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A label-free photoelectrochemical immunosensor for detection of the milk allergen β -lactoglobulin based on Ag_2S -sensitized spindle-shaped $\text{BiVO}_4/\text{BiOBr}$ heterojunction by an in situ growth method

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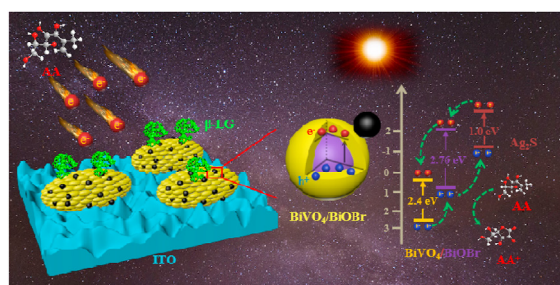
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HIGHLIGHTS

- A label-free PEC immunosensor was developed for rapid quantitative characterization of β -lactoglobulin.
- Spindle-shaped $\text{BiVO}_4/\text{BiOBr}$ with large specific surface area was synthesized by in situ corrosion.
- Ag_2S quantum dot sensitized $\text{BiVO}_4/\text{BiOBr}$ by in-situ growth demonstrated good PEC performance.
- This PEC immunosensor showed wide range and a low detection limit.

GRAPHICAL ABSTRACT



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ABSTRACT

Food allergies have become a nonnegligible food safety issue, and milk allergies are one of the most common food allergies, that has attracted large consumer attention. In this work, a novel label-free photoelectrochemical (PEC) immunosensor for the detection of the allergen β -lactoglobulin (β -LG) in dairy products was designed that used the specific recognition of allergen β -LG and antibodies in dairy products in combination with biosensing technology. Here, Ag_2S -sensitized spindle-shaped $\text{BiVO}_4/\text{BiOBr}$ heterojunction was fixed on the surface of the ITO electrode as an excellent photoactive substrate and effectively improved the photocurrent responses and sensitivity. Thioglycolic acid (TGA) was used as a linker to immobilize the β -LG antibody on the surface of the electrode. The photocurrent was detected at different antigen concentrations, which realized the quantitative testing of β -LG. Under the optimal experimental conditions, the PEC immunosensor proved an ideal linear relationship ranging from 10 pg/mL to 100 ng/mL, with a low detection limit of 3.7 pg/mL. The designed immunosensor showed good stability, a wide linear range, high sensitivity and good reproducibility and could be used for the detection of actual samples. The PEC immunosensor had a strong ability to specifically recognize β -LG, which was not affected by other proteins in the milk without pretreatment. Meanwhile, the developed

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immunosensor provided a promising PEC detection platform and reference idea for the detection of other proteins in milk.

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1. Introduction

Cows' milk allergy (CMA) is one of the most important allergies; it is the most common allergen in foods and is characterized by a strong IgE response to milk proteins [1]. The prevalence of CMA in children under two years old is approximately 2.5% [2]. CMA is a serious problem for children because milk is an important source of protein, and β -lactoglobulin is one of the main allergens in milk. It is infeasible to strictly avoid the use of milk, and it is impractical to replace milk [3]. In addition, severe allergies may persist into adulthood [4], which has attracted the attention of consumers [5].

β -Lactoglobulin (β -LG), a primary whey protein (approximately 20% of whey protein content) [6], is a globular protein with a molecular weight of 18,400 as a monomer [7]. It is considered the main whey allergen in milk, which is most frequently and strongly combined by human IgE [8]. β -LG is a nutrient-rich protein with a variety of rich functions (foaming, gelation, binding properties, emulsifying and food additives) [9]. Since children are extremely sensitive to trace allergens, the number of allergens in milk must be closely monitored.

Different β -LG allergen detection methods have been proposed, such as liquid chromatography-tandem mass spectrometry [10], gel electrophoresis [11], liquid chromatography [12], cell-based fluorescence sensors [13], lateral flow immunoassays (LFIA) [2], ELISA [8], and microcantilever resonator arrays [14]. At present, alternative methods with faster response, better selectivity, higher sensitivity and lower cost speed urgently need to be developed. Photoelectrochemical (PEC) sensing has now evolved as a powerful testing platform with high sensitivity and rapidity [15,16]. PEC immunosensors have attracted wide attention in the field of biological analysis due to their advantages, such as excellent selectivity and sensitivity, and fast analysis speed, low background influence [17].

For the PEC immunosensor, sensitivity is an indicator of performance. Various strategies have been used to improve sensitivity, of which increasing the initial signal is one of the most effective strategies. BiVO_4 is an important photoelectric active material with a good visible light response [18,19]. It has attracted strong interest on account of its stability and low cost of the appropriate band gap [20]. However, the photoelectric activity of BiVO_4 is limited by poor electron-hole pair separation ability, small specific surface area, and weak surface adsorption capacity [21,22]. Therefore, it is necessary to further optimize the photoelectric active properties of BiVO_4 . In our work, *in situ* acid etching was performed with hydrobromic acid as an etching agent. The $\text{BiVO}_4/\text{BiOBr}$ heterojunction, which has been synthesized by this method is more conducive to the separation and transfer of charges [23,24]. The $\text{BiVO}_4/\text{BiOBr}$ heterojunction with a smooth and large specific surface area could increase the binding site and facilitate binding with more particles.

In this research, a novel label-free PEC immunosensor for milk allergen β -LG detection was prepared using an Ag_2S [25]-sensitized spindle-shaped $\text{BiVO}_4/\text{BiOBr}$ heterojunction by an *in situ* growth method. Here, Ag_2S was fixed on the surface of spindle-shaped $\text{BiVO}_4/\text{BiOBr}$ through the strong coordination interaction between the bismuth atom of $\text{BiVO}_4/\text{BiOBr}$ and thiol group of TGA. The label-free PEC immunosensor platform showed good selectivity, sensitivity and excellent specificity.

2. Experimental

2.1. Reagents and solutions

Indium tin oxide (ITO) glass was obtained from Zhuhai Kaivo Electronic Components Co., Ltd, China. Ammonium vanadate (NH_4VO_3), Hydrobromic acid (anhydrous) (HBr), silver nitrate (AgNO_3) and ethanol ($\text{C}_2\text{H}_6\text{O}$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Bismuth nitrate pentahydrate ($\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$), ethylene glycol ($(\text{CH}_2\text{OH})_2$), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and ascorbic acid (AA) were obtained from Macklin Reagent Co., Ltd. (Shanghai, China). Mercapto acetic acid ($\text{C}_2\text{H}_4\text{O}_2\text{S}$), sodium sulfide (Na_2S), Albumin from bovine serum (BSA) and N-hydroxysuccinimide (NHS) were obtained from Aladdin Reagent Dology Co., Ltd. (Shanghai, China). Polyvinyl pyrrolidone, β -lactoglobulin, α -Lactalbumin, Casein and IgE were obtained from Sigma-Aldrich (Beijing, China). The rabbit anti-bovine β -LG capture antibody (A10–125A) was purchased from Bethyl Laboratories, Inc.

Phosphate buffered solutions (PBS, 1/15 mol/L KH_2PO_4 and 1/15 mol/L Na_2HPO_4) containing AA was used as an electrolyte for the photoelectrochemical measurements. Ultrapure water (Milli-Q, Millipore) used in all experiments was deionized to 18.25 M Ω ·cm. Other chemical reagents were analytical reagents grade and were directly used without further purification.

2.2. Apparatus

Transmission electron microscope (TEM) images were obtained from a FEI Tecnai G2 F20 field emission high resolution transmission electron microscope. Scanning electron microscope (SEM) images and energy dispersive spectrometry (EDS) were obtained using a field emission SEM (quanta 250, America). UV–vis diffuse reflectance spectrum measurements were performed with a Shimadzu UV3600 plus spectrometer (Japan). All photoelectrochemical measurements were performed with an electrochemical workstation (CHI760E, Chenhua Instrument Shanghai Co., Ltd, China). A three-electrode system was used, with a platinum wire as a counter-electrode, saturated calomel electrode as a reference electrode and modified ITO electrode ($2.5 \times 0.8 \text{ cm}^2$) as the working electrode. A 100 W LED lamp was used as an irradiation source. Electrochemical impedance spectroscopy (EIS) analysis was performed with a PGSTAT302 N Autolab electrochemical workstation (Metrohm, Switzerland). X-ray diffraction (XRD) patterns were obtained by using D8 advance focus diffractometer (Bruker AXS, Germany).

2.3. Synthesis of spindle-shaped $\text{BiVO}_4/\text{BiOBr}$

$\text{BiVO}_4/\text{BiOBr}$ was synthesized by a hydrothermal method with minor modifications [26]. First, 1.5 mmol $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ was dispersed in 50 mL ethylene glycol, and PVP (MW ~ 40 K) was slowly added to the above solution under magnetic stirring and ultrasonicated for 15 min, which was marked as solution A. NH_4VO_3 (1.5 mmol) was dispersed in 30 mL ultrapure water for 10 min under ultrasound and labeled solution B. Then, solution A was slowly added to solution B under magnetic stirring (drop 50 mL of solution A in 5 min), and the color changed from transparent to

orange. The final mixture was transferred to a 100 mL Teflon-lined stainless-steel autoclave and maintained at 180 °C for 10 h. The collected yellow product was alternately washed three times with excessive ultrapure water and ethanol. Finally, the BiVO_4 products were dried at 60 °C for 10 h in vacuum.

The prepared 1 mmol BiVO_4 and 1 g PVP were dispersed in 80 mL ultrapure water under magnetic stirring. After 20 min of ultrasonic dispersion, 0.4 mL HBr was dropped into the above mixture under magnetic stirring. Finally, the mixture was transferred to a 100 mL Teflon-lined stainless-steel autoclave and reacted at 180 °C for 10 h. The collected yellow product was alternately washed three times with excessive ultrapure water and ethanol and then dried at 60 °C under vacuum.

2.4. Assembled of label-free PEC immunosensor

The construction of the label-free PEC immunosensor is shown in Scheme 1. First, 5 mg/mL $\text{BiVO}_4/\text{BiOBr}$ was dispersed and sonicated for 10 min. Then, 10 μL of the $\text{BiVO}_4/\text{BiOBr}$ suspension was dropped on a clean ITO electrode. The $\text{BiVO}_4/\text{BiOBr}$ -coated ITO electrode was dried under an infrared baking lamp and calcined at 400 °C for 30 min. Ag_2S was deposited on the surface of $\text{BiVO}_4/\text{BiOBr}$ [27], which was reacted and adsorbed according to successive ionic layer adsorption [28]. The 3 μL TGA (0.1 mol/L) solution was placed on the surface of the ITO/ $\text{BiVO}_4/\text{BiOBr}$ electrode with a pipette and dried in air for approximately 30 min. After rinsing with ultrapure water, 3 μL of 0.1 mol/L AgNO_3 solution was covered with the ITO/ $\text{BiVO}_4/\text{BiOBr}$ /TGA electrode and reacted at room temperature for 30 min, then rinsed with ultrapure water to remove unbound solution. Three

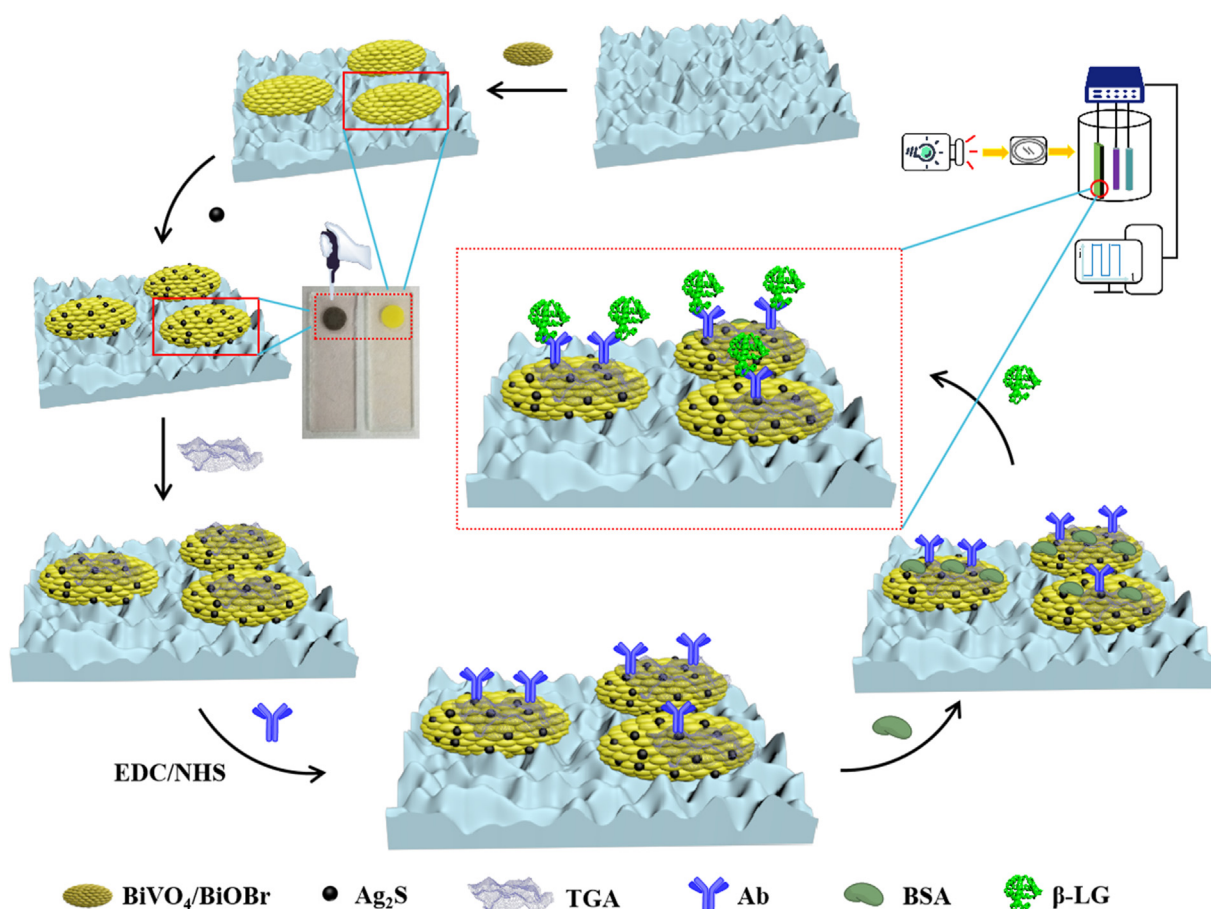
microliters of 0.12 mol/L Na_2S solution was added to the above electrode and reacted for approximately 30 min to complete the growth of Ag_2S at room temperature. Then, the excess Ag_2S was washed thoroughly with ultrapure water. The illustrations in Scheme 1 were $\text{BiVO}_4/\text{BiOBr}$ and $\text{BiVO}_4/\text{BiOBr}@ \text{Ag}_2\text{S}$ electrodes. The sample changed from yellow to black, which proved the generation of the new substance Ag_2S .

TGA (3 mmol/L, 3 μL) solution was coated on the ITO/ $\text{BiVO}_4/\text{BiOBr}@ \text{Ag}_2\text{S}$ electrode and naturally dried, followed by rinsing with ultrapure water. Three microliters of freshly prepared EDC/NHS solution were embellished on the ITO/ $\text{BiVO}_4/\text{BiOBr}@ \text{Ag}_2\text{S}$ /TGA electrodes to activate the carboxyl group at room temperature for 30 min. After washing to flush out the excess EDC/NHS, 4 μL of 10 $\mu\text{g}/\text{mL}$ anti- β -LG solution was assembled into an ITO/ $\text{BiVO}_4/\text{BiOBr}@ \text{Ag}_2\text{S}$ /TGA/(EDC/NHS) electrode and incubated for 30 min at 4 °C via a classic amidation protocol. The electrode was washed to remove uncombined anti- β -LG, incubated in 1 wt % BSA solution to close nonspecific sites and then washed thoroughly. Subsequently, 4 μL β -LG at different concentrations was dropped on the surface of the electrode and incubated for 30 min at 4 °C. After rinsing, the prepared ITO/ $\text{BiVO}_4/\text{BiOBr}@ \text{Ag}_2\text{S}$ /TGA/(EDC/NHS)/Ab/BSA/ β -LG electrodes were stored at 4 °C until use.

3. Results and discussion

3.1. Characterization of prepared materials

The nanomaterials were characterized by scanning electron microscopy (SEM). As shown in Fig. 1A, the spindle-shaped BiVO_4



Scheme 1. Fabrication process of the label-free PEC immunosensor.

scanning electron microscope image had a novel structure and uniform size. Fig. 1B is a further enlarged SEM image of BiVO_4 . BiVO_4 was a spindle-like structure with a rough surface, which increased the specific surface area and could bind more quantum dots. As shown in Fig. 1C, the $\text{BiVO}_4/\text{BiOBr}$ composite was formed by an in situ corrosion reaction of BiVO_4 with HBr . As shown in Fig. 2D, Ag_2S nanoparticles were grown in situ on the surface of TGA-modified $\text{BiVO}_4/\text{BiOBr}$ hierarchical microspheres, which were completely covered by Ag_2S nanoparticles. The EDS image (Fig. 2I) also proved the successful synthesis of the $\text{BiVO}_4/\text{BiOBr}/\text{Ag}_2\text{S}$ composite. From Fig. 1E, it could be concluded that a $\text{BiVO}_4/\text{BiOBr}$ structure was formed, in which BiOBr was coated on the outer layer of BiVO_4 under the action of HBr . The HRTEM image in Fig. 1F shows the lattice spacing of the substances in $\text{BiVO}_4/\text{BiOBr}/\text{Ag}_2\text{S}$, which corresponded to the lattice planes in the XRD results.

The energy dispersive spectrometry (EDS) test results of the synthesized BiVO_4 , $\text{BiVO}_4/\text{BiOBr}$ and $\text{BiVO}_4/\text{BiOBr}/\text{Ag}_2\text{S}$ materials are shown in Fig. 1 [29]. The spindle-like BiVO_4 was strongly confirmed to be successfully synthesized by the presence of Bi, V, and O elements in the EDS image of BiVO_4 (Fig. 1G). The presence of Bi, V, Br, and O in the EDS image of $\text{BiVO}_4/\text{BiOBr}$ (Fig. 1H) demonstrated the successful synthesis of hollow spindle-shaped $\text{BiVO}_4/\text{BiOBr}$ synthesized by in situ etching. It is worth mentioning that the

low content of BiOBr resulted in fewer diffraction peaks.

To prove the success of the synthetic materials, the X-ray diffraction (XRD) patterns of BiVO_4 and $\text{BiVO}_4/\text{BiOBr}$ were further studied [26,30]. There were no other interference diffraction peaks compared with the XRD pattern of BiVO_4 , and one-to-one correspondence with the standard card indicated that BiVO_4 was successfully prepared (Fig. 2A). The diffraction peak of BiVO_4 was completely present in the XRD pattern of $\text{BiVO}_4/\text{BiOBr}$. The diffraction peaks of BiOBr are located at 11.00° , 25.32° , 31.87° and 32.37° (Fig. 2B), indicating that the loading of BiOBr was successful.

The UV–visible diffuse reflectance spectra of $\text{BiVO}_4/\text{BiOBr}$ and $\text{BiVO}_4/\text{BiOBr}/\text{Ag}_2\text{S}$ are shown in Fig. 2C. The absorption range of $\text{BiVO}_4/\text{BiOBr}$ was below 500 nm, which indicated that $\text{BiVO}_4/\text{BiOBr}$ had a certain absorption of visible light (curve a), but the absorption of the visible light region was weak [31]. The absorption area and visible light absorption were significantly enhanced by the in situ growth of Ag_2S , which proved that the $\text{BiVO}_4/\text{BiOBr}/\text{Ag}_2\text{S}$ composite had significant visible light activity (curve b). The narrow band gap of Ag_2S produced by in situ growth resulted in less required electron transition energy (Fig. 2D). Therefore, the $\text{BiVO}_4/\text{BiOBr}/\text{Ag}_2\text{S}$ composite was prone to photogenic electron holes under visible light irradiation, which significantly enhanced the visible light activity.

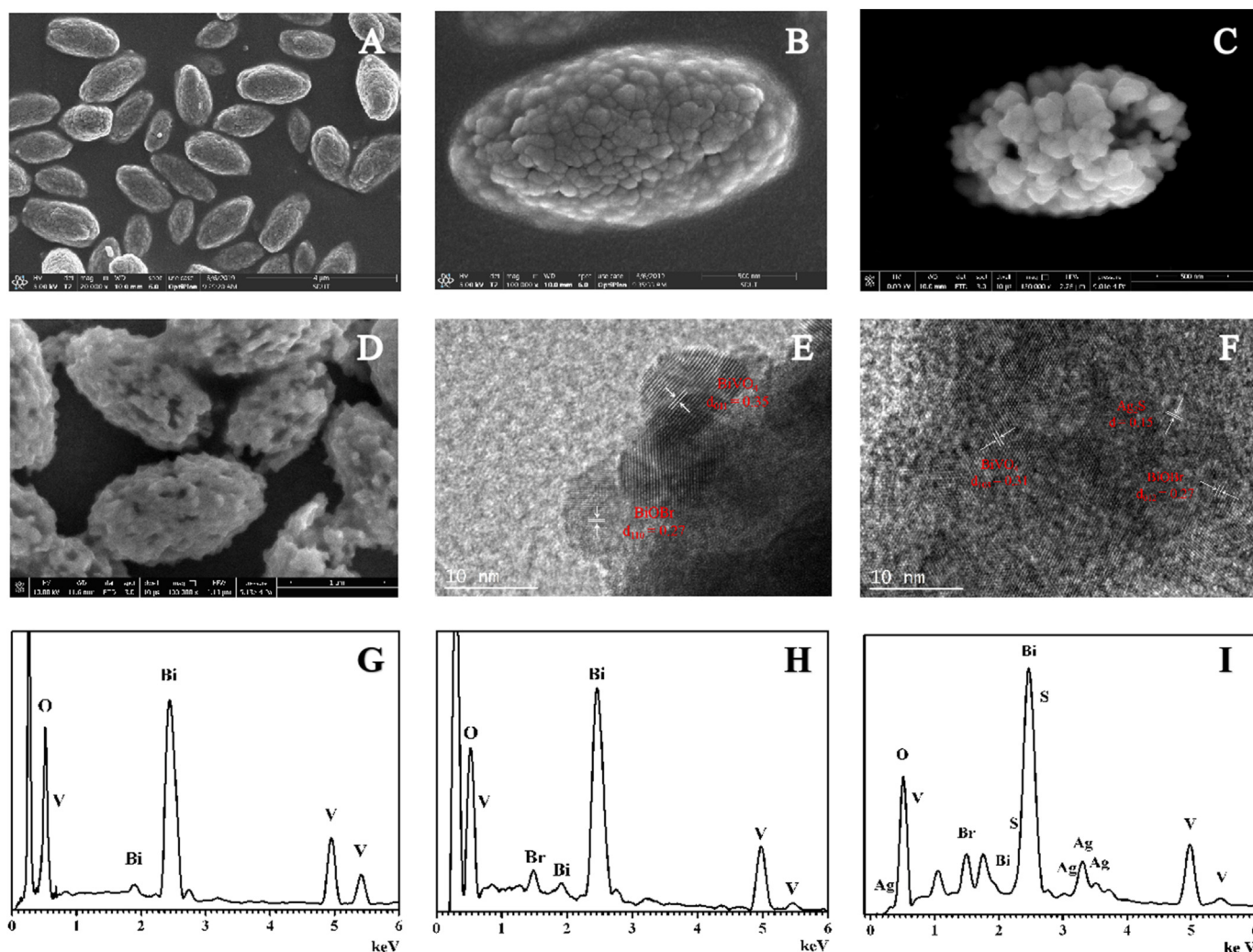


Fig. 1. SEM images of BiVO_4 (A and B), $\text{BiVO}_4/\text{BiOBr}$ (C) and $\text{BiVO}_4/\text{BiOBr}/\text{Ag}_2\text{S}$ (D), HRTEM images of $\text{BiVO}_4/\text{BiOBr}$ (E) and $\text{BiVO}_4/\text{BiOBr}/\text{Ag}_2\text{S}$, EDS images of BiVO_4 (G), $\text{BiVO}_4/\text{BiOBr}$ (H) and $\text{BiVO}_4/\text{BiOBr}/\text{Ag}_2\text{S}$ (I).

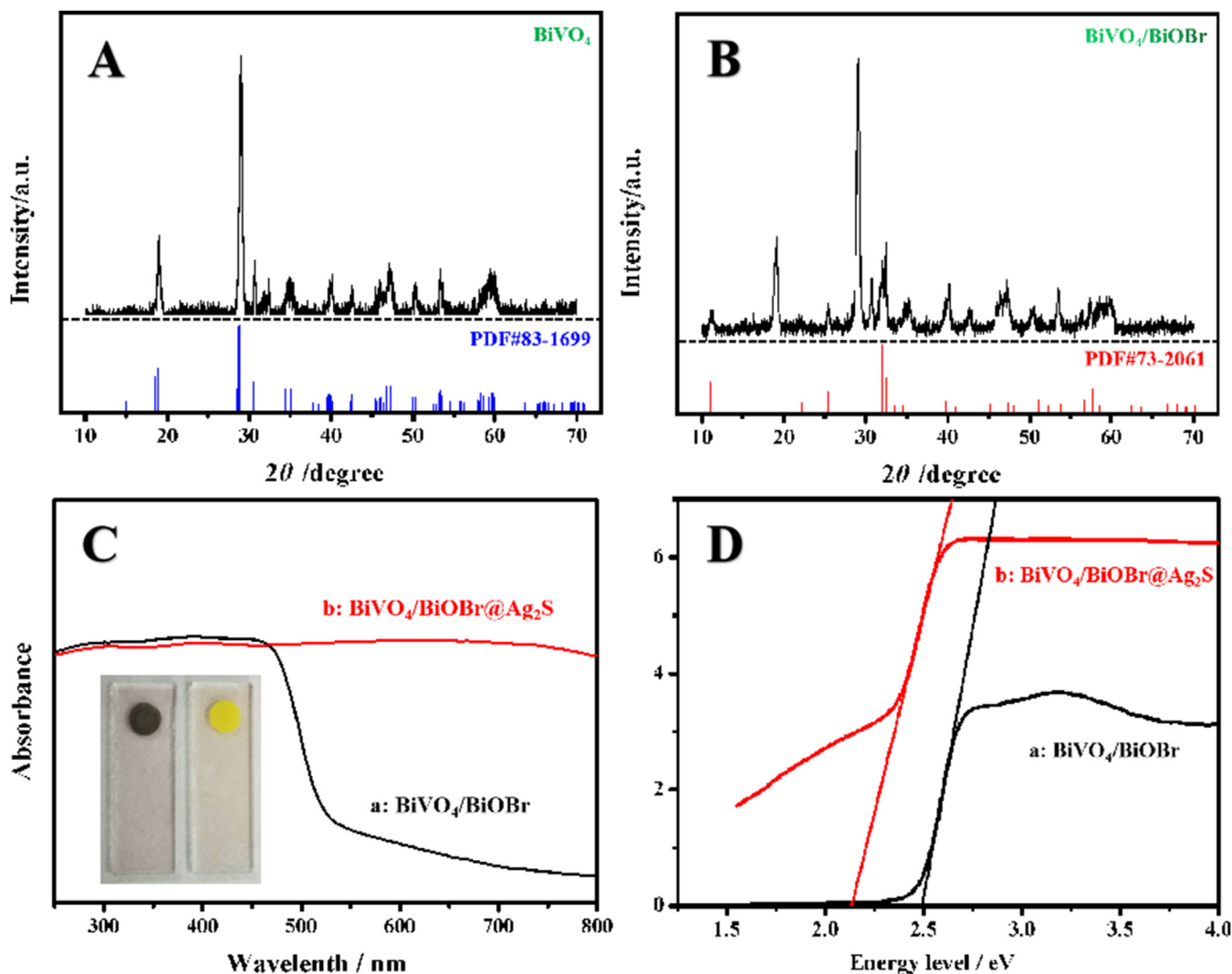


Fig. 2. XRD images of BiVO_4 (A) and $\text{BiVO}_4/\text{BiOBr}$ (B). (C) UV–vis diffuse reflectance spectra and the photon energy (D) of (a) $\text{BiVO}_4/\text{BiOBr}$ and (b) $\text{BiVO}_4/\text{BiOBr}@Ag_2S$.

3.2. Characterization of the label-free immunosensor

The layer-by-layer photocurrent response diagrams and electrochemical impedance spectroscopy (EIS) of the PEC immunosensor are an effective way to characterize the PEC immunosensor. As shown in Fig. 3A, the blank ITO electrode (curve a, 0.1 μA) and $\text{BiVO}_4/\text{BiOBr}$ (curve b, 0.3 μA) have almost no photocurrent signal because of the wide band gap energy of $\text{BiVO}_4/\text{BiOBr}$. Ag_2S NPs were grown in situ on $\text{BiVO}_4/\text{BiOBr}$ through strong coordination between the sulfhydryl and Bi atoms of $\text{BiVO}_4/\text{BiOBr}$ modified by TGA. When Ag_2S (curve c, 115 μA) is fixed to ITO/ $\text{BiVO}_4/\text{BiOBr}$, the photocurrent increases significantly due to the sensitization of Ag_2S with improvement of photocurrent conversion efficiency. β -LG antibody was immobilized on the electrode by classical amidation EDC/NHS (curve d, 97 μA). The $-\text{COOH}$ of Ag_2S could be obtained by coordinating the sulfhydryl group of TGA with the Ag atom. EDC/NHS was used as the linker between the $-\text{COOH}$ of TGA modified Ag_2S and the $-\text{NH}_2$ of anti- β -LG via an amidation reaction. After the Ab (curve e, 78 μA) and BSA (curve f, 52 μA) were fixed on the ITO/ $\text{BiVO}_4/\text{BiOBr}@Ag_2S/\text{EDC}/\text{NHS}$ electrode, the photocurrent was decreased because of the steric hindrance of bulky and insulated biomolecules. Finally, β -LG (curve g, 29 μA) was connected to

electrodes through a specific immune response. All photoelectric chemical measurements were made in PBS solution (pH = 7.4) containing 0.1 mol/L AA, with a bias voltage of 0 V and excitation wavelength of 405 nm. Each step variation of the photocurrent responses indicated that the prepared PEC immunosensor was effectively and successfully assembled.

EIS is an effective method to reflect electrode surface performance [32] and describe the electron transfer resistance of the electrode, which is measured in 5.0 mmol/L $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.10 mol/L KCl solution. Fig. 3B and Table 1 show the EIS Nyquist diagram of the layer-by-layer modified electrode. R_{et} values were reflected by the semicircle diameter of the EIS Nyquist diagram, which played an important role in interface characteristics. Therein, based on the impedance of the bare ITO electrode (curve a), a semicircular arc at the high frequency was displayed with almost a straight line at low frequency, indicating that the electrode surface was controlled by diffusion. With the modification of the semiconductor material $\text{BiVO}_4/\text{BiOBr}$ and quantum dot Ag_2S (curves b and c), the semicircular region increased due to the hindrance of electron transfer by the modified layer to a large extent, indicating the successful modification of the substrate material. The EDC/NHS of the insulating organic layer with amidation reaction

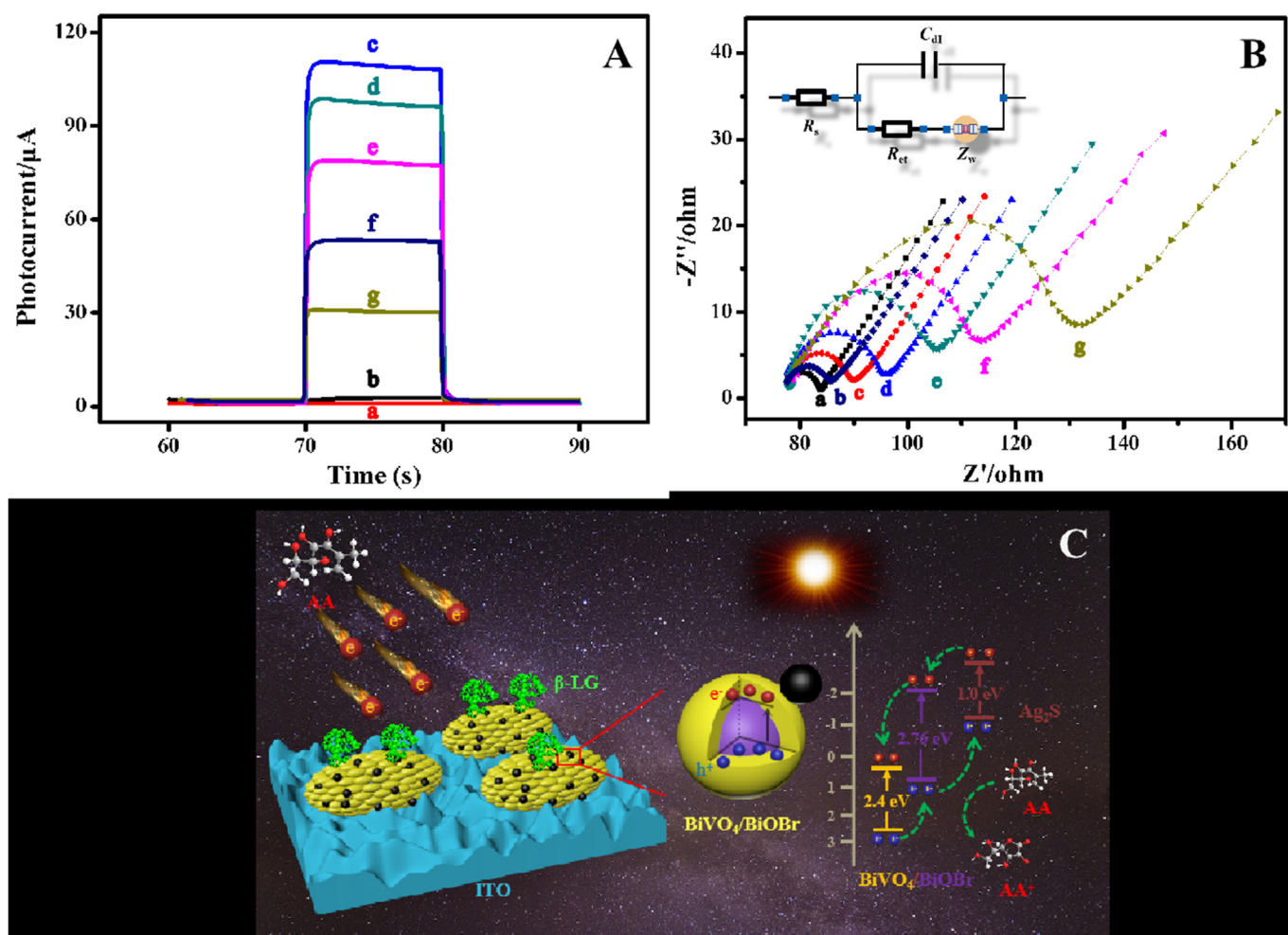


Fig. 3. (A) Photocurrent responses and (B) EIS Nyquist plots: (a) ITO, (b) ITO/BiVO₄/BiOBr, (c) ITO/BiVO₄/BiOBr@Ag₂S, (d) ITO/BiVO₄/BiOBr@Ag₂S/TGA/EDC-NHS, (e) ITO/BiVO₄/BiOBr@Ag₂S/TGA/EDC-NHS/Ab, (f) ITO/BiVO₄/BiOBr@Ag₂S/TGA/EDC-NHS/Ab/BSA, (g) ITO/BiVO₄/BiOBr@Ag₂S/TGA/EDC-NHS/Ab/BSA/β-LG. All measurements were made in a solution containing 5.0×10^{-3} mol/L [Fe(CN)₆]^{3-/4-}, 0.10 mol/L KCl. (C) The electron transfer mechanism diagram of PEC immunosensor based on Ag₂S sensitized BiVO₄/BiOBr heterojunction in AA-containing PBS solution.

Table 1
Simulation parameters of the equivalent circuit components.

Electrode	R_s (Ω)	R_{et} (Ω)	C_{dl} (F)	Z_w
ITO	64.18	5.957	3.629×10^{-6}	0.02448
ITO/BiVO ₄ /BiOBr	70.71	8.062	4.384×10^{-6}	0.01364
ITO/BiVO ₄ /BiOBr@Ag ₂ S	60.71	11.48	3.762×10^{-6}	0.0208
ITO/BiVO ₄ /BiOBr@Ag ₂ S/TGA/EDC-NHS	89.11	16.87	3.193×10^{-6}	0.01688
ITO/BiVO ₄ /BiOBr@Ag ₂ S/TGA/EDC-NHS/Ab	81.77	23.92	4.418×10^{-6}	0.01143
ITO/BiVO ₄ /BiOBr@Ag ₂ S/TGA/EDC-NHS/Ab/BSA	78.61	28.79	6.466×10^{-6}	0.01201
ITO/BiVO ₄ /BiOBr@Ag ₂ S/TGA/EDC-NHS/Ab/BSA/β-LG	114.3	46.15	4.255×10^{-6}	0.01041

was used as a linker between Ag₂S and the antibody, and the R_{et} value was further increased (curve d). With the layer-by-layer modification of Ab, BSA, and β-LG, impedance values increased significantly (curves e, f, and g) because the presence of BSA, antibody and antigen bioprotein molecules hindered electron transfer. In summary, every step change in the semicircular region (R_{et} value) was effective evidence for the successful construction of the PEC immunosensor.

Fig. 3C shows the possible electron transfer mechanism of the hierarchical structure BiVO₄/BiOBr p-n heterojunction. When electrons were excited to the conduction band of BiVO₄ and BiOBr

under visible light irradiation, electrons and holes would be transferred to the conduction band of BiVO₄ and the valence band of BiOBr due to the higher valence band and conduction band of BiOBr. The valence band and conduction band of Ag₂S were higher than those of the BiVO₄/BiOBr p-n heterojunction. Therefore, the photogenic electrons generated by Ag₂S were quickly transferred to the conduction band of the BiVO₄/BiOBr p-n heterojunction and injected into the external circuit through ITO glass. At the same time, the holes generated by the p-n heterojunction would be transferred to the valence band of the Ag₂S and sacrificed by the electron donor AA in the solution [33,34]. This synergistic effect

between the $\text{BiVO}_4/\text{BiOBr}$ p-n heterojunction and Ag_2S could reduce the photogenerated electron/hole (e^-/h^+) recombination efficiency of the $\text{BiVO}_4/\text{BiOBr}$ p-n heterojunction and greatly increase the photocurrent signal. The specific binding of the β -LG antibody to β -LG blocked the transfer of electrons from AA to the $\text{BiVO}_4/\text{BiOBr}@ \text{Ag}_2\text{S}$ composite material, resulting in decreased photocurrent due to e^-/h^+ recombination. With increasing β -LG concentration, the photocurrent intensity gradually decreased. Therefore, the assembled PEC immunosensor could be used for the quantitative detection of β -LG.

3.3. Optimization of the experimental conditions of the label-free immunosensor

To obtain the best performance of the sensor for analyzing β -LG, the optimized experimental conditions in Fig. 4 include the concentration of $\text{BiVO}_4/\text{BiOBr}$ and AgNO_3 , AA and the pH of PBS.

To optimize the thickness of the $\text{BiVO}_4/\text{BiOBr}$ heterojunction, ITO/ $\text{BiVO}_4/\text{BiOBr}$ electrodes with different concentrations of $\text{BiVO}_4/\text{BiOBr}$ suspensions were researched. It can be seen from Fig. 4A that the photocurrent signal increased until it reached 5 mg/mL with the increase in the spindle-shaped $\text{BiVO}_4/\text{BiOBr}$, but the photocurrent

decreased as the concentration further increased. Hence, 5 mg/mL was chosen as the optimal concentration of spindle-shaped $\text{BiVO}_4/\text{BiOBr}$.

Fig. 4B shows that as the concentration of the AgNO_3 solution increased from 0.02 to 0.1 mol/L, the photocurrent signal of the ITO/ $\text{BiVO}_4/\text{BiOBr}@ \text{Ag}_2\text{S}$ electrode increased continuously. When the concentration of the AgNO_3 solution was 0.1 mol/L, the maximum photocurrent was approximately 115.90 μA . However, the photocurrent signal decreased significantly as the solution concentration continued to increase. In this experiment, Ag^+ could be completely converted to Ag_2S precipitation by excessive Na_2S solution and deposited on the ITO/ $\text{BiVO}_4/\text{BiOBr}$ electrode. When the AgNO_3 solution concentration exceeded 0.1 mol/L, Ag_2S was excessively deposited on the ITO/ $\text{BiVO}_4/\text{BiOBr}$ electrode, which impeded the transport of electrons, could not absorb external light energy, and reduced the yield of e^-/h^+ . Therefore, 0.1 mol/L AgNO_3 solution was selected as the best concentration for subsequent experiments.

Fig. 4C shows the photocurrent response of the immunosensor in PBS solution with different pH values ranging from 5.6 to 8.0. The test environment of PBS solution with a pH of 7.4 was similar to the physiological environment of proteins and was suitable for fixing proteins.

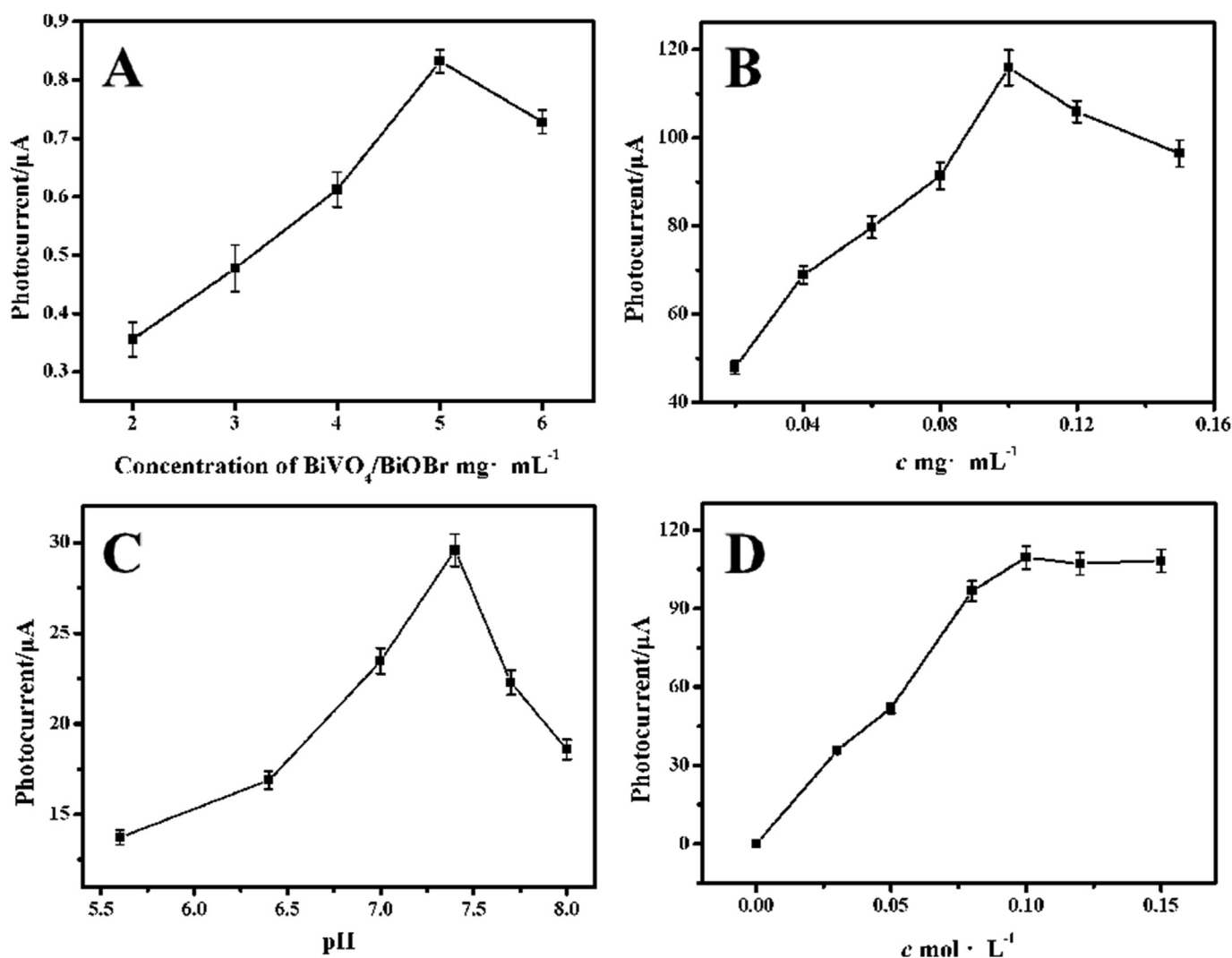


Fig. 4. Effects of (A) concentration of $\text{BiVO}_4/\text{BiOBr}$ heterojunction, (B) concentration of the AgNO_3 solution, (C) pH on the photocurrent response ITO/ $\text{BiVO}_4/\text{BiOBr}@ \text{Ag}_2\text{S}/\text{TGA}/\text{EDC}-\text{NHS}/\text{Ab}/\text{BSA}/\beta\text{-LG}$ electrodes and (D) concentration of AA in the PBS buffer solution on the photocurrent response of ITO/ $\text{BiVO}_4/\text{BiOBr}@ \text{Ag}_2\text{S}$ electrodes.

As shown in Fig. 4D, AA, as an effective electron donor, inhibited the e^-/h^+ combination of $\text{BiVO}_4/\text{BiOBr}/\text{Ag}_2\text{S}$ composites. The photocurrent response of the $\text{ITO}/\text{BiVO}_4/\text{BiOBr}/\text{Ag}_2\text{S}$ electrode increased gradually as the concentration increased. When the concentration was 0.1 mol/L, the electron donor's ability to provide electrons tended to be saturated. Therefore, in terms of consumable consumption and photocurrent response, 0.1 mol/L was chosen as the optimal PBS concentration.

In summary, the optimal conditions for this experiment were determined. A 5 mg/mL $\text{BiVO}_4/\text{BiOBr}$ suspension, 0.1 mol/L AgNO_3 solution, PBS solution (7.4 pH) and 0.1 mol/L AA were the final experimental conditions.

3.4. Label-free PEC immunosensor determination of β -LG

Under the optimal experimental conditions, different concentrations of β -LG antigen were detected by the sensor. As seen from the 3D photocurrent response diagram (Fig. 5A), the photocurrent signal was highly correlated with the concentration of the β -LG antigen. As shown in Fig. 5B, the photocurrent response decreased linearly with the logarithm of β -LG antigen concentration from 0.01 to 100 ng/mL. All photoelectric chemical measurements were made in PBS solution (pH = 7.4) containing 0.1 mol/L AA, with a bias voltage of 0 V.

The linear equation was $I = 33.64 - 6.86 \lg c(c_{\beta\text{-LG}}, \text{ng/mL})$ with a correlation coefficient $R^2 = 0.9934$. The detection limit of the prepared label-free photoelectrochemical immunosensor was 3.7 pg/mL (according to the equation $C_L = 10^{(xb1+3sb1-b)/a}$, where C_L was the detection limit).

This sensor was superior to other reported detection methods, as listed in Table 2. The results showed that the PEC immunosensor had good application prospects in dairy product detection.

3.5. Selectivity, stability and reproducibility

To test the stability of the sample, the prepared immunosensor was stored in a 4 °C refrigerator. The photocurrent response of the incubated 5 ng/mL β -LG immunosensor was evaluated at 15 on/off irradiation cycles of 300 s (Fig. 5C). There was no significant change in the photocurrents, indicating that the prepared immunosensor had a stable photocurrent response.

In order to study the repeatability of the sensor, several electrodes were modified under the same conditions to test the photocurrent signal. As shown in Fig. 5D, the relative standard deviation (RSD) of the immunosensor was less than 2.69%, which provided the manufactured PEC immunosensor with obvious high reproducibility.

As shown in Fig. 5E, the assembled PEC immunosensor was evaluated for selectivity to assess the prospect of practical application value. The mixed photocurrent responses were studied including 1 ng/mL β -LG mixed separately with 100 ng/mL casein, 100 ng/mL α -lactalbumin, and 100 ng/mL cow's milk immunoglobulin (IgE). The photocurrent was not affected by the added interferences. In summary, the sensor had high sensitivity, good stability, good selectivity and specificity.

3.6. Real sample analysis

To explore the practical application value of the prepared immunosensor, the recovery rate of milk was tested. The actual samples were tested by dilution from commercially available milk. The relevant data are shown in Table 3. Under optimal experimental conditions (PBS solution (pH = 7.4) containing 0.1 mol/L AA), the RSD data were between 1.02% and 1.94% and the recovery rate was between 96.7% and 104.2%. Therefore, the prepared immunosensor has potential application value in practical applications.

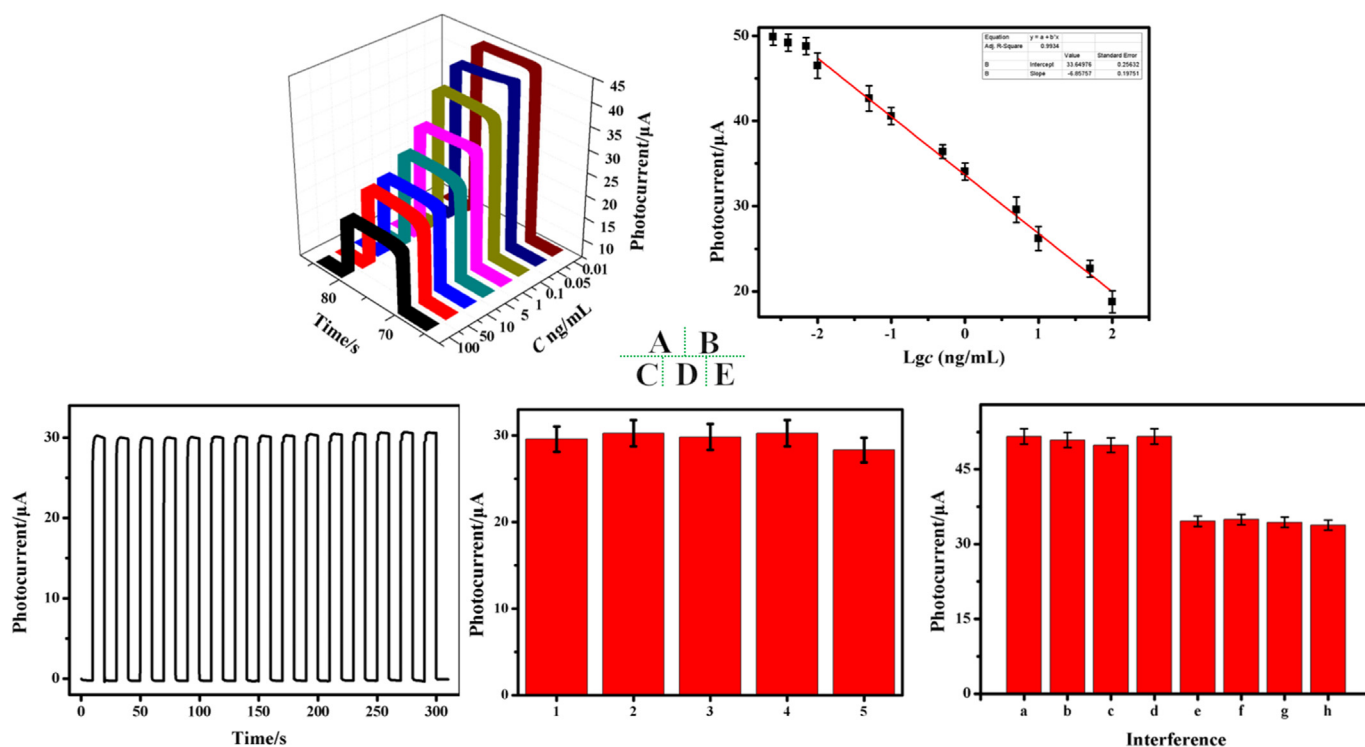


Fig. 5. (A) Photocurrent response curve and (B) Logarithmic calibration curve for immunosensors for β -LG detection from 0.01 to 100 ng/mL. (C) Stability assessment of the immunosensor at 15 on/off irradiation cycles, $c_{\beta\text{-LG}} = 5$ ng/mL, (D) Study on the reproduction of the immunosensor. Error bar = SD ($n = 5$). (E) Selectivity of the immunosensor to detect β -LG, (a) Blank, (b) 100 ng/mL casein, (c) 100 ng/mL α -lactalbumin, (d) 100 ng/mL cow's milk IgE, (e) 1 ng/mL β -LG, (f) 1 ng/mL β -LG + 100 ng/mL casein, (g) 1 ng/mL β -LG + 100 ng/mL α -lactalbumin, (h) 1 ng/mL β -LG + 100 ng/mL cow's milk IgE.

Table 2
Comparison of immunosensor methods for β -LG detection.

Method	Linear range	Detection limit	Reference
ELISA	0.078–10 μ g/mL	114 ng/mL	8
Graphene-based biosensor	$0.1-1 \times 10^4$ ng/mL	20 pg/mL	[35]
Sandwich enzyme-linked immunosorbent assay	$0.49-1.6 \times 10^4$ ng/mL	0.12 ng/mL	[36]
Ultra-high performance liquid chromatography method	20–560 μ g/mL	20 μ g/mL	[37]
Liquid chromatography-tandem mass spectrometry	0.48–31.25 μ g/mL	0.2 μ g/mL	10
Electrochemical magnetosensing platform	2.8–100 ng/mL	0.8 ng/mL	[38]
Label-free photoelectrochemical immunosensor	0.01–100 ng/mL	3.7 pg/mL	This method

Table 3
The results of the β -LG determination in milk sample.

β -LG Concentration (ng/mL)	Added β -LG (ng/mL)	Measured concentration (ng/mL, n = 3)	Relative standard deviation (%)	Recovery (%)
3.84	0.1	4.02, 3.87, 3.92	1.94%	96.7%
	2	5.94, 5.82, 5.89	1.02%	104.2%
	5	8.72, 8.82, 8.98	1.48%	100.8%

4. Conclusion

In summary, a novel label-free photoelectrochemical immunosensor based on BiVO₄/BiOBr@Ag₂S was fabricated to detect allergen β -LG in dairy products. The spindle-like BiVO₄/BiOBr heterojunction photoactive matrix sensitized by Ag₂S could reduce the recombination rate of e[−]/h⁺ and thereby effectively improve the photoelectric conversion rate and its sensitivity. The immunosensor could accurately detect the β -LG allergen in the range of 0.01–100 ng/mL with a detection limit of 3.7 pg/mL. The constructed sensor had the advantages of good stability, wide linear range, high sensitivity, and good reproducibility. This work provides a new perspective for future PEC construction strategies and supplies a reference for the detection and analysis of other allergens in foods.

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CRediT authorship contribution statement

Xiaokai Sun: Data curation, Investigation, Visualization, Writing - original draft. **Canguo Li:** Formal analysis, Methodology. **Qiyang Zhu:** Formal analysis, Methodology. **Haowei Huang:** Investigation. **Wei Jing:** Investigation. **Zhiwei Chen:** Project administration, Resources. **Ling Kong:** Supervision, Writing - review & editing. **Lei Han:** Supervision, Writing - review & editing. **Jun Wang:** Writing - review & editing. **Yueyun Li:** Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aca.2020.10.021>.

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1. Screen captures of the original data

Fig. 1 showed a summary of the original current graph (Fig. 2-10). The data was selected according to the fourth part of the spectrum, which included three parallel samples with three concentrations. Table 1 showed the current measured by the BIN format file and the calculated concentration. According to the experimental equation: $I = 33.64 - 6.86 \lg c$ ($c_{\beta\text{-LG}}$, ng/mL). The concentration of β -lactoglobulin in the actual sample could be calculated.

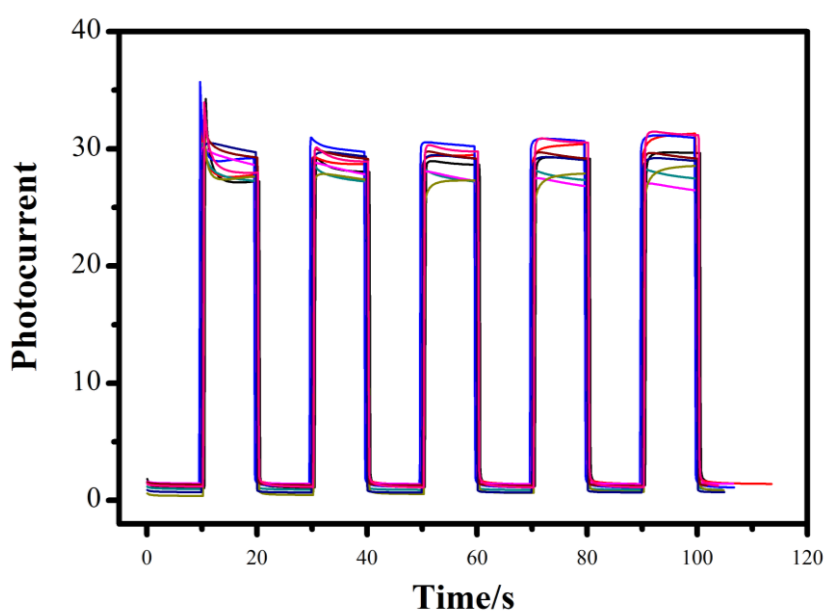


Fig. 1. Summary of raw photocurrent signals

Table 1. Photocurrent and corresponding concentration

Measured current (μA)	Measured concentration (ng/mL)
29.50, 29.61, 29.57	4.02, 3.87, 3.92
28.33, 28.40, 28.36	5.94, 5.82, 5.89
27.19, 27.15, 27.10	8.72, 8.82, 8.98

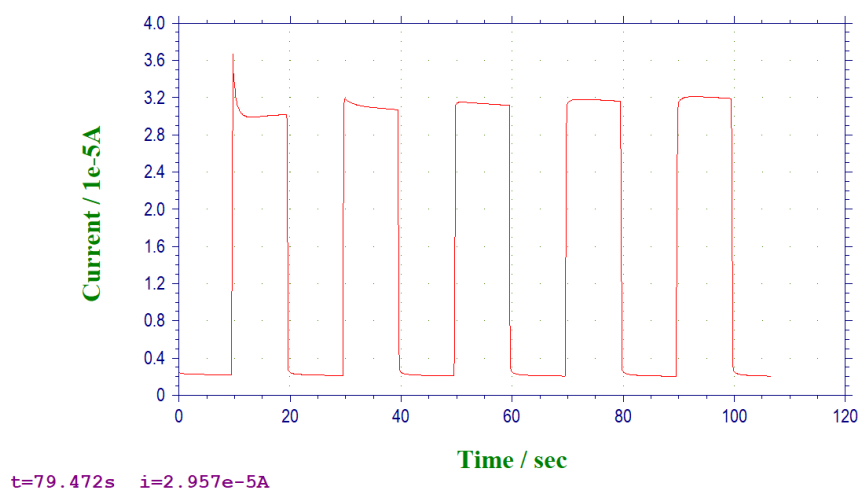


Fig. 2. The original photocurrent response curve

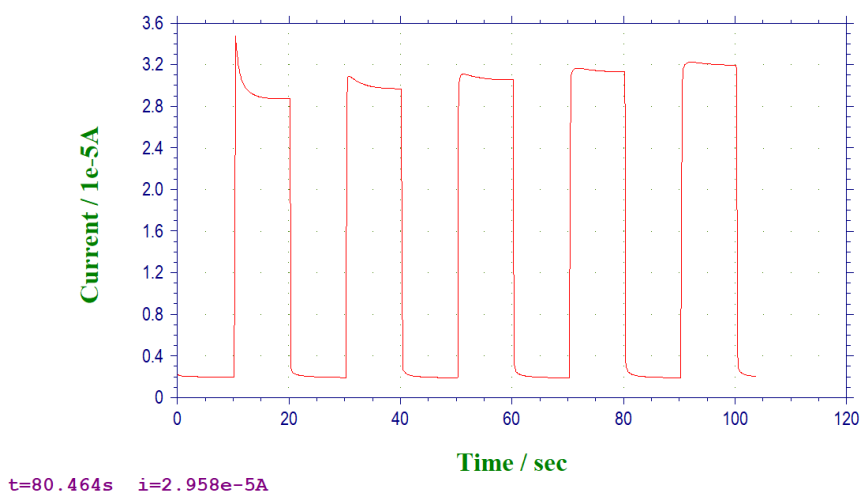


Fig. 3. The original photocurrent response curve

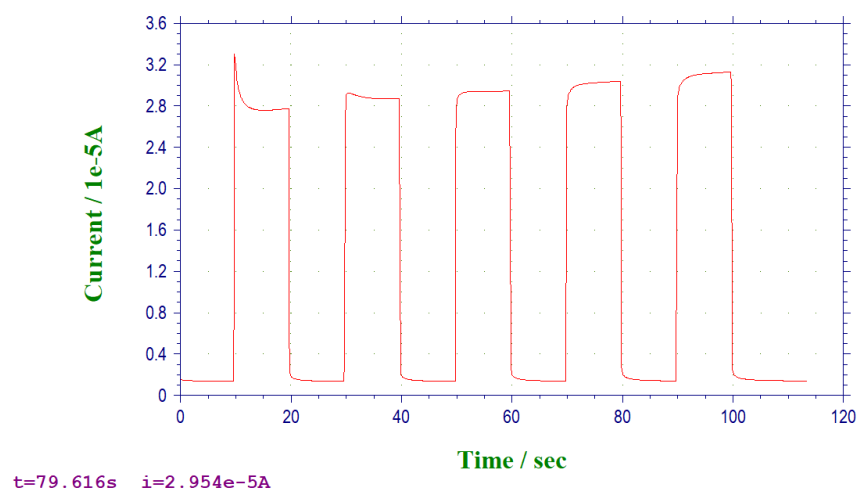


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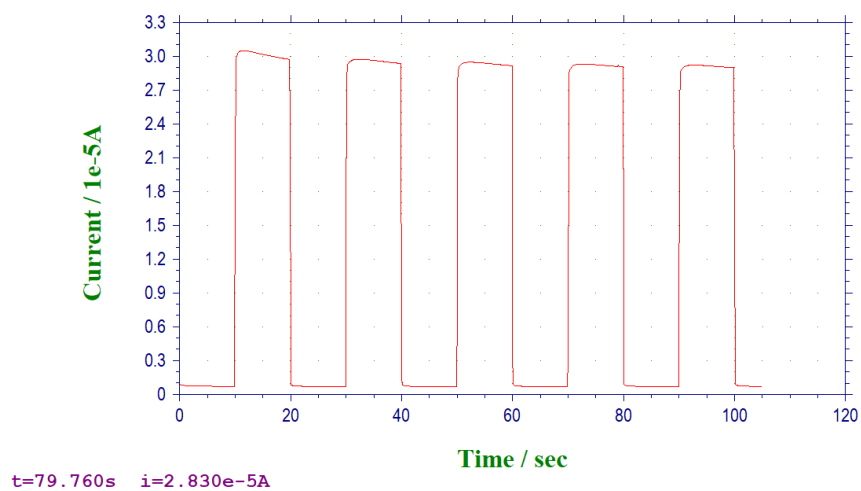


Fig. 5. The original photocurrent response curve

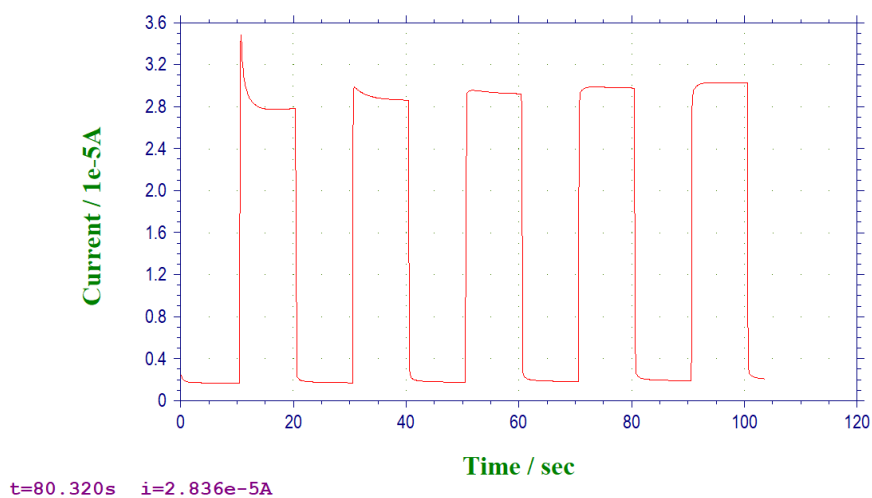


Fig. 6. The original photocurrent response curve

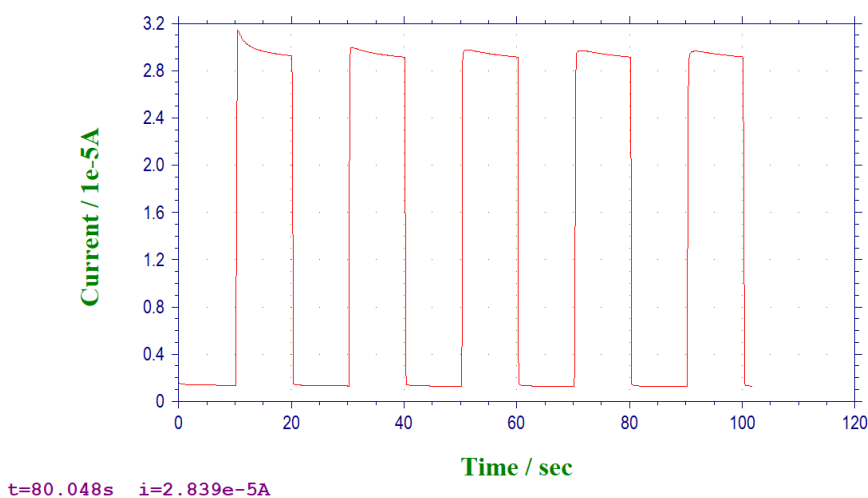


Fig. 7. The original photocurrent response curve

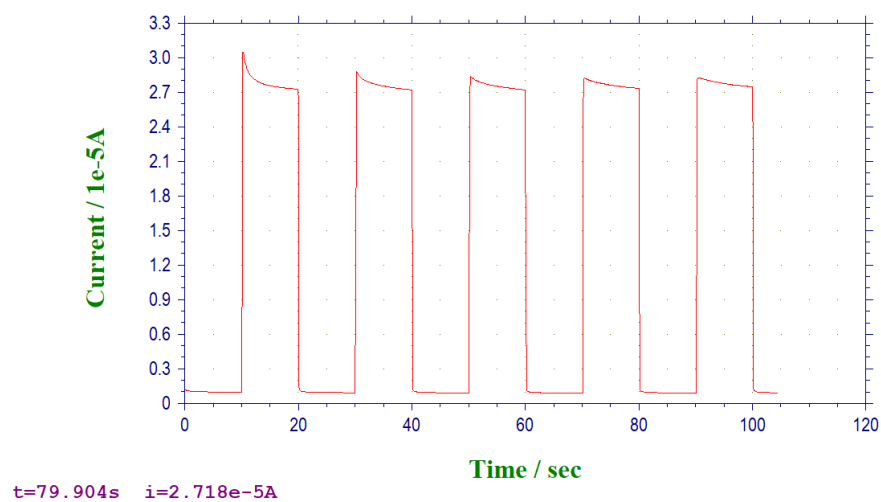


Fig. 8. The original photocurrent response curve

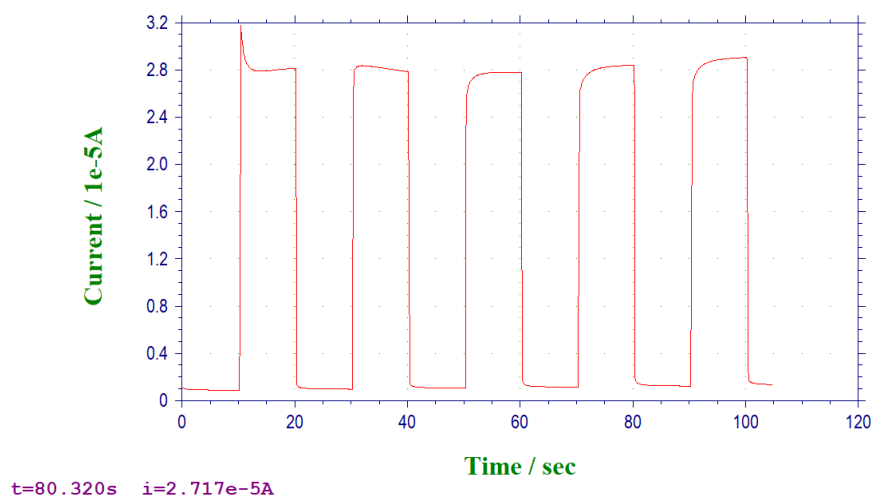


Fig. 9. The original photocurrent response curve

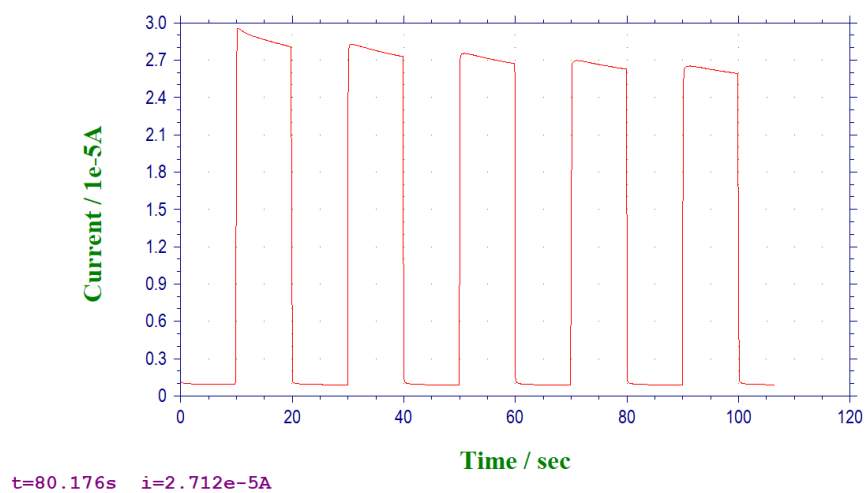


Fig. 10. The original photocurrent response curve