



Short communication

A novel multi-amplification photoelectrochemical immunoassay based on copper(II) enhanced polythiophene sensitized graphitic carbon nitride nanosheet



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ABSTRACT

A new sandwich photoelectrochemical (PEC) sensing strategy was proposed for the first time based on the increasing photocurrent of water-soluble polythiophene sensitized g-C₃N₄ nanosheet (PT-Cl/g-C₃N₄) in the presence of copper(II) (Cu²⁺), which was doped on the surface of titanium dioxide as labels for multi-amplification. Herein, the photoactive films of PT-Cl/g-C₃N₄ is employed as the photoactive antibody (Ab₁) immobilization matrix for the subsequent sandwich-type antibody–antigen affinity interactions. Upon the presence of antigen (Ag), greatly enhanced photocurrent could be triggered in the PEC platform by the labels of second antibody (Ab₂) of Cu²⁺ doped titanium dioxide (Cu²⁺-TiO₂). As a result of the multi-amplification in this Cu²⁺-TiO₂ enhanced PT-Cl/g-C₃N₄-based PEC immunoassay, it possesses excellent analytical performance. The antigen could be detected from 0.01 pg mL⁻¹ to 100.0 ng mL⁻¹ with a detection limit of 5 fg mL⁻¹. This work opens up g-C₃N₄ nanosheet applied in PEC sensing. More importantly, the strategy of specific positive effect of Cu²⁺ on the photocurrent of g-C₃N₄ opens an alternative horizon for PEC sensing.

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1. Introduction

Recently, polymer-like semiconductor material graphitic carbon nitride (g-C₃N₄) has attracted great interest in numerous fields including photocatalytic water splitting, photodegradation of environmental organic pollutants and electrochemical sensors, because the stable and metal-free g-C₃N₄ possess energy band characteristics of metal oxide semiconductors (Wang et al., 2012a, 2012b, 2009a, 2009b). However, low carrier mobility, insufficient sunlight absorption and high recombination rate of photogenerated electron–hole pairs in the bare g-C₃N₄ system, limit its potential application in the area of photoelectric conversion (Sun et al., 2012). To solve these problems, some effective approaches have been developed to further optimize the photoelectric performance of g-C₃N₄, including doping it with metallic or nonmetallic elements, and copolymerizing it with other organic compounds (Zhang et al., 2010; Wang et al., 2009a, 2009b; Cheng et al., 2013). Even though, significant improvement of its photoelectric conversion efficiency has been shown by the methods mentioned above,

the more effective and simple photosensitizers to improve the performance of g-C₃N₄-based photovoltaic devices are highly deserved.

Conjugated polymers (CPs) are attracting more and more interest because of their wide absorption coefficients leading to efficient light harvesting properties, which could reduce the thickness of the semiconductor film (Yanagida et al., 2004). In quest of commercially viable conducting polymers, polythiophene and its derivatives have always been the most promising candidates because they have high absorption efficiency and charge-carrier mobility, good chemical stability and thermal stability, easy modification and controlling for electrochemical and photoelectrochemical (PEC) behavior (Gazotti et al., 2001; Schilinsky et al., 2002; Valaski et al., 2001; Huynh et al., 2002; Song et al., 2003; Breeze et al., 2001). Although obviously improved performance were obtained through the coupling of CPs and inorganic semiconductors (e.g. TiO₂) (Smestad et al., 2003; Arango et al., 1999; Grant and Schwartzberg, 2002; Yanagida et al., 2004) and carbon nanotubes (CNT) (Sgobba et al., 2009), to the best of our knowledge, little work has been done about these CPs as photosensitizers (“dye” molecules) in the g-C₃N₄ systems. In the present study, a novel water-soluble cationic conjugated polymer denoted

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as poly 2,5-[3-(1,1-dimethyl-4-piperidine methylene) thiophene] chloride (PT-Cl) was used to be an efficient photo-sensitizer for enhancing PEC sensing of $g\text{-C}_3\text{N}_4$. The novel PT-Cl-sensitized $g\text{-C}_3\text{N}_4$ photoactive films (PT-Cl/ $g\text{-C}_3\text{N}_4$) with super-photoelectric performance were fabricated by assembling of positively charged PT-Cl and negative charged carboxylated $g\text{-C}_3\text{N}_4$. Additionally, in order to further improve the crystal morphology of the photoactive films to acquire higher photoelectric conversion efficiency as well as better stability of modified electrode, a method of calcination at 450 °C was employed in this work.

In recent reports, $g\text{-C}_3\text{N}_4$ has been applied to the PEC selective sensing of copper (II) (Cu^{2+}) in aqueous solutions, which were based on the photocurrent intensity of the $g\text{-C}_3\text{N}_4$ increased with the increasing concentration of Cu^{2+} (Xu et al., 2013, 2014; She et al., 2014). Inspired by this mind, in present work, the Cu^{2+} -loaded nanomaterial was explored as labels for the first time to enhance the photocurrent of $g\text{-C}_3\text{N}_4$ -based materials. Nanoporous titanium dioxide (TiO_2) has been particularly investigated in recent decades since they have high photo-current conversion efficiency, superior optical and electrical properties (Gajjela et al., 2010; Lutz et al., 2012; Wang et al., 2011; Leshuk et al., 2012; Liu et al., 2010). In this study, copper ions surface-doped nanoporous titanium dioxide microspheres ($\text{Cu}^{2+}\text{-TiO}_2$) was employed as labels of secondary antibodies (Ab_2) to obtain multi-amplification by using the excellent photoelectric conversion efficiency of TiO_2 as well as the specific positive effect of Cu^{2+} on the photocurrent of $g\text{-C}_3\text{N}_4$. The multi-amplification in this $\text{Cu}^{2+}\text{-TiO}_2$ enhanced PT-Cl/ $g\text{-C}_3\text{N}_4$ -based PEC immunoassay possesses excellent analytical performance and opens an alternative horizon for PEC sensing.

2. Experimental section

2.1. Chemicals and reagents

Ethylenediamine ($\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2$, $\geq 99\%$), glutaraldehyde (25%), Bovine serum albumin (BSA) (96–99%) were obtained from Sigma-Aldrich (Beijing, China). All aqueous solutions were prepared using ultrapure water (Milli-Q, Millipore). The details are shown in [Supplementary information](#).

2.2. Apparatus

Fourier Transform Infrared Spectrometer (FT-IR Spectrometer) was recorded by VERTEX70 spectrometer (Bruker Co., Germany). The details are shown in [Supplementary information](#).

2.3. Preparation and characterization of materials

In this work, carboxylated $g\text{-C}_3\text{N}_4$ was synthesized according to the reference with some slight modifications (Cheng et al., 2013). As shown in [Fig. S1](#), the SEM ([Fig. S1A](#)) and TEM images ([Fig. S1B](#)) of the carboxylated $g\text{-C}_3\text{N}_4$ illustrated the nanosheet structure, which could provide excellent photoelectric properties and large surface area. [Fig. S1C](#) shows the FT-IR spectrum of $g\text{-C}_3\text{N}_4$ (blank line) and carboxylated $g\text{-C}_3\text{N}_4$ (red line) in the wavelength range of 500–4000 cm^{-1} . The peak of 1720 cm^{-1} is corresponding to the C=O bending band and the absorption band for -COO at 1575 cm^{-1} and 1380 cm^{-1} , indicating successful functionalization of carboxyl group on $g\text{-C}_3\text{N}_4$ (Dong et al., 2013). The fabrication of TiO_2 and $\text{Cu}^{2+}\text{-TiO}_2$ was conducted following a literature procedure with some modifications (Li et al., 2008). As the TEM images of TiO_2 and $\text{Cu}^{2+}\text{-TiO}_2$ show ([Fig. S1D](#) and [E](#)), the as-prepared TiO_2 possess uniform size with an average diameter of 250 nm ([Fig. S1D](#)). The photo of [Fig. S1E](#) showed that there was an obvious change in the color of the powder after the assembling of Cu^{2+} ,

which proved the successful loading of Cu^{2+} onto the TiO_2 . The details of preparation of $\text{Cu}^{2+}\text{-TiO}_2\text{-Ab}_2$ was in [Supplementary materials](#). The utilized water-soluble Poly 2,5-[3-(1,1-dimethyl-4-piperidine methylene) thiophene] chloride (PT-Cl) was synthesized according to literature (Li et al., 2012).

2.4. Immunoassay development

[Scheme 1](#) shows the developing process of the multi-amplified PEC immunoassay. Firstly, 4 μL negative charged $g\text{-C}_3\text{N}_4$ solution and 4 μL positively charged PT-Cl solution was dropped onto the pretreated ITO surface sequentially and kept for 20 min. After each dropping step, the ITO sample zone was rinsed thoroughly, dried in room temperature and then annealed at 450 °C for 5 min in a muffle furnace to obtain desired film stability and photocurrent intensity. Afterwards, 4 μL of 0.5 mg mL^{-1} Ab_1 was immobilized onto the PT-Cl/ $g\text{-C}_3\text{N}_4$ modified ITO electrode via physical process, after incubation for 1 h, the electrode was rinsed with the washing buffer to remove physically adsorbed Ab_1 . The electrode was then blocked with 4 μL blocking solution for 1 h at 4 °C to block non-specific binding sites and washed with the washing buffer thoroughly. Next, 4 μL of NMP-22 antigen with different concentrations were dropped onto the Ab_1 modified electrodes and incubated for 1 h at 37 °C followed by washing with washing buffer. After the binding reaction between Ab_1 and the antigen, the electrodes were allowed for labeling by additional incubation with 4 μL diluted $\text{Cu}^{2+}\text{-TiO}_2\text{-Ab}_2$ bioconjugates solution for 1 h. Thereafter, the electrodes were washed thoroughly with washing buffer to remove nonspecifically bound conjugations, and immersed in the PBS (pH 5.4) for 5 min before measurements. Then, the photocurrent was measured by the current–time technique at a bias voltage of -0.2 V following a 200 W LED excitation.

3. Results and discussion

[Fig. 1A](#) reveals the stepwise photocurrent responses of corresponding assembly. Curve a presented the photocurrent of PT-Cl/ $g\text{-C}_3\text{N}_4$ modified ITO electrode. Even though, the photocurrent decreased gradually with Ab_1 anchoring, BSA blocking and Ag specific binding (curves b–d), which was due to the steric hindrance of the hydrophobic protein layer (curves c–e, [Fig. S3](#)). However, after the final amplification via $\text{Cu}^{2+}\text{-TiO}_2$ by the formation of $\text{Cu}^{2+}\text{-TiO}_2\text{-Ab}_2\text{-Ag}$ conjugations, the photocurrent intensity was 3 times of the initial intensity (curve e). Compared with pure $g\text{-C}_3\text{N}_4$ (curve a, [Fig. 1B](#)), the coupling of PT-Cl and $g\text{-C}_3\text{N}_4$ resulted in a dramatic enhanced photocurrent (curve b, [Fig. 1B](#)). This may be ascribed to the wide absorption coefficients of PT-Cl that leading to efficient light harvesting properties (Yanagida et al., 2004), and the fast separation of charge carriers in the PT-Cl/ $g\text{-C}_3\text{N}_4$ system caused by PT-Cl (Sgobba et al., 2009). Moreover, as shown in [Fig. 1B](#), the photocurrent of $\text{Cu}^{2+}\text{-TiO}_2$ (curve d) as labels was clearly higher than that of TiO_2 (curve c), indicating the prominent synergy enhancement of TiO_2 and Cu^{2+} .

In comparison with the previous PEC immunoassay protocols, the prominent signal enhancement should be attributed to the $\text{Cu}^{2+}\text{-TiO}_2$ labeling-induced multi-signal amplification. Specifically, the addition of Cu^{2+} would significantly increase the photocurrent of $g\text{-C}_3\text{N}_4$. The possible mechanism (see [Fig. S2](#)) may be that photo-generated electron of $g\text{-C}_3\text{N}_4$ transmitted to the ITO electrode, and then to the Pt reference electrode and formed loops (Xu et al., 2014). The photoinduced electron could be captured by Cu^{2+} that released from $\text{Cu}^{2+}\text{-TiO}_2\text{-Ab}_2$ in weakly acid solution, thus promoted the separation rate of electron and hole and led to the increment of the photocurrent response. Besides, TiO_2 would expand the light absorption of the underlying

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