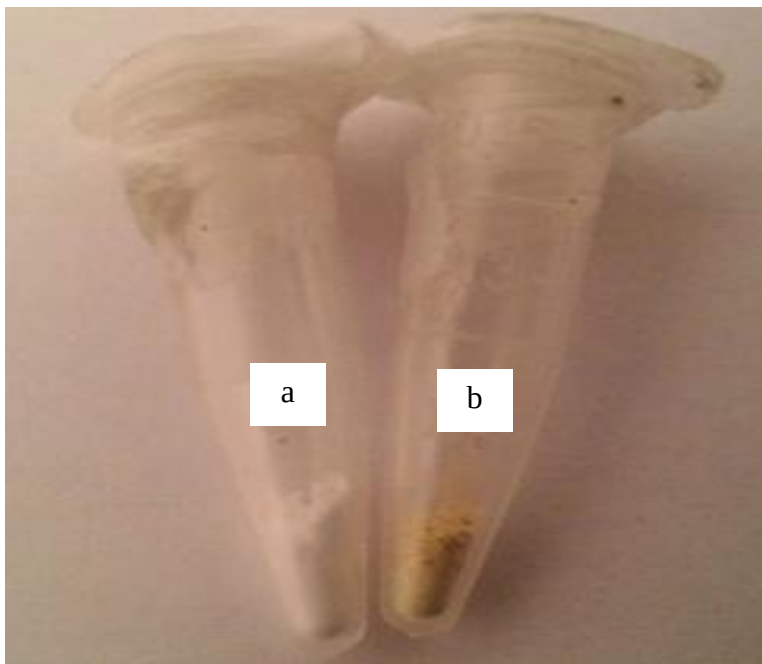
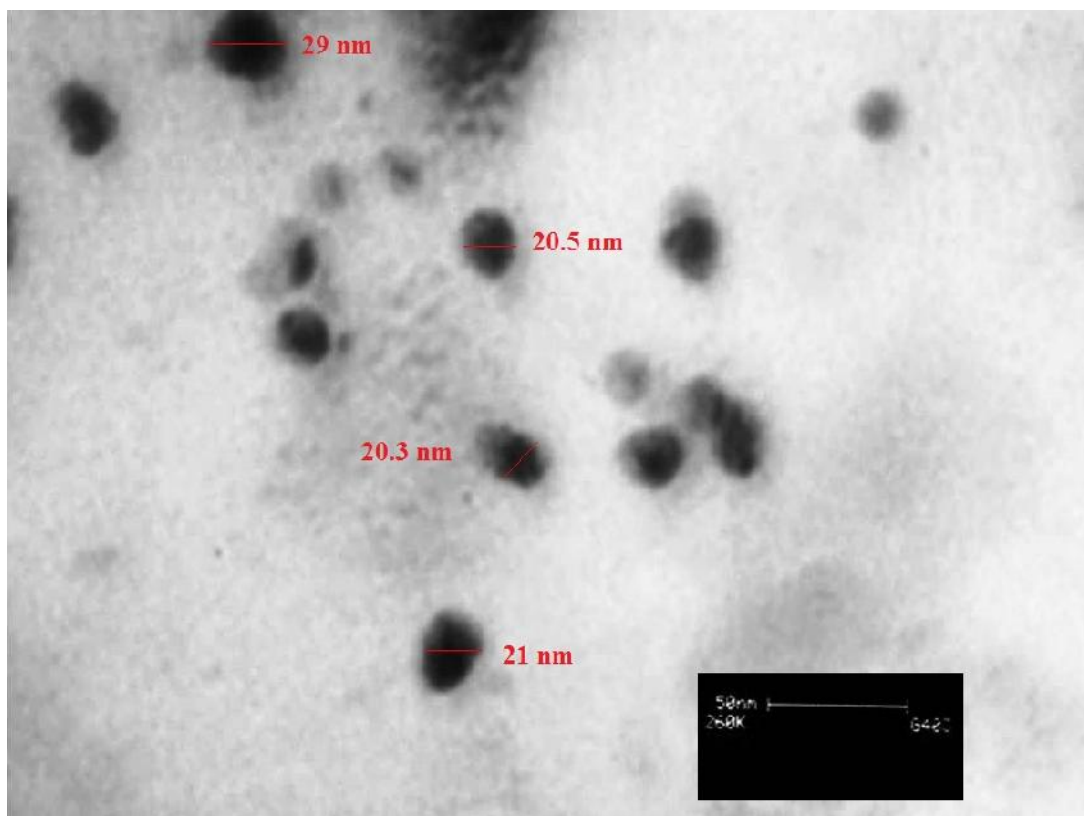
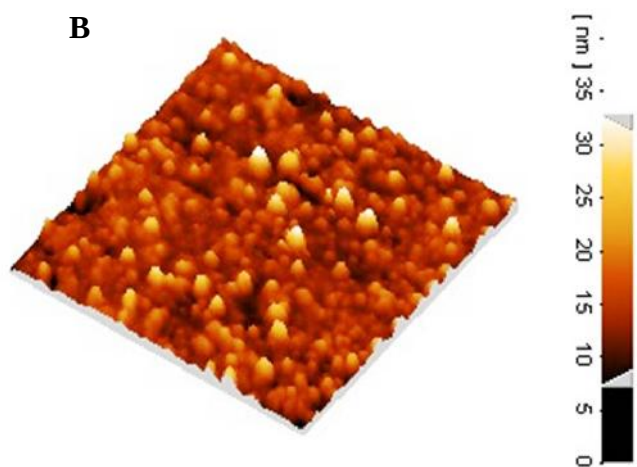


Figure S1. Photograph showing the change in colour of melamine before (a) and after (b) pyrolysis process.

A



B



C

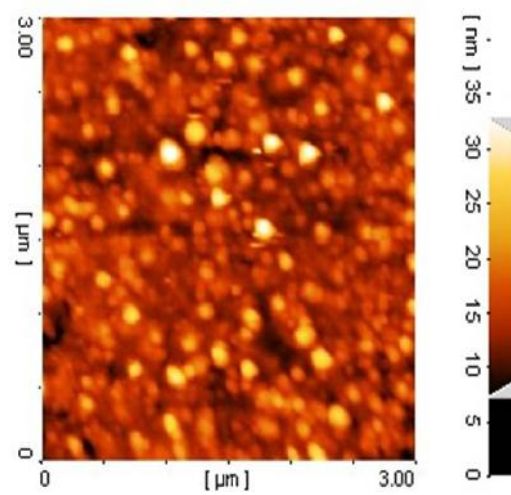


Figure S2. (A) TEM and (B, C) AFM images of TiO_2 nanoparticles.

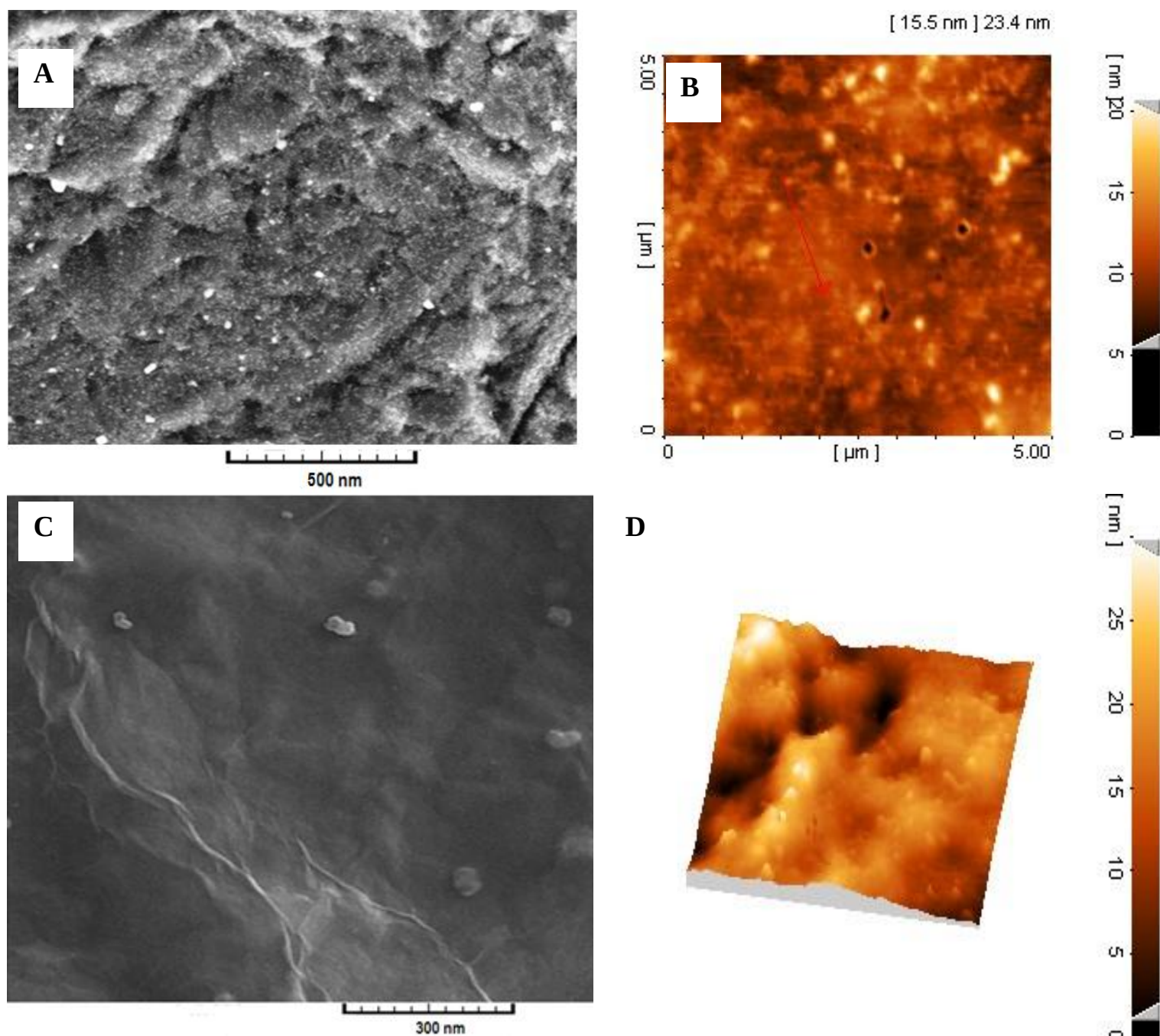


Figure S3. SEM (A,C) and AFM (B, D) images of ITO/g-C₃N₄-TiO₂ (A, B) and ITO/g-C₃N₄-TiO₂/PEI (C, D).

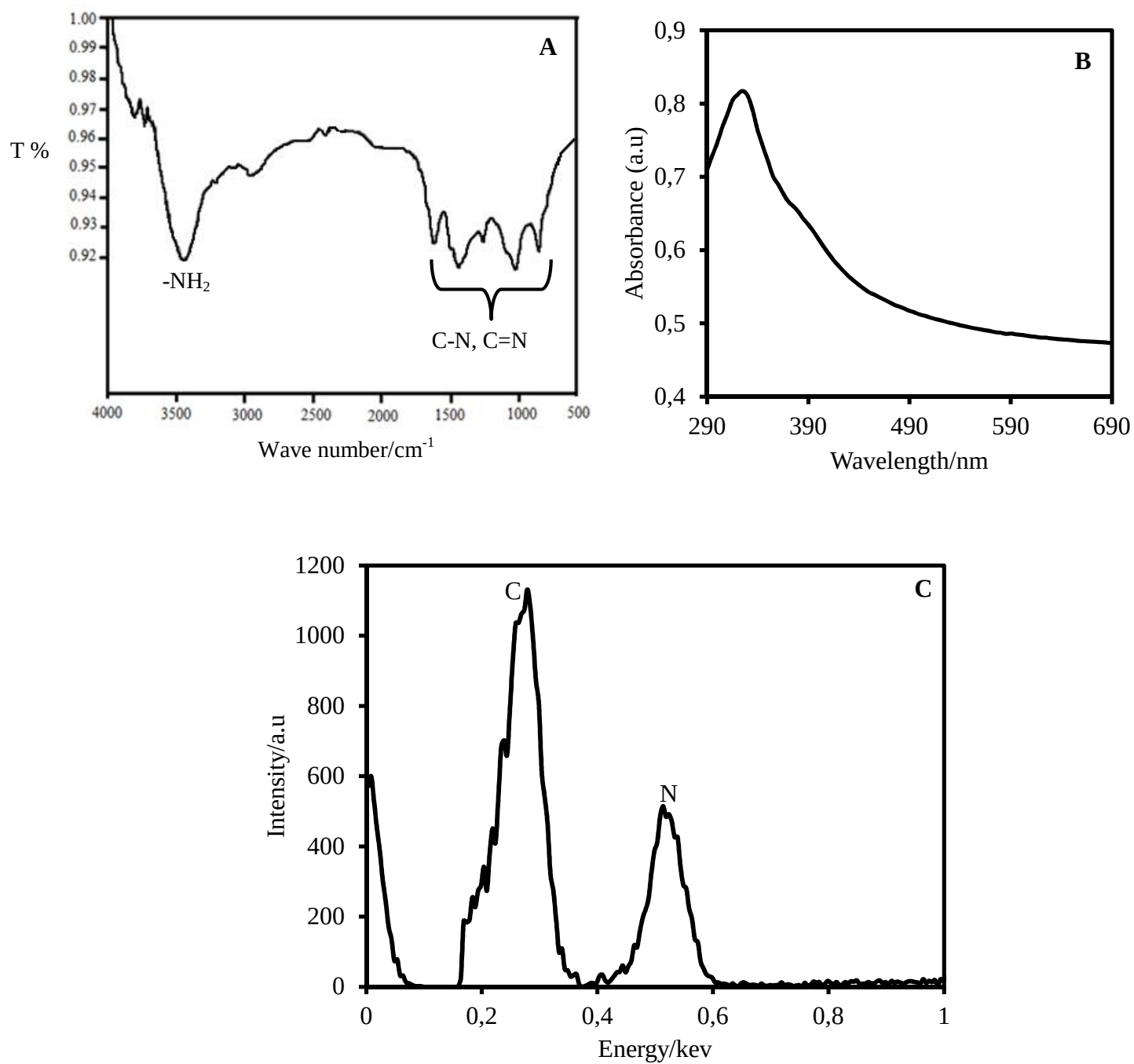


Figure S4. (A) FTIR, (B) UV-vis absorption, and (C) EDX spectra of g-C₃N₄.

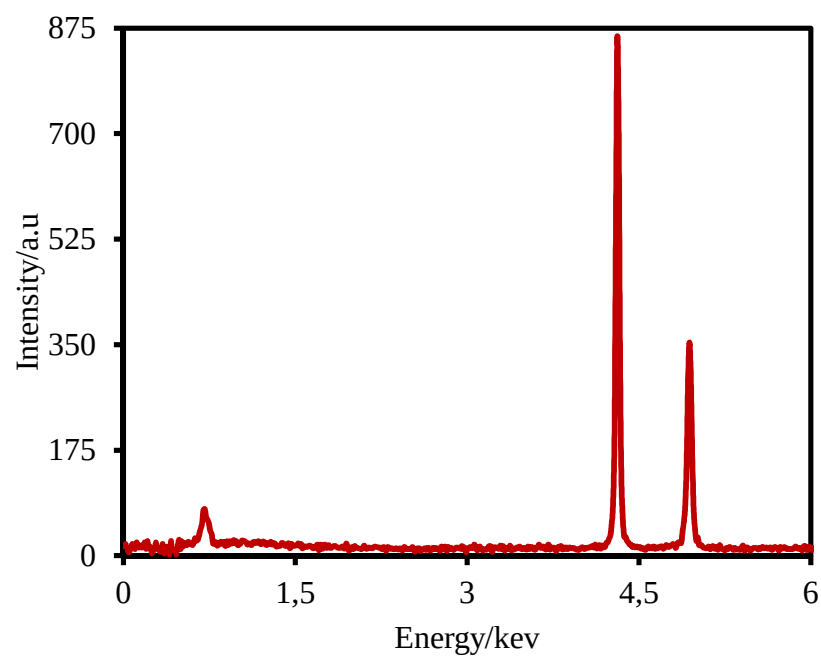


Figure S5. EDX spectra of TiO₂ nanoparticles.

Table S1. Determination of TROP cancer cells in human serum sample (n=2).

Sample	Added (ng mL ⁻¹)	Found (cell mL ⁻¹)	Recovery (%)
1	25	26.25	105.0
2	25	26.62	106.5

n: Determination times